

Glutamine kinetics and protein turnover in end-stage renal disease

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Raj, Dominic S. C., Tomas Welbourne, Elizabeth A. Dominic, Debra Waters, Robert Wolfe, and Arny Ferrando. Glutamine kinetics and protein turnover in end-stage renal disease. *Am J Physiol Endocrinol Metab* 288: E37–E46, 2005. First published July 20, 2004; doi:10.1152/ajpendo.00240.2004.—Alanine and glutamine constitute the two most important nitrogen carriers released from the muscle. We studied the intracellular amino acid transport kinetics and protein turnover in nine end-stage renal disease (ESRD) patients and eight controls by use of stable isotopes of phenylalanine, alanine, and glutamine. The amino acid transport kinetics and protein turnover were calculated with a three-pool model from the amino acid concentrations and enrichment in the artery, vein, and muscle compartments. Muscle protein breakdown was more than synthesis ($\text{nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$) during hemodialysis (HD) (169.8 ± 20.0 vs. 125.9 ± 21.8 , $P < 0.05$) and in controls (126.9 ± 6.9 vs. 98.4 ± 7.5 , $P < 0.05$), but synthesis and catabolism were comparable pre-HD (100.7 ± 15.7 vs. 103.4 ± 14.8). Whole body protein catabolism decreased by 15% during HD. The intracellular appearance of alanine (399.0 ± 47.1 vs. 243.0 ± 34.689) and glutamine (369.7 ± 40.6 vs. 235.6 ± 27.5) from muscle protein breakdown increased during dialysis ($\text{nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$, $P < 0.01$). However, the de novo synthesis of alanine ($3,468.9 \pm 572.2$ vs. $3,140.5 \pm 467.7$) and glutamine ($1,751.4 \pm 82.6$ vs. $1,782.2 \pm 86.4$) did not change significantly intradialysis ($\text{nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$). Branched-chain amino acid catabolism (191.8 ± 63.4 vs. -59.1 ± 42.9) and nonprotein glutamate disposal (347.0 ± 46.3 vs. 222.3 ± 43.6) increased intradialysis compared with pre-HD ($\text{nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg}^{-1}$, $P < 0.01$). The mRNA levels of glutamine synthase (1.45 ± 0.14 vs. 0.33 ± 0.08 , $P < 0.001$) and branched-chain keto acid dehydrogenase-E2 (3.86 ± 0.48 vs. 2.14 ± 0.27 , $P < 0.05$) in the muscle increased during HD. Thus intracellular concentrations of alanine and glutamine are maintained during HD by augmented release of the amino acids from muscle protein catabolism. Although muscle protein breakdown increased intradialysis, the whole body protein catabolism decreased, suggesting central utilization of amino acids released from skeletal muscle.

amino acids; protein catabolism; nitrogen carriers; hemodialysis

ALANINE AND GLUTAMINE play a central role in substrate recycling and interorgan nitrogen metabolism. Together, they comprise <15% of amino acid content of protein, yet they account for ~60% of the amino nitrogen leaving the muscle (10). In disease states, glutamine consumption exceeds the release from the muscle (19). Branched-chain amino acids (BCAA) include valine, leucine, and isoleucine. Metabolism of BCAA, alanine, glutamine, and glutamate are interrelated (Fig. 1). Ferrando et

al. (16) demonstrated an inverse relationship between alanine and glutamine production in the muscle in patients with burn. The precursors for glutamine synthesis include glutamate, α -ketoglutarate, free ammonia, and amino nitrogen derived from catabolism of BCAA. On the other hand, pyruvate is transaminated with glutamate to generate alanine and α -ketoglutarate. In catabolic states, net synthesis of glutamine is decreased, but de novo alanine synthesis is increased. Thus alanine becomes the major carrier of nitrogen from skeletal muscle to body tissues in conditions of prolonged severe illness (5). The transported nitrogen may be utilized for protein synthesis in the splanchnic region.

A number of alterations in plasma and intracellular amino acid levels, as well as amino acid exchange across peripheral tissues in postabsorptive (12) and fed state (13), have been described in chronic renal failure. Preliminary evidence indicates that metabolism of BCAA, alanine, and glutamine is altered in end-stage renal disease (ESRD) (3). Results from our laboratory (34, 35) and from other investigators (23, 29) have shown that hemodialysis (HD) induces muscle protein catabolism. BCAA and glutamine have been shown to have a regulatory effect on protein turnover (25, 32). Despite their qualitative and quantitative importance, the kinetics of alanine and glutamine in ESRD are largely unknown. Examining the metabolism of glutamine, alanine, and muscle protein simultaneously in ESRD patients compared with normal volunteers will allow better understanding of their metabolism and its relationship, if any, to muscle wasting in ESRD. We sought to determine the relationship between skeletal muscle and whole body protein turnover during HD and the resultant effects on glutamine and alanine metabolism. Our previous work focused on protein turnover and amino acid transport kinetics. The present study is designed specifically to examine the metabolism of alanine and glutamine in ESRD.

MATERIALS AND METHODS

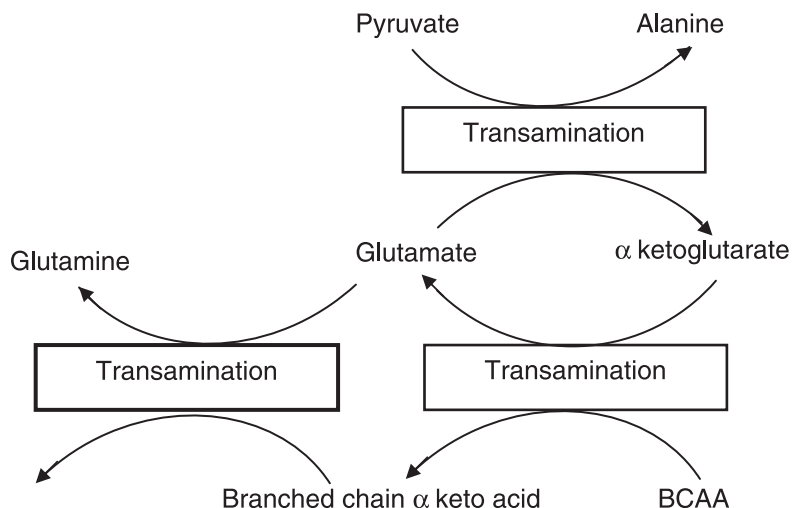
Study Subjects and Methods

The study was approved by the Institutional Review Board at the University of New Mexico Health Sciences Center. Informed consent was obtained from all the study participants. Study subjects included nine stable ESRD patients and eight healthy volunteers. There were two and three subjects with type 2 diabetes mellitus (T2DM) among controls and ESRD patients, respectively. Studies were performed in

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Fig. 1. Glutamate plays a central role in the synthesis of alanine and glutamine. BCAA, branched-chain amino acids.



a postabsorptive state. The participants were admitted to the General Clinical Research Center at the University of New Mexico Health Sciences Center 1 day before the study. Leg volume was estimated as described previously (6, 14). A 3-day dietary history was obtained by an experienced dietician. Polyethylene catheters were inserted in the femoral artery and vein on the same side. The femoral arterial catheter was used for infusion of indocyanine green (ICG). Catheters were also placed in the nonaccess forearm veins for infusion of labeled amino acids and in the right wrist vein for arterialized blood sampling.

After a blood sample was obtained for background amino acid enrichment, a primed continuous infusion of L-[ring- $^{13}\text{C}_6$]phenylalanine (prime 2 $\mu\text{mol/kg}$, infusion 0.1 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), L-[1- ^{13}C]alanine (prime 35 $\mu\text{mol/kg}$, infusion 0.35 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), and L-[5- ^{15}N]glutamine (prime 63 $\mu\text{mol/kg}$, infusion 0.35 $\mu\text{mol/kg}$) was initiated and continued

throughout the experiment (Fig. 2). Blood flow to the lower extremity was measured by dye dilution technique (26). Leg plasma flow was calculated from steady-state ICG concentrations in the femoral artery and arterialized wrist vein.

Controls were not exposed to dialysis. ESRD patients were studied before (pre-HD) and during HD. HD was initiated at 300 min and continued for 4 h. Patients were dialyzed using their usual blood and dialysate flow rates by means of a new polysulfone membrane (F70; Fresenius, Hemoflow). Dialysate composition was as follows: sodium (Na^+) 139 meq/l, bicarbonate (HCO_3^-) 35 meq/l, calcium (Ca^{2+}) 2.5 meq/l, magnesium (Mg^{2+}) 1 meq/l, dextrose 200 mg/dl, and potassium (K^+) per patient's need. A representative spent-dialysate sample was collected throughout the dialysis. At the end of treatment, the dialysate samples were mixed well and stored at -70°C for analysis of amino acids.

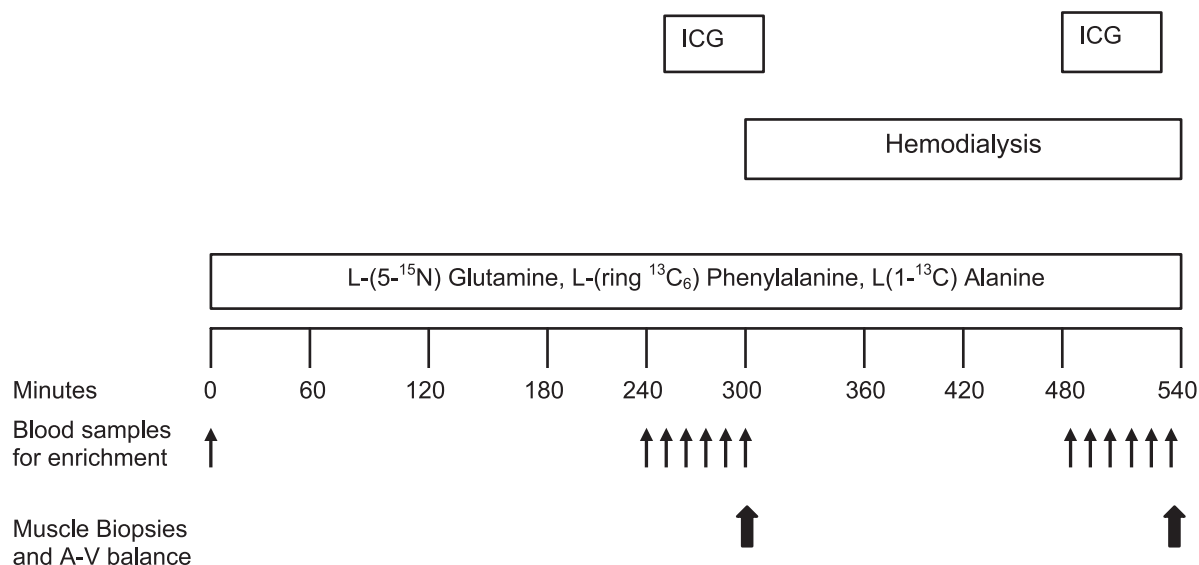


Fig. 2. Study design. End-stage renal disease (ESRD) patients were studied before the hemodialysis phase (pre-HD; postabsorptive phase, 0–300 min) and during HD phase (HD; 300–540 min). After blood samples were obtained for background at 0 min, a primed continuous infusion of L-[5- ^{15}N]glutamine, L-[ring- $^{13}\text{C}_6$]phenylalanine, and L-[1- ^{13}C]alanine was started and continued throughout the experiment. From 240 to 300 min and from 480 to 540 min, blood samples were obtained from artery and vein to estimate enrichment and arteriovenous balance and measure protein kinetics. Muscle biopsies were obtained at 300 and 540 min. A continuous infusion of indocyanine green (ICG) was administered into the femoral artery between 240 and 270 min and 480 and 510 min to estimate the blood flow rate to the leg. Blood samples were taken every 10 min from femoral and wrist veins from 240 to 270 and from 480 and 510 min to estimate ICG concentration. Controls were studied only during postabsorptive phase.

Muscle biopsies were performed at 300 min (pre-HD) and 540 min (HD) in ESRD patients. Controls had only one biopsy at 300 min. Biopsies were taken from the lateral portion of vastus lateralis muscle ~20 cm above the knee with a Bergstrom biopsy needle. Fat and connective tissue were removed, and the samples were frozen in liquid nitrogen and stored at -80°C for future analysis. Blood urea nitrogen, creatinine, electrolytes, and hemoglobin concentrations were measured. Serum albumin was measured by the bromocresol green method, catecholamines by RIA, and insulin and cortisol by immulite chemiluminescence.

Analytic Procedures

Blood samples for the measurement of amino acid concentrations and enrichment were collected as previously described (6). A known amount of an internal standard mixture (100 µl/ml blood) was added for measurement of amino acid concentrations. The composition of the internal standard was as follows: 1.13 mmol/l [U-¹³C₉,¹⁵N]phenylalanine, 21.55 mmol/l [²H₄]alanine, and 10.00 mmol/l [U-¹³C₅]glutamine. Blood amino acids were separated using cation exchange chromatography (41). The enrichment of free amino acids in the arterial and venous samples was determined by gas chromatography-mass spectrometry (GC-MS; GC HP 5890, MSD HP 5989; Hewlett Packard, Palo Alto, CA) by selected ion [mass-to charge ratio (*m/z*)] monitoring. Chemical ionization was used for nitrogen-acetyl-*n*-propyl ester derivatives of phenylalanine (*m/z* 336, 342, and 346) and alanine (*m/z* 260, 261, and 264). A separate aliquot of the sulfosalicylic acid supernatant extract was processed to obtain the *t*-butyldimethylsilyl (*t*-BDMS) derivative of glutamine (41). Electron impact ionization was used for the *t*-BDMS derivative of glutamine (*m/z* 431, 432, and 436).

Blood samples from femoral artery and vein were collected into lithium-heparin tubes for estimating the arteriovenous balance. Sera-prep (Pickering Laboratories) was added to the samples to prevent hydrolysis of L-glutamine, and samples were stored at -70°C until analysis. Free amino acid concentrations were determined by HPLC

(2960 system; Waters, Milford, MA) and precolumn derivatization and *o*-phthalaldehyde and 3-mercaptopropionic acid.

Muscle samples were weighed and the proteins precipitated with 450 µl of 14% perchloric acid. An internal standard solution (2 µl/mg muscle tissue) was added to measure the intracellular concentrations of the traced amino acids. The internal standard solution contained 3.00 µmol/l [U-¹³C₉,¹⁵N]phenylalanine, 92.3 µmol/l [²H₄]alanine, and 775.0 µmol/l [U-¹³C₅]glutamine. The tissue was homogenized and centrifuged, and the supernatant was collected. The enrichment and concentration of amino acids were determined as described before (6).

Kinetic Modeling

Muscle parameters. Muscle amino acid kinetics were calculated using a three-pool model (6, 34) (Fig. 3). Amino acids enter the leg through the femoral artery (*F_{in}*) and leave the leg via the femoral vein (*F_{out}*):

$$F_{in} = C_a \cdot BF$$

$$F_{out} = C_v \cdot BF$$

where *C_a* and *C_v* are free amino acid concentrations in the artery and vein, respectively. *BF* is the blood flow to the leg.

Net balance (*NB*) was calculated as

$$NB = (C_a - C_v) \cdot BF$$

Data derived from muscle biopsy were used to modify the traditional arteriovenous balance approach to calculate inward (*F_{ma}*) and outward transport (*F_{vm}*) rates:

$$(C_a - C_v) \cdot BF = F_{ma} - F_{vm}$$

$$(C_a \cdot E_a - C_v \cdot E_v) \cdot BF = F_{ma} \cdot E_a - F_{vm} \cdot E_m$$

where *E_a*, *E_v*, and *E_m* are amino acid enrichments in the femoral artery, vein, and muscle, respectively. The equation was solved to

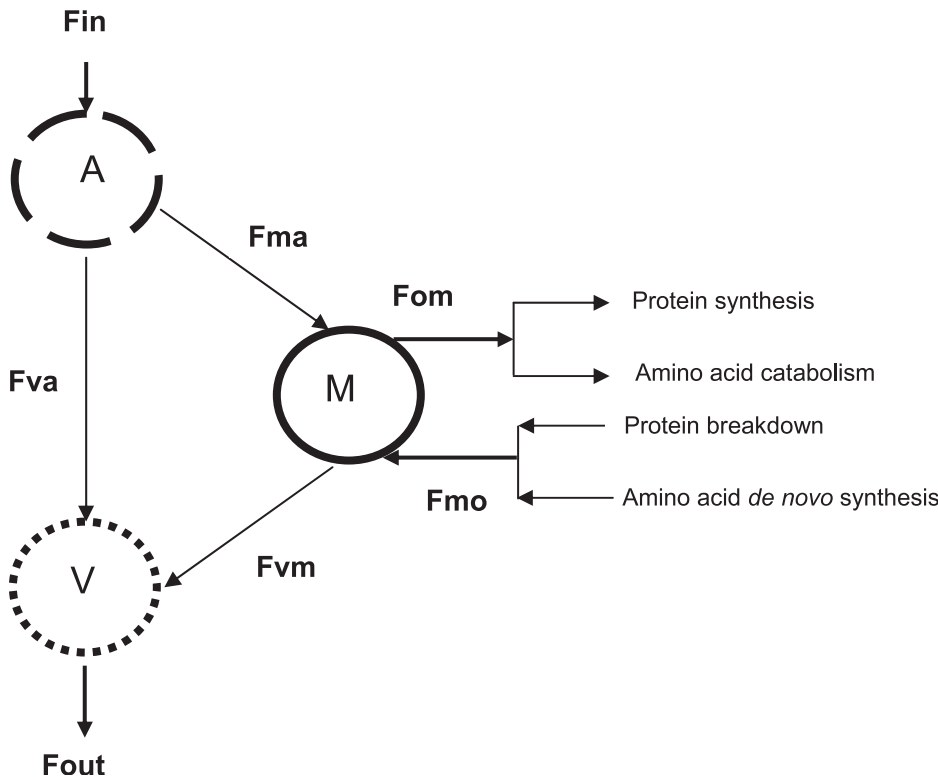


Fig. 3. Three-pool model for calculation of amino acid transport kinetics: A 3-compartmental model with unidirectional flow of amino acids between artery (A), vein (V), and muscle (M) compartments is shown. Amino acids enter the leg through femoral artery (*F_{in}*) and exit through the femoral vein (*F_{out}*). *F_{va}*, shunt to the vein from the artery; *F_{ma}*, inward transport of amino acid to the muscle from the artery; *F_{vm}*, outward transport of amino acids to the vein from muscle intracellular free pool. *F_{mo}*, rate of intracellular appearance of amino acids in the intracellular compartment; it represents protein breakdown for phenylalanine, but proteolysis and *de novo* synthesis for glutamine and alanine. *F_{om}*, rate of utilization of amino acids in the intracellular compartment; it represents incorporation of amino acids into protein for phenylalanine and protein synthesis, also intracellular catabolism for alanine and glutamine.

derive F_{ma} (inward transport) and F_{vm} (outward transport):

$$F_{ma} = \left[\left(\frac{E_m - E_v}{E_a - E_m} \cdot C_v \right) + C_a \right] \cdot BF$$

$$F_{vm} = \left[\left(\frac{E_m - E_v}{E_a - E_m} \cdot C_v \right) + C_v \right] \cdot BF$$

The intracellular amino acid appearance [from protein breakdown, and/or de novo synthesis (F_{mo})] was derived as follows:

$$F_{mo} = F_{ma} \cdot (E_q/E_m - 1)$$

The rate of intracellular amino acid utilization (F_{om}) was estimated as

$$F_{om} = (C_a \cdot E_a - C_v \cdot E_v) \cdot BF/E_m$$

F_{om} and F_{mo} represent different metabolic pathways for each amino acid. Phenylalanine is neither synthesized de novo nor degraded in the muscle. Hence, F_{om} represents protein synthesis, and F_{mo} reflects intracellular appearance from protein degradation for phenylalanine.

Protein synthesis efficiency, an estimate of the fraction of amino acid that appears in the intracellular pool that is directed to protein synthesis is calculated as

$$\text{protein synthesis efficiency} = F_{om}/(F_{ma} + F_{mo})$$

For alanine and glutamine, F_{mo} represents the sum of de novo synthesis and appearance from proteolysis. We have assumed that amino acids are released from proteolysis in proportion to their relative content in muscle protein. The rates of alanine and glutamine appearance from protein breakdown (PB) were calculated as

$$PB_{Ala} = F_{moPhe} \cdot MR_{Ala/Phe}$$

$$PB_{Gln} = F_{moPhe} \cdot MR_{Gln/Phe}$$

where $MR_{Ala/Phe}$ and $MR_{Gln/Phe}$ are the ratios between alanine and phenylalanine and between glutamine and phenylalanine content in muscle protein, respectively. Each 100 g of muscle protein contains ~23 mmol of phenylalanine and 65 mmol of alanine (molar ratio 2.8) and 46 mmol of glutamine (molar ratio 2.0) (6).

The rates of alanine and glutamine de novo synthesis (DNS) were calculated as follows:

$$DNS_{Ala} = F_{moAla} - PB_{Ala}$$

$$DNS_{Gln} = F_{moGln} - PB_{Gln}$$

The rates of release of glutamate and the BCAA from protein catabolism were calculated from the rate of phenylalanine release, corrected for the molar ratios of glutamate to phenylalanine, leucine to phenylalanine, isoleucine to phenylalanine, and valine to phenylalanine (7):

$$\text{Net nonprotein glutamate disposal} = -[\text{net glutamate balance} \\ - (\text{net phenylalanine balance} \cdot 1.87)]$$

$$\text{Leucine catabolism} = [\text{net leucine balance} \\ - (\text{net phenylalanine balance} \cdot 3.10)]$$

$$\text{Isoleucine catabolism} = [\text{net isoleucine balance} \\ - (\text{net phenylalanine balance} \cdot 2.55)]$$

$$\text{Valine catabolism} = [\text{net valine balance} \\ - (\text{net phenylalanine balance} \cdot 1.77)]$$

$$\text{Total BCAA catabolism} = \text{leucine catabolism} \\ + \text{isoleucine catabolism} + \text{valine catabolism}$$

The calculated rate of BCAA catabolism (i.e., nonprotein BCAA disposal) is not equivalent to the actual rate of BCAA oxidation.

BCAA are deaminated to the corresponding keto acids before undergoing irreversible decarboxylation and oxidation. Some of the keto acid could escape into the blood stream. However, the difference between the rates of BCAA catabolism and oxidation is likely to be very small, because the arteriovenous difference of the leucine keto acid α -ketoisocaproic acid across the leg is small (38).

Because the tracer and the tracee are lost in the dialysate in equal amounts, it should not have any influence on the modeling equations (34).

Whole body parameters. The whole body appearance rate (R_a) of each amino acid was calculated from the rate of tracer infusion ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) divided by the arterial enrichment of the amino acid.

Whole body de novo synthesis rate was estimated as follows:

$$\text{for glutamine} = R_{aGln} - (2.0 \cdot R_{aPhe})$$

$$\text{for Alanine} = R_{aAla} - (2.8 \cdot R_{aPhe})$$

Gene expression of glutamine synthase and branched-chain keto acid dehydrogenase in the muscle. Muscle biopsy material (20 mg) was homogenized in Qiagen's RNeasy lysis buffer containing 2-mercaptoethanol at room temperature. RNA was isolated and purified using the Qiagen RNeasy miniprotocol for isolation of total RNA from muscle, which included proteinase K digestion and DNase treatment (Qiagen 74104, 19131, and 79254). By use of 500 ng of total RNA as template, cDNA was prepared by reverse transcriptase reaction using the ABI high-capacity cDNA archive kit (ABI 4322171). mRNA expression was assessed by the real-time PCR (RT-PCR) method using Applied Biosystems TaqMan Assays-on-Demand Gene Expression Products. Assays-on-Demand is a comprehensive collection of predesigned primer and probe sets available "off the shelf," and each assay consists of two unlabeled PCR primers and a FAM dye-labeled TaqMan MGB probe. PCR was performed on the MJ Research Opticon 2 instrument. Negative controls for RT-PCR included reactions containing no template. The analysis of relative gene expression data was performed using the relative gene curve method (30). The amount of target gene was normalized to an endogenous control (GAPDH).

Statistical Analysis

Data are presented as means \pm SE. Alpha was set at 0.05. Paired and unpaired *t*-tests were used when applicable. Repeated measures of analysis of variance (ANOVA) were used with pre- and post-HD as the repeating factor and ESRD vs. control as the grouping factor with a post hoc Tukey test. Linear regression analysis was used to identify the relation between variables.

RESULTS

The characteristics of the participants are described in Table 1. The etiology of ESRD was hypertension in two, diabetic nephropathy in two, glomerulonephritis in three, and unknown in two subjects. Hemoglobin concentration was significantly lower in ESRD patients. The glycated hemoglobin (Hb A_{1c}) levels in diabetic ESRD and controls were 7.4 ± 0.3 and $7.6 \pm 0.4\%$, respectively. Patients were adequately dialyzed, as evidenced by a urea reduction ratio of $73.6 \pm 2.1\%$. Plasma insulin level decreased and the cortisol level increased during HD compared with pre-HD levels.

The mean total amino acid concentration in the dialysate was $461.1 \pm 28.8 \mu\text{mol/l}$ (Table 2). The total amino acid concentrations ($\mu\text{mol/l}$) in the artery and vein during HD ($1,930.8 \pm 102.5$ vs. $2,239.3 \pm 118.5$) were lower than the levels in ESRD patients before dialysis ($2,697.4 \pm 114.9$ vs. $2,790.4 \pm 116.8$)

Table 1. Clinical and biochemical profile of study participants

	Control	Pre-HD	HD
Age, yr	46.2±3.9	44.4±3.7	
Sex, no. of males	7	8	
Weight, kg	75.9±4.8	83.5±3.9	
Diabetics, no.	2	3	
Hemoglobin, g/l	42.0±0.7	35.1±0.7 ^a	
Blood flow rate, ml·min ⁻¹ ·100 ml leg ⁻¹	3.9±0.2	4.1±0.2	4.1±0.1
Glucose, mg/dl	111.4±8.3	103.1±8.1	90.3±7.9 ^b
Urea, mg/dl	12.7±1.4	71.2±6.4 ^c	19.2±1.9
Creatinine, mg/dl	0.9±0.04	10.6±1.13 ^d	5.3±0.56 ^e
Albumin, g/dl	3.8±0.2	3.2±0.2	3.6±0.2 ^f
Bicarbonate, meq/l*	22.6±0.6	23.1±1.1	27.2±0.7 ^e
Insulin, μU/dl	11.4±4.2	22.4±2.5	15.9±2.0 ^f
Cortisol, μg/dl	14.6±3.1	9.5±1.6	17.5±3.0 ^f
Epinephrine, pg/ml	33.7±8.5	31.7±9.2	25.9±6.9
Norepinephrine, pg/ml	229.1±49.2	219.6±52.6	343.3±91.6
Protein intake, g·kg ⁻¹ ·day ⁻¹	1.36±0.05	1.43±0.08	
Calorie intake, kcal·kg ⁻¹ ·day ⁻¹	35.2±1.32	33.4±4.2	

Values are means ± SE. HD, hemodialysis. *Normal range 22–27 meq/l. ^aESRD (end-stage renal disease) vs. control, $P < 0.01$; ^bHD vs. pre-HD, $P < 0.01$; ^cpre-HD vs. control and HD, $P < 0.01$; ^dpre-HD vs. control and HD, $P < 0.001$; ^eHD vs. control, $P < 0.001$; ^fHD vs. pre-HD, $P < 0.05$; ^gHD vs. pre-HD, $P < 0.01$.

and in controls ($2,791.2 \pm 215.3$ vs. $2,985.8 \pm 241.7$) ($P < 0.001$). The magnitudes of the decrease in the concentrations of total, nonessential, essential, and branched-chain amino acids during HD were more pronounced in the artery than the decrease in the vein. During HD, arteriovenous balance became more negative compared with pre-HD. The arteriovenous balance and the dialysate concentration of total amino acid were positively correlated ($r^2 = 0.527$, $P < 0.05$). The enrich-

Table 3. Enrichment of free amino acids in femoral artery, vein, and muscle compartments

	Control	Pre-HD	HD
Phenylalanine			
E _a	0.165±0.011	0.120±0.014	0.140±0.0085
E _v	0.130±0.01	0.101±0.0084	0.100±0.008
E _m	0.090±0.003 ^a	0.071±0.005	0.071±0.006
Alanine			
E _a	0.079±0.004	0.071±0.004	0.092±0.003 ^b
E _v	0.051±0.005	0.043±0.002	0.041±0.003
E _m	0.011±0.001	0.008±0.001	0.009±0.001
Glutamine			
E _a	0.093±0.006	0.073±0.004	0.091±0.006 ^c
E _v	0.072±0.005	0.061±0.003	0.062±0.003
E _m	0.013±0.001	0.014±0.001	0.016±0.001

Values are means ± SE. E_a, E_v, and E_m, enrichment in artery, vein, and muscle, respectively. ^aControl vs. pre-HD and HD, $P < 0.01$; ^bHD vs. control and pre-HD, $P < 0.01$; ^cHD vs. pre-HD, $P < 0.001$.

ment of the free amino acids in the artery increased during HD, reflecting a decrease in amino acid concentration (Table 3). Amino acid enrichment in the vein was lower than that in the artery due to dilution of tracer by the efflux of amino acids from the muscle into the vein.

The concentration of phenylalanine, alanine, and glutamine in the artery and vein decreased during HD ($P < 0.01$). The intradialytic decrease in the venous concentration was less pronounced (Table 4). The intracellular concentration of all the traced amino acids, however, remained relatively stable during HD. Inward (F_{ma}) and outward (F_{vm}) transport rates of phenylalanine increased by 27 and 61%, respectively, during HD ($P < 0.01$). Amino acids are transported by independently regulated active transport systems in the cell membranes (4,

Table 2. Arteriovenous balance across the leg in controls and ESRD patients

	Control	Pre-HD	HD	Dialysate
Nonessential				
Alanine	-90.3±12.0	-55.0±15.7	-91.0±23.2 ^a	49.9±3.9
Arginine	-6.8±2.7	-5.2±1.8	-14.9±2.5 ^b	19.9±3.7
Glutamine	-69.3±15.5	-28.2±5.7	-83.5±15.5	126.3±11.0
Glutamate	49.0±8.8	42.7±9.0	51.0±9.3	15.9±2.8
Glycine	-19.0±7.6	-15.3±3.5	-31.8±4.2 ^c	42.7±5.8
Histidine	-4.0±2.2	-2.9±0.6	-7.8±1.9 ^c	11.3±0.6
Serine	2.0±2.6	1.1±1.2	-3.4±2.0	18.5±2.0
Tyrosine	-8.7±4.2	-9.1±1.8	-16.1±2.8 ^c	7.1±0.7
Citrulline	-3.3±2.0	-3.6±4.3	-1.7±0.81	10.4±0.6
Ornithine	-4.2±1.4	-0.5±1.9	5.1±2.3	7.4±0.9
1-Methylhistidine	0.0±0.9	-3.8±3.8	-10.3±5.1	5.7±0.8
3-Methylhistidine	-1.6±2.0	1.0±2.5	-5.4±1.4 ^a	10.8±1.9
Cysteine	-3.9±1.1	-0.5±2.0	-4.2±2.8	
Essential				
Isoleucine	-2.6±1.2	-2.2±0.8	-7.0±1.3 ^a	10.2±0.8
Leucine	-5.4±3.2	-2.5±1.2	-15.7±3.9 ^a	22.6±1.3
Lysine	-11.4±5.5	-6.3±2.6	-19.5±3.7 ^b	21.5±1.4
Phenylalanine	-5.1±1.8	0.16±1.4	-10.9±2.3 ^b	14.8±1.3
Threonine	-5.1±1.4	-2.7±0.8	-4.1±1.5	17.3±1.4
Valine	-4.5±1.6	-3.2±1.1	13.1±3.1 ^d	24.3±2.4
Histidine	-4.0±2.1	-2.9±0.6	-7.8±1.9 ^c	11.3±0.6
Methionine	-3.2±1.0	-1.9±0.7	-5.2±0.9 ^b	3.6±0.4
BCAA	-12.5±5.1	-7.9±5.3	-35.8±7.6 ^d	57.4±3.6
TAA	-194.5±55.3	-116.8±23.5	-344.7±50.8	461.1±28.8

Values are means ± SE in μmol/l. BCAA, branched-chain amino acids; TAA, total amino acids. Negative numbers indicate net release. ^aHD vs. pre-HD, $P < 0.01$; ^bHD vs. pre-HD, $P < 0.001$; ^cHD vs. pre-HD, $P < 0.05$; ^dHD vs. pre-HD and control, $P < 0.01$.

Table 4. Amino acid transport kinetics in muscle derived from 3-pool model

	Control	Pre-HD	HD
Phenylalanine			
Concentration in artery (C_a)*	101.2±2.1	90.1±4.5	73.3±3.5 ^a
Concentration in vein (C_v)*	109.0±2.5 ^b	91.3±4.9	83.9±4.8 ^{a,b}
Concentration in muscle free pool (C_m)*	112.6±3.6 ^c	98.2±3.3	95.9±3.6
Inward transport (F_{ma})	189.2±20.4	137.1±13.5	171.4±19.8 ^d
Outward transport (F_{vm})	218.0±19.5 ^f	136.8±12.5 ^e	217.4±23.3 ^f
Intracellular utilization (F_{om})	98.4±7.5	100.7±15.7	125.9±21.8 ^g
Intracellular appearance (F_{mo})	126.9±6.9 ^h	103.4±14.8	169.8±20.0 ^{d,h}
Net balance (NB)	-28.6±3.6	-4.6±4.7	-48.3±10.8 ^d
Alanine			
Concentration in artery (C_a)*	328.0±14.8	285.0±19.2	201.2±16.2 ⁱ
Concentration in vein (C_v)*	417.7±20.2 ^b	336.3±30.4	297.1±31.1 ^{i,b}
Concentration in muscle free pool (C_m)*	1,997.5±100.6	2,460.6±264.9	2,348.1±239.1
Inward transport (F_{ma})	482.6±38.2 ^j	373.9±26.6	372.6±27.5
Outward transport (F_{vm})	735.6±60.3 ^f	575.5±52.7 ^f	743.8±84.7 ^{d,f}
Intracellular utilization (F_{om})	2,750.7±310.9	3,177.1±515.8	3,867.9±604.5 ^k
Intracellular appearance (F_{mo})	2,996.5±309.9	3,383.5±498.8	4,252.8±593.8 ^d
Net balance (NB)	-329.0±54.8	-179.6±65.7	-396.5±76.2 ^k
Glutamine			
Concentration in artery (C_a)*	543.6±27.2	646.7±33.8	464.2±38.7 ^d
Concentration in vein (C_v)*	595.6±31.1	675.3±35.1	551.3±44.8 ^d
Concentration in muscle free pool (C_m)*	15,989.9±515.4	15,822.9±438.2	15,081.8±520.4
Inward transport (F_{ma})	347.7±27.1	460.5±28.3	512.0±34.0 ⁱ
Outward transport (F_{vm})	539.1±33.6 ^f	576.8±34.2	868.4±80.2 ^{i,f}
Intracellular utilization (F_{om})	1,986.6±176.2	1,830.8±71.1	2,119.9±120.4 ^k
Intracellular appearance (F_{mo})	2,095.1±200.7	1,957.1±74.8	2,490.6±123.1 ^{d,h}
Net balance (NB)	-188.6±21.8	-117.1±60.1	-364.6±61.4 ^a

Kinetic data are expressed as means ± SE in $\text{nmol}\cdot\text{min}^{-1}\cdot 100\text{ ml leg}^{-1}$; * $\mu\text{mol/l}$. F_{om} , index of amino acid utilization (protein synthesis); F_{mo} , measure of protein degradation ± de novo synthesis. ^aHD vs. control and pre-HD, $P < 0.001$; ^b C_a vs. C_v , $P < 0.05$; ^ccontrol vs. pre-HD and HD, $P < 0.01$; ^dHD vs. pre-HD, $P < 0.001$; ^epre HD vs. control and HD, $P < 0.01$; ^f F_{ma} vs. F_{vm} , $P < 0.01$; ^gHD vs. pre-HD, $P < 0.05$; ^h F_{mo} vs. F_{om} , $P < 0.05$; ⁱHD vs. control and pre-HD, $P < 0.01$; ^jcontrol vs. pre-HD and HD, $P < 0.05$; ^kHD vs. pre-HD, $P < 0.01$; ^lHD vs. control, $P < 0.05$.

11). The transport rate into the muscle of each amino acid is increased in direct proportion to its concentration in the plasma (2). To eliminate the impact of differences in the concentrations of amino acids on the transport kinetics, we corrected the inward transport to amino acid inflow (F_{in}) and the outward transport to the intracellular concentration (C_m) of amino acids (6). The corrected inward transport (F_{ma}/F_{in}) of phenylalanine during HD was comparable to that of controls but higher than pre-HD. The outward transport of phenylalanine expressed as a ratio of intracellular concentration (F_{vm}/C_m) was higher intradialysis compared with pre-HD but not different from controls. Inward transport (F_{ma}) of alanine was faster in controls than in ESRD patients (pre- and intradialysis; $P < 0.05$). The corrected inward transport (F_{ma}/F_{in}) of alanine, however, increased during HD. The outward transport (F_{vm}) of alanine increased by 27.5% during HD. The ratio of outward transport to intracellular concentration (F_{vm}/C_m) of alanine increased during dialysis and became comparable to that of controls. The magnitude of increase in outward transport of glutamine intradialysis was more than the increase in inward transport (50.6 vs. 12%, $P < 0.01$). The ratio of inward transport to arterial inflow of glutamine (F_{ma}/F_{in}) during HD was more than that of controls and pre-HD. Also, the outward transport rate of glutamine expressed as F_{vm}/C_m during HD was faster compared with pre-HD and controls.

The F_{om} and F_{mo} indicate different metabolic pathways for phenylalanine, alanine, and glutamine (6). F_{om} and F_{mo} represent protein synthesis and intracellular appearance from protein catabolism for phenylalanine. Muscle protein catabolism was more than synthesis in both controls and during HD ($P <$

0.05). Muscle protein synthesis (F_{om}) increased by 24.9%, but breakdown (F_{mo}) increased by 74.3% during HD, resulting in net protein loss (Fig. 4). Muscle protein synthesis efficiency decreased from 41 to 30% during HD ($P < 0.01$). Both intracellular appearance (F_{mo}) and utilization (F_{om}) of alanine and glutamine increased during HD ($P < 0.01$). F_{mo} for alanine and glutamine represents the sum of de novo synthesis and appearance from proteolysis. Although the de novo syntheses of alanine and glutamine in controls and ESRD patients were comparable (Fig. 5), the release of these amino acids from

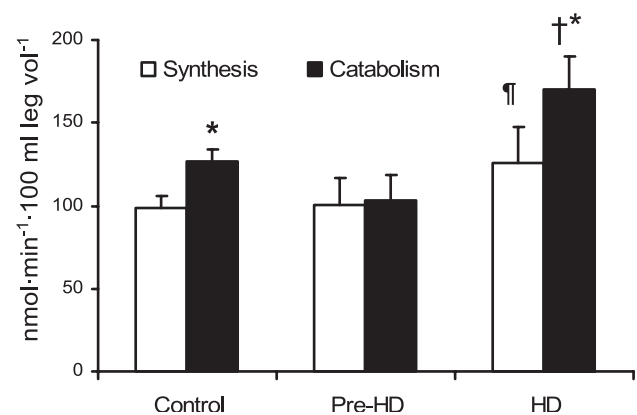


Fig. 4. Muscle protein synthesis and catabolism estimated by compartmental model in controls and ESRD patients pre-HD and during HD. Protein breakdown was more than synthesis in controls and during HD. †HD vs. pre-HD, $P < 0.05$; ‡HD vs. pre-HD, $P < 0.001$; *catabolism vs. synthesis, $P < 0.05$.

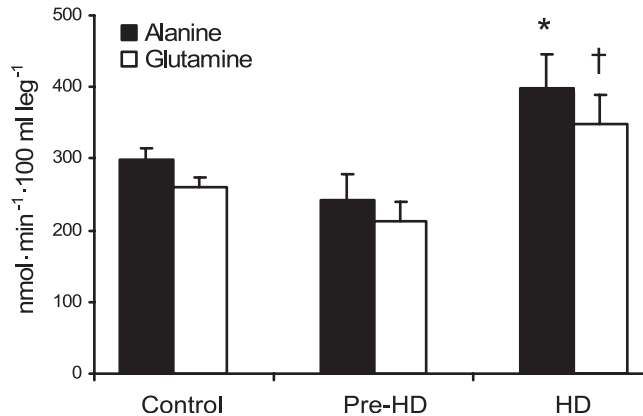


Fig. 5. Intracellular release of alanine and glutamine from protein breakdown increased in ESRD patients during HD compared with pre-HD. *HD vs. pre-HD, $P < 0.01$; †HD vs. pre-HD and control, $P < 0.01$.

protein breakdown increased intradialysis ($P < 0.01$) (Fig. 6). Net balance became more negative during HD compared with pre-HD for all the traced amino acids ($P < 0.01$).

Concentrations of BCAA in the artery and vein were higher in controls than in ESRD patients ($P < 0.01$; Table 5). Release of BCAA from the muscle into the vein increased the net negative balance during dialysis. Catabolism (nmol·min⁻¹·100 ml leg⁻¹) of leucine (76.9 ± 28.6 vs. -16.7 ± 18.2 , $P < 0.01$), isoleucine (87.6 ± 20.5 vs. -17.0 ± 13.6 , $P < 0.001$), and valine (27.3 ± 16.9 vs. -25.3 ± 13.5 , $P < 0.05$) increased during HD. The net nonprotein disposal of glutamate also increased during HD ($P < 0.01$). Whole body R_a values for phenylalanine, alanine, and glutamine were higher pre-HD than during HD and in controls ($P < 0.001$; Table 6). Whole body alanine and glutamine synthesis, however, decreased by 26.8 and 18.8%, respectively, during HD.

The gene expressions of glutamine synthase and branched-chain keto acid dehydrogenase (BCKAD)-E2 were measured in only six controls and seven ESRD patients. The glutamine synthase gene expression increased during HD (1.45 ± 0.14) compared with pre-HD (0.33 ± 0.08) and controls (0.62 ± 0.13) ($P < 0.001$). The mRNA level of BCKAD-E2 was higher during HD compared with pre-HD (3.86 ± 0.48 vs. 2.14 ± 0.27 , $P < 0.05$) but not different from controls (2.98 ± 0.48).

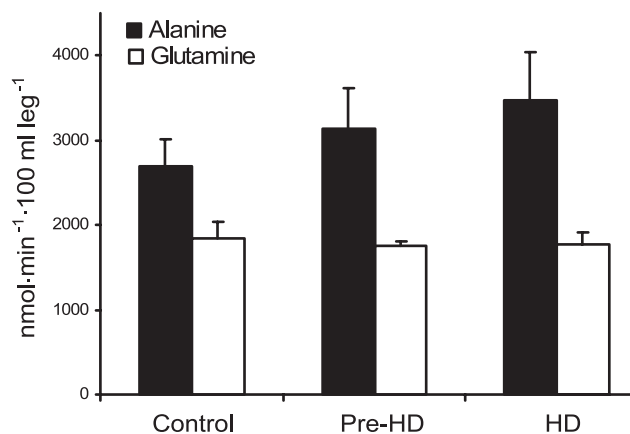


Fig. 6. De novo synthesis of alanine and glutamine in muscle pre-HD and during HD in ESRD patients and in controls.

Table 5. BCAA and glutamate metabolism in controls and ESRD patients

	Control	Pre-HD	HD
Concentration in artery (C_a)*	476.7 ± 66.0^a	282.9 ± 30.4^b	209.7 ± 20.4
Concentration in vein (C_v)*	489.2 ± 63.9^a	291.3 ± 28.8^b	243.4 ± 25.8
Net balance (NB)	-44.1 ± 17.8	-34.8 ± 8.2	-144.4 ± 32.5^c
BCAA catabolism	102.9 ± 55.3	-59.1 ± 42.9^b	191.8 ± 63.4
Net nonprotein glutamate disposal	238.0 ± 46.5	222.3 ± 43.6^b	347.0 ± 46.3

Kinetic data are means \pm SE expressed as nmol·min⁻¹·100 ml leg⁻¹; * C_a and C_v are in μ mol/l. ^aControl vs. pre-HD and HD, $P < 0.001$; ^bHD vs. pre-HD, $P < 0.01$; ^cHD vs. control and pre-HD.

DISCUSSION

Consistent with our previous report (34), this study also demonstrates that HD induces significant alterations in protein turnover and amino acid transport kinetics. Despite the declining plasma amino acid concentrations and accelerated efflux of amino acids from the muscle, the intracellular concentrations of amino acids remained relatively stable during HD, due to augmented protein catabolism and altered amino acid kinetics. There was a general tendency for the muscle protein breakdown to increase and for the whole body protein turnover to decrease during HD, which is not unlike the pattern in controls (Tables 4 and 6). The direction of changes in degradation and synthesis rates of alanine and glutamine in ESRD patients pre- and during HD mirrors the changes observed in phenylalanine kinetics. However, the turnover rates of alanine and glutamine are many times higher than that of phenylalanine in both the muscle and whole body (Table 4). Alanine and glutamine release from muscle protein breakdown increased during HD without significant change in de novo synthesis (Figs. 5 and 6).

Loss of lean body mass is common (21) and is one of the most important predictors of increased mortality and morbidity in patients with ESRD (28, 31). Despite variations in protein turnover (17, 18), a balance between protein synthesis and catabolism is maintained in patients with uncomplicated chronic renal failure (40). Our findings also confirm that muscle protein catabolism is not increased in ESRD patients pre-HD. Protein balance was maintained in ESRD patients pre-HD because the patients did not have metabolic acidosis and were consuming adequate protein and calories. We demonstrated earlier (36) that the genes promoting protein catabolism are downregulated pre-HD but increased during HD. Modeled data showed that muscle protein breakdown is more than synthesis in controls, which is not unlike the results reported by Biolo et al. (6) using a similar technique. Although

Table 6. Whole body metabolism of phenylalanine, alanine, and glutamine in controls and ESRD patients

	Control	Pre-HD	HD
R_a phenylalanine	0.67 ± 0.03	0.86 ± 0.05^a	0.73 ± 0.02
R_a alanine	4.92 ± 0.20	5.16 ± 0.21^a	4.01 ± 0.15
R_a glutamine	4.15 ± 0.25	5.06 ± 0.20^a	4.17 ± 0.22
Alanine synthesis	3.26 ± 0.18	3.13 ± 0.18	2.29 ± 0.14^b
Glutamine synthesis	2.56 ± 0.19	3.29 ± 0.15	2.69 ± 0.21^c

Data are means \pm SE expressed as μ mol·kg⁻¹·min⁻¹. ^aPre-HD vs. control and HD, $P < 0.001$; ^bHD vs. control and pre-HD, $P < 0.001$; ^cpre-HD vs. HD, $P < 0.01$.

investigators have reported that protein synthesis is decreased (29), unchanged (23), or increased (34) during HD, there is consensus that HD induces net muscle protein catabolism. The etiology of intradialytic protein catabolism could be due to loss of amino acids in the dialysate (29), activation of cytokines (35, 36), or altered amino acid transport kinetics (34). The plasma insulin level was higher in ESRD patients pre-HD compared with controls. This might be due to alterations in insulin levels due to renal failure and decreased clearance of insulin. We did not find any significant differences in protein turnover or amino acid transport kinetics in patients with T2DM compared with nondiabetics. Other investigators have also observed that protein breakdown and amino acid kinetics in patients with T2DM are not different from those in nondiabetics (8, 22).

Arteriovenous balance studies have shown that there is net release of all the amino acids from the muscle during HD, except glutamate, for which there was a net uptake (Table 2). We observed a positive correlation between dialysate loss of amino acids and the arteriovenous balance, suggesting a possible etiological relationship. The decrease in phenylalanine concentration in the artery (18.5%) was more than the change in venous (6.6%) and muscle (2.2%) free amino acid concentrations (Table 4). Similarly, the alanine and glutamine concentration in the artery (29.8 vs. 28.8%) declined more than that in the vein (12.3 vs. 19.1%) and muscle compartments (4.1 vs. 4.7%). Human and animal studies have suggested that the muscle glutamine pool positively influences protein synthesis (24, 27). Furthermore, it is claimed that this correlation is specific for glutamine and has not been observed with any other amino acid (32). However, Gore and Wolfe (20) demonstrated that muscle protein synthesis is elevated despite depletion of muscle glutamine. Furthermore, exogenous amino acid supplementation did not did not replenish the glutamine availability within the muscle due to failure of glutamine transport into muscle (20). In our study, the intracellular glutamine concentration remained stable despite variations in protein turnover.

Amino acids can appear in the cell via transport from arterial blood, from protein breakdown and/or from de novo synthesis in the case of nonessential amino acids. HD increased the protein turnover in the muscle, with net increase in protein catabolism. Increase in the intracellular appearance from protein breakdown (F_{mo}) was more than the rate of intracellular amino acid utilization (F_{om}) for alanine and glutamine (Table 4). The intracellular appearance of alanine and glutamine could be from protein breakdown and/or from intracellular de novo synthesis. During HD, intracellular appearance of alanine and glutamine derived from protein breakdown increased by 74.3 and 60%, respectively, but the de novo synthesis of alanine and glutamine did not change (Figs. 5 and 6). The increase in BCAA catabolism during HD could theoretically provide enough nitrogen for glutamine synthesis (Table 5). Glutamate is necessary for the synthesis of both alanine and glutamine. Increased alanine synthesis could limit the glutamate available for glutamine synthesis. In catabolic states, alanine becomes the major carrier of nitrogen from muscle to other tissues (5). We found that HD augments the release of both alanine and glutamine from the leg. Considering the fact that glutamine carries both amino and amide nitrogens, glutamine remains the principal nitrogen carrier during HD.

In contrast to alanine, which is formed through reversible transamination reactions operating near equilibrium, the formation of glutamine is determined by a balance of distinct synthetic and degradative pathways. Glutamine synthesis is regulated by the enzyme glutamine synthase and degradation by glutaminase. In vitro studies have demonstrated that the activity of the enzyme increases in response to a decrease in glutamine concentration in the culture medium and increased availability of glutamate (37). In response to an intradialytic decrease in plasma glutamine, the glutamine synthase gene expression increased. The rate-limiting enzyme for BCAA degradation in muscle is BCKAD (15). The mRNA level of BCKAD-E2 increased during HD, which is consistent with the augmented BCAA catabolism observed during HD.

The amino acids released from protein catabolism can have any one of three fates: to be reutilized for protein synthesis, to be used for synthesis of other amino acids, or to exit the muscle pool into the vein. Augmented intradialytic amino acid efflux could be a compensatory mechanism to maintain the plasma amino acid concentration in the face of intradialytic loss of amino acids, be due to ineffective utilization of amino acids, or both. The fraction of amino acid that appears in the intracellular pool that is directed to protein synthesis (synthesis efficiency) decreased during HD. The effectiveness of reutilization of amino acid released from protein catabolism can be estimated by the ratios F_{om}/F_{mo} and F_{mo}/F_{vm} . The former is the fraction of the amino acid released from protein breakdown that is reutilized for protein synthesis; the latter is the estimate of the fraction of amino acid released from muscle catabolism that exits the intracellular compartment. The ratio of protein synthesis to catabolism (F_{om}/F_{mo}) for phenylalanine and alanine decreased during HD. However, the ratio of protein breakdown to outward transport (F_{mo}/F_{vm}) did not change intradialysis, because of a balanced increase in protein breakdown and efflux of phenylalanine and alanine. The reutilization of glutamine decreased during HD, as evidenced by the decrease in the ratio F_{om}/F_{mo} and increase in F_{mo}/F_{vm} .

In humans, skeletal muscle is an important site for glutamine production and the splanchnic bed the site of its removal (33). With fasting, the liver switches from net glutamine utilization to that of production, whereas skeletal muscle does not enhance production of glutamine (9). Tessari et al. (38) demonstrated that the contribution and protein balance in muscle, splanchnic region, and kidney vary widely. Although the muscle protein turnover decreased pre-HD, whole body R_a for phenylalanine, alanine, and glutamine increased. Dissociation between muscle and whole body protein turnover has been reported from our laboratory (36) and also by other investigators (1, 29, 39). Amino acids released from intradialytic muscle protein breakdown are used for synthesis of new proteins at the whole body level. Consistent with this hypothesis, we demonstrated previously (35) that the amino acids released from muscle protein catabolism facilitate increased hepatic protein synthesis intradialysis.

To summarize, muscle protein catabolism is increased due to intradialytic loss of amino acids and increased net efflux of amino acids from the muscle. The intracellular appearance of alanine and glutamine from protein catabolism is increased during HD, without significant change in de novo synthesis. Glutamine remains an important nitrogen carrier during HD. Muscle protein breakdown is increased, but the whole body

protein catabolism is decreased intradialysis. Although muscle protein breakdown during HD provides amino acid substrate for splanchnic protein synthesis, it will lead to progressive muscle wasting with duration. Intradialytic replacement of amino acids would increase the substrate availability for protein synthesis, but the utilization of the amino acids may be limited by altered amino acid transport kinetics. Future studies should be aimed at improving reutilization of amino acids and protein synthesis efficiency in ESRD.

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