Compartmental distribution of amino acids during hemodialysis-induced hypoaminoacidemia

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Børshheim, Elisabet, Hisamine Kobayashi, Daniel L. Traber, and Robert R. Wolfe. Compartmental distribution of amino acids during hemodialysis-induced hypoaminoacidemia. Am J Physiol Endocrinol Metab 290: E643–E652, 2006. First published November 8, 2005; doi:10.1152/ajpendo.00267.2005.—The intracellular concentrations of essential amino acids (EAA) in muscle are maintained relatively constant under a variety of conditions. However, the effect of a decrease in blood amino acid concentrations on intracellular concentrations is not clear. Similarly, the relation between intracellular and interstitial concentrations has not been determined in this circumstance. Thus the aim of this study was to determine the effect of hypoaminoacidemia on intracellular, interstitial, and plasma concentrations of EAA and the mechanisms responsible for the respective changes. Twelve normal pigs were investigated before and during 120 min of hemodialysis by use of stable-isotope tracer methodology, microdialysis technique, and muscle biopsies. During hemodialysis, there was a decrease in the interstitial fluid concentrations of phenylalanine, leucine, alanine, and lysine that corresponded to their decrease in plasma concentration. Nonetheless, the intracellular concentrations of these amino acids were maintained at the basal levels throughout the entire period due principally to a reduction in the rate of incorporation of amino acids into protein that was approximately equivalent to the decrease in uptake from the plasma. In conclusion, intracellular concentrations of amino acids are regulated to maintain relatively constant values, even when plasma and interstitial concentrations fall as a consequence of hemodialysis.

INTRACELLULAR CONCENTRATIONS of essential amino acids (EAA) in muscle are relatively constant under a variety of conditions, e.g., during a moderate increase in plasma EAA concentrations caused by ingestion (25) or infusion (5, 7) of amino acids. The intracellular concentrations increase only when the plasma concentrations exceed the maximal stimulatory effect of an increase in amino acid availability on muscle protein synthesis (6). Thus it has been suggested that the free intramuscular concentrations of EAA are regulated by the integrated responses of protein synthesis, breakdown, movement of EAA into and out of the intracellular pool from/to the extracellular fluid, and (if applicable) oxidation (30).

An alternative explanation of the constant intracellular EAA pool during hyperaminoacidemia is that it occurs coincidently as a result of the combination of accelerated uptake and stimulated synthesis. If in fact intramuscular EAA concentrations are regulated, then they should be maintained not only when plasma concentrations rise but also when they fall. However, the response to a reduction in concentrations has not previously been tested. Thus the principal aim of this study was to assess the effect of a reduction in blood amino acid concentrations below the basal level on muscle intracellular EAA.

Furthermore, the relation between intracellular and interstitial amino acid concentrations has not been investigated in a circumstance in which blood amino acid concentrations are reduced. Therefore, we have combined microdialysis technique with blood samples to address this issue. Because the exchange between the interstitial fluid and blood occurs by diffusion, the interstitial concentration of EAA would be expected to parallel reductions in plasma concentrations, and thus the ratio between them should not change during hemodialysis. The exchange between intracellular and interstitial fluid, on the other hand, occurs by active transport. A variety of amino acid transporters with different characteristics has been described on the basis of in vitro studies (23). On the basis of these in vitro studies, it would be predicted that specific amino acids have different transport kinetics. However, the in vivo kinetics of transport of different amino acids in response to a fall in plasma (or interstitial fluid) concentrations has not previously been determined. Therefore, a second goal of this study was to determine the changes when blood amino acid concentrations fall in the relation between interstitial and intracellular concentrations of certain amino acids with either similar or different transport characteristics.

The intracellular concentrations of EAA result not only from the balance between inward and outward rates of transport but also the extent to which they appear/disappear from the free intracellular pool as a result of muscle protein breakdown and synthesis, respectively, and, in some cases, as a result of oxidation of the particular amino acid (e.g., leucine). Prior studies suggest that muscle protein synthesis may be regulated in a manner that results in maintenance of the intracellular free pool of EAA. Thus, in circumstances of either an absence or deficiency (10) of amino acids (protein) in the diet, muscle protein synthesis has been reported to be decreased in rats whereas intracellular free EAA were maintained. Rates of breakdown have not been measured in this circumstance. Furthermore, certain EAA, such as leucine, can disappear irreversibly from the intracellular pool as a result of oxidation. Thus the third goal of this study was to determine changes in rates of appearance and disappearance of EAA into the intracellular pool when blood amino acid concentrations are reduced [parts of the protein synthesis and breakdown data have been reported in Kobayashi et al. (12)].

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Finally, the fourth goal of the study was to relate the regulation of the intracellular EAA pool to the regulation of non-EAA, such as alanine, that can both be synthesized de novo, as well as catabolized within muscle.

MATERIALS AND METHODS

Animals. Twelve normal Yorkshire swine (K-bar livestock; Sabinal, TX) were studied [weight: 47.6 ± 11.8 kg (means ± SD); age: 4.2 ± 1.4 mo]. The experimental protocol was approved by the Animal Care and Use Committee of the University of Texas Medical Branch (IACUC 00-03-017).

Experimental protocol. The animals were studied after an overnight fast and were weighed in the morning before the experiment. The study was performed under general anesthesia of isoflurane inhalation. The depth of anesthesia was monitored throughout the experiment by heart rate and blood pressure, and appropriate modifications in the rate of anesthesia administration were made to ensure a stable physiological condition throughout the experiment.

The experimental protocol is shown in Fig. 1. There was a 4-h basal period without any hemodialysis followed by 2 h of hemodialysis. Surgical procedures. Before the start of the experiment, a Swan-Ganz catheter was inserted into the right external jugular vein and the tip advanced to the pulmonary artery for infusions of isotopes tracers and monitoring of body temperature. The body temperature was maintained at 37°C throughout the study with the use of a heating blanket. A dual-lumen catheter was placed in the left external jugular vein for hemodialysis. A third catheter was inserted in the carotid artery for arterial blood sampling as well as for blood pressure and heart rate monitoring. An indwelling catheter was placed in the femoral vein for sampling of venous blood from the leg. Patency of the catheters was maintained by saline infusion. A flow probe was placed around the femoral artery and connected to a flowmeter (T106; Transonic Systems, Ithaca, NY) to measure femoral arterial blood flow.

Isotope infusion. After a blood sample had been obtained for measurement of background amino acid enrichment, a primed, constant infusion of [ring,1-15N]phenylalanine, [1-1-13C]leucine, [1-15C]alanine, and [1-15N]lysine, 2HCl was started at time 0, PD 4 μmol/kg; [1-1-13C]leucine: IR 0.10 μmol·kg⁻¹·min⁻¹; PD 4 μmol/kg; [1-15C]alanine: IR 0.16 μmol·kg⁻¹·min⁻¹; PD 9.6 μmol/kg; [1-1-13C]leucine: IR 0.35 μmol·kg⁻¹·min⁻¹; PD 35 μmol/kg; and [1-15N]lysine:2HCl: IR 0.16 μmol·kg⁻¹·min⁻¹, PD 14.4 μmol/kg. Isotopes were purchased from Cambridge Isotope Laboratories (CIL, Andover, MA). They were dissolved in 0.9% saline and filtered through a 2-μm filter before infusion.

Microdialysis. Three microdialysis catheters (40 × 0.2 mm, 13,000 molecular weight cut-off; Spectrum; Spectrum Laboratories, Laguna Hills, CA) were placed in the gracilis muscle in one leg for determination of interstitial concentrations of amino acids. The polyimide tubing inlets (MicroMumen; MicroMumen, Tampa, FL) of the catheters were connected to a microinjection pump (CMA/100; Carnegie Microdialysis, Stockholm, Sweden). After the insertion, each probe was continually perfused at a rate of 5.0 μl/min. The perfusion fluid consisted of saline with 3.0 mmol/l lactate, 0.5 mmol/l glucose, 14C]alanine (0.108 μCi/ml), and [5H]phenylalanine (0.108 μCi/ml). The radioactive isotopes were included for calculation of relative recovery of the microdialysis probe and were purchased from Amersham Pharmacia Biotech (Piscataway, NJ). After 60 min of equilibration, dialysate was collected in intervals from the outlet of the tubing.

Hemodialysis. After the 4-h basal period (time 0), hemodialysis was started and continued for the rest of the experiment. A polymethylmethacrylate dialyzer (Filtryzer B3-1.6A; Toray, Tokyo, Japan) was used. The flow rate of blood through the dialyzer was 450 ml/min, and dialysate flow rate was 900 ml/min. The composition of dialysate (NaturaLyte; Fresenius Medical Care, Lexington, MA; and Renasol BC-1-L; Minntech Renal Systems, Minneapolis, MN) was as follows: 139–145 meq/l sodium, 2.0–3.0 meq/l potassium, 3.0–3.5 meq/l calcium, 1.0 meq/l magnesium, 4.0 meq/l acetate, 107 meq/l chloride, and 2.0–2.5 g/l dextrose. Sampling. Blood samples to determine total amino acid and electrolyte concentration and pH were drawn throughout the experiment from the carotid artery. Blood samples to determine phenylalanine, leucine, alanine, and lysine enrichments and concentrations were taken simultaneously from the carotid artery and femoral vein throughout the experiment. In addition, muscle biopsies were obtained from the gracilis muscle of the opposite leg of the microdialysis catheters. The biopsies were −1 g each, and the biopsy sites were separated by ≥3 cm to ensure that they were not affected by a local response to the previous biopsy. An incision was made in the skin, and the biopsy was sampled with a scalpel. The samples were quickly rinsed, blotted, and immediately frozen in liquid nitrogen and stored at −80°C for later analysis. The skin was immediately closed. Femoral arterial blood flow rate was recorded at each sampling.

Microdialysate was collected in triplicate in periods during the study. Microdialysate was collected in 1-h periods the first half-hour after start of hemodialysis. Thereafter, microdialysate was collected over 10-min periods the first half-hour after start of hemodialysis and then over 30-min periods for the remainder of the experiment.

Sample analyses. Blood samples for determination of amino acid enrichment and concentrations were immediately precipitated in preweighed tubes containing 15% sulfosalicylic acid, and a weighed amount of an appropriate internal standard, consisting of amino acids labeled differently from the infused amino acids, was added (3, 4, 21). The supernatant was passed over a cation exchange column (Dowex AG 50W-8X, 100–200 mesh H⁺ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum with a Speed Vac (Savant Instruments, Farmingdale, NY). Enrichments of free amino acids were then determined on the tertiary-butyldimethylsilyl derivatives using gas chromatography mass spectrometry (GC-MS; Hewlett-Packard 5973, Palo Alto, CA) and selected ion monitoring (29). Enrichments were expressed as tracer-to-tracee ratio. Appropriate corrections were made for overlapping spectra (29). Concentrations of phenylalanine, leucine, alanine, and lysine were calculated by an internal standard method (3).

To determine muscle intracellular enrichment of infused tracers and intracellular concentrations of phenylalanine, leucine, alanine, and lysine, muscle tissue was weighed (−30–40 mg), and the proteins were precipitated with 800 μl of 14% perchloroacetic acid. The tissue was washed, and the supernatant was passed over a cation exchange column (Dowex AG 50W-8X, 100–200 mesh H⁺ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum with a Speed Vac (Savant Instruments, Farmingdale, NY). Enrichments of free amino acids were then determined on the tertiary-butyldimethylsilyl derivatives using gas chromatography mass spectrometry (GC-MS; Hewlett-Packard 5973, Palo Alto, CA) and selected ion monitoring (29). Enrichments were expressed as tracer-to-tracee ratio. Appropriate corrections were made for overlapping spectra (29). Concentrations of phenylalanine, leucine, alanine, and lysine were calculated by an internal standard method (3).

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was then homogenized and centrifuged, and the supernatant was collected. The procedure was repeated, and the pooled supernatant was passed through a cation exchange column and processed in the same way as the supernatant from the blood samples.

Microdialysis samples for determination of interstitial amino acid enrichment and concentrations were weighed, and a weighted amount of an appropriate internal standard was added. The subsequent processing was the same as for the blood supernatant, except that cation exchange columns with higher mesh were used (200–400 mesh H⁺ form). Except for the samples that were collected over 1 h, the samples collected simultaneously from the three probes were pooled before analysis.

To determine radioactivity in the microdialysate perfusate and dialysate (for determination of relative recovery, see Calculations), 10 μl of sample were mixed with 15 ml of scintillation fluid (Ecolite; ICN Biomedicals, Costa Mesa, CA) and measured in a liquid scintillation counter (1219 Rackbeta; Wallac, Gaithersburg, MD).

Plasma samples separated from arterial blood and muscle intracellular fluid were also analyzed for all the individual amino acid concentrations by high-performance liquid chromatography (HPLC System 2690; Waters Alliance, Milford, MA). For this procedure, plasma or ~20–30 mg of muscle were mixed with 100 μl of internal standard and 100 μl of acetonitrile (HPLC grade) and for muscle also 400 μl of distilled H₂O. This was thoroughly mixed before being incubated for 30 min (plasma) to 1 h (muscle) on ice. The muscle samples were homogenized. Thereafter, 400 μl (muscle) or 750 μl (plasma) of distilled H₂O were added before the sample was centrifuged at 1,500 g for 10 min. Finally, 100 μl of the supernatant were transferred to a 0.2-cm Ultrafree-MC Centrifugal Filter and spun at 3,000 g for 4 h at 4°C.

Plasma glucose concentration was determined enzymatically by an automated system (YSI 1500; Yellow Spring Instruments, Yellow Springs, OH). Plasma insulin concentration was determined by a radioimmunoassay method (Linco Research, St. Charles, MO). Arterial blood pH was determined immediately after sampling with a blood gas analyzer (Instrumentation Laboratory, Lexington, MA). Electrolyte concentrations were determined in serum samples separated from arterial blood by a VITROS 250 Chemistry System (Ortho-Clinical Diagnostics, Raritan, NJ).

Calculations: amino acid concentrations. Concentrations of free amino acids in blood and muscle intracellular water were calculated as follows: \( Q_{IS}/(V \cdot E_{IS}) \), where \( Q_{IS} \) (nmol) is the amount of internal standard added to the sample, \( V \) is the volume of blood or intracellular water, and \( E_{IS} \) is the internal standard tracer-to-tracer ratio in blood or intracellular water as measured by GC-MS (1, 29). The muscle intracellular concentration was corrected according to the equation in Wagenmakers (28): \( C_m = [C_t - (0.15 \times C_e)]/0.85 \), where \( C_m \) is the concentration in muscle intracellular fluid, \( C_t \) is the concentration in total muscle water (measured), and \( C_e \) is the concentration in extracellular water (i.e., measured by microdialysis).

Relative recoveries of muscle interstitial amino acids were calculated with the internal reference technique (14). \[^{14}C\]Leucine (0.108 μCi/ml) and \[^{1}H\]phenylalanine (0.108 μCi/ml) were added to the microdialysate perfusate. Relative recovery (R) of tissue phenylalanine and leucine in the microdialysate was calculated according to the formula \( R = \frac{F_{ol}}{(P_e - D_e)/P_e} \), where \( F_{ol} \) is the fractional output of labeled phenylalanine or leucine, respectively; \( P_e \) is counts in perfusate \(^{1}H\) for phenylalanine and \(^{14}C\) for leucine); and \( D_e \) is counts in the dialysate \(^{1}H\) for phenylalanine and \(^{14}C\) for leucine) (14).

Interstitial phenylalanine and leucine concentrations were then calculated as \( C_D/R \), where \( C_D \) is phenylalanine or leucine concentration, respectively, in the microdialysate. On the basis of their molecular weights, the relative recovery of alanine was assumed to be similar to the relative recovery of phenylalanine, and the relative recovery of lysine was assumed to be similar to the relative recovery of leucine.

Average concentrations in the blood, microdialysate, and muscle samples collected over the last hour of the basal period are presented and were used to calculate gradients between the blood, the interstitial fluid, and the intracellular fluid in the basal state before hemodialysis. Correspondingly, the average concentrations in the samples collected over the time period 30–120 min after the start of hemodialysis are also presented and were used to calculate the gradients during the hemodialysis period.

Amino acid kinetics. Figure 2 shows a model of muscle amino acid metabolism. Amino acids enter the leg via the artery and leave the leg via the vein. Some of the amino acids are directly shunted from the artery to the vein, whereas “inward transport” represents the rate of net amino acid movement from the artery into the free amino acid pool in the muscle. Amino acids also enter the intracellular pool as a result of release from protein breakdown and/or de novo synthesis, depending on the specific type of amino acid. Amino acids can disappear from the free intracellular pool either because of intracellular utilization (protein synthesis and other metabolic fates), or intracellular appearance or from protein breakdown or de novo synthesis.

The different fluxes presented above were calculated for phenylalanine, leucine, alanine, and lysine according to a three-pool compartment model previously published (1, 3). The model enables quantification of muscle amino acid transmembrane transport, as well as de novo amino acid appearance or disappearance into the free amino acid pool in the muscle cell. Assumptions and derivations of the model are discussed by Biolo et al. (3). Protein kinetics were calculated for two specific intervals: in the basal period before start of dialysis and from 30 to 120 min after start of hemodialysis. Averages for blood flow and blood and muscle amino acid concentrations and enrichments, which were calculated from individual samples drawn during these periods, were used for the model calculation.

In the model described above, it is the total appearance and disappearance of the specific amino acid that is calculated. For phenylalanine and lysine, irreversible loss from the intracellular pool can only be to protein synthesis, since it is not oxidized in the muscle. Because leucine and alanine can be oxidized in muscle, the irreversible loss is due to synthesis plus oxidation and, in the case of alanine, metabolic conversion. Because phenylalanine, lysine, and leucine cannot be synthesized in muscle, de novo appearance of these amino acids is due entirely to breakdown. On the other hand, de novo appearance of alanine can also be due to de novo synthesis of the amino acid in the muscle cell.

![Fig. 2. Model of muscle protein kinetics. See MATERIALS AND METHODS for explanations. AA, amino acids.](http://ajpendo.physiology.org/10.2203.36.6)
The rate of incorporation of leucine or alanine into protein was calculated from the rate of protein synthesis, as calculated using the phenylalanine tracer, and the ratio between leucine and phenylalanine or between alanine and phenylalanine, respectively, in mixed muscle protein. A ratio of 2.4 between leucine and phenylalanine and a ratio of 2.47 between alanine and phenylalanine were used (22). The rate of leucine oxidation was then calculated from the difference between the rate of total utilization of intracellular leucine and the rate at which leucine was used for protein synthesis. The difference between the rate of utilization of intracellular alanine and the rate at which it was used for protein synthesis was taken to represent alanine oxidation plus metabolic conversion. Correspondingly, the rate of appearance from protein breakdown for alanine was calculated from the rate of protein breakdown as calculated using the phenylalanine tracer and the ratio of alanine and phenylalanine in mixed muscle protein (22). The rate of its production was then calculated from the difference between the total rate of appearance of intracellular alanine and the rate at which it was released from protein breakdown.

The results for the amino acid kinetics are expressed in nanomoles per minute per 100 ml of leg. The leg mass was measured in eight of the pigs, weighing the surgically removed leg after termination of the study. On average, leg weight constituted 11.59 ± 0.39% (SE) of the total body weight in these animals. Because the animals were of the same breed, age, and thus body weight and the variation in leg mass as a percentage of total body mass was small, this value (11.59% of total body mass) was used to estimate leg mass in the last four pigs.

Statistical methods. All statistical calculations were performed using the software package SigmaStat 2.03 (SPSS, Chicago, IL). Overall significance of concentration differences in phenylalanine, leucine, alanine, and lysine, respectively, in response to time was tested by two-way [time (i.e., basal and hemodialysis) × site (i.e., arterial blood, venous blood, interstitial fluid, and intracellular fluid)] repeated-measures analysis of variance (ANOVA) followed by Tukey’s test. Significance of changes in concentration ratios, kinetics, and plasma concentrations for individual amino acids was tested by paired t-test. Results were considered significant if P < 0.05. The results are presented as means ± SE unless otherwise noted.

RESULTS
Physiological parameters. The pigs were in a relatively physiological steady state during the experiments. Mean arterial pressure was 79 ± 2 mmHg before the start of the hemodialysis compared with average mean arterial pressure of 77 ± 3 mmHg between 30 and 120 min after start of hemodialysis [not significant (NS) vs. basal]. Heart rate was 105 ± 4 beats/min both before and after the start of dialysis (NS). From the basal insulin value of 1.81 ± 0.27 μIU/ml, there was a small increase during hemodialysis to 2.85 ± 0.36 μIU/ml (P = 0.018, n = 11). There was also a small increase in arterial pH from 7.397 ± 0.015 to 7.457 ± 0.011 during dialysis (P < 0.001). A slight decrease from the basal value was observed during dialysis for sodium (143 ± 1 to 141 ± 0.5 mmol/l, P = 0.01), potassium (4.8 ± 0.1 to 4.0 ± 0.1 mmol/l, P < 0.001), and chloride (113 ± 1 to 109 ± 0.5 mmol/l, P < 0.001). No change was observed in leg blood flow during dialysis (3.20 ± 0.38 ml·min⁻¹·100 ml leg⁻¹) vs. basal (3.78 ± 0.31 ml·min⁻¹·100 ml leg⁻¹, NS).

Plasma, interstitial, and intracellular amino acid concentrations. Total amino acid concentration in arterial plasma decreased from a basal value of 5,413 ± 219 to 3,372 ± 137 nmol/ml during hemodialysis (P < 0.001 vs. basal). Nonetheless, there was no change in the total intracellular EAA concentration from the basal period (2,192 ± 217 nmol/ml) to the hemodialysis period (2,108 ± 246 nmol/ml, NS vs. basal). Nor was any change seen in total intracellular nonessential amino acid (NEAA) concentration (basal: 12,088 ± 1,523, hemodialysis: 13,065 ± 1,832 nmol/ml, NS), and thus total intracellular amino acid concentration was maintained (basal: 14,280 ± 1,490, hemodialysis: 15,173 ± 1,833 nmol/ml, NS). Furthermore, there were no significant changes in intracellular concentrations of individual amino acids when the HPLC analyses of all amino acids were analyzed statistically (Fig. 3), except for a small drop in valine (basal: 387 ± 56, hemodialysis: 343 ± 51 nmol/ml, P = 0.004).

The concentration measurements of phenylalanine, leucine, alanine, and lysine made by means of tracer methodology (Figs. 4 and 5) were consistent with the corresponding results from the HPLC analyses (Fig. 3). Figure 4 shows the time course of phenylalanine concentration in blood, interstitial fluid, and intracellular fluid. For all of the amino acids, the concentrations in blood and interstitial fluid decreased rapidly during the first 30 min of hemodialysis. Thereafter, they reached a new steady state (Fig. 4). Thus average concentrations in the blood, microdialysate, and muscle samples collected over the last hour of the basal period and over the time period 30–120 min after the start of hemodialysis are presented. In the basal period, the relative recoveries in the microdialysate were 0.20 ± 0.01 for both phenylalanine and leucine. In the hemodialysis period, relative recoveries were 0.23 ± 0.02 and 0.24 ± 0.01 for phenylalanine and leucine, respectively (NS between tracers).

Whereas the arterial and venous blood concentrations of phenylalanine, leucine, alanine, and lysine all decreased during hemodialysis compared with the basal period, the intracellular concentrations were maintained, or even increased, in the case of the alanine (Fig. 5). In addition, it was found that the interstitial concentrations followed the changes in blood concentrations. In summary, for each of the amino acids, ANOVA revealed strong, significant effects of both time (hemodialysis) and site (arterial blood, venous blood, interstitial fluid, intracellular fluid) and also an interaction effect, meaning that the effect of hemodialysis was different on different sites.

In the case of phenylalanine, interstitial concentrations were not different from muscle concentrations during basal, but during hemodialysis there was a significant difference (P < 0.001; Fig. 5, top left), since the interstitial concentration mirrored the changes in plasma concentrations and decreased during hemodialysis (P < 0.001 vs. basal), whereas the intracellular muscle concentration was maintained. Interstitial phenylalanine concentration was higher than both arterial (P = 0.001) and venous (P = 0.009) concentrations during basal, but during hemodialysis interstitial concentration was no longer statistically different from the venous.

For leucine, there were no differences in concentrations between intracellular fluid, interstitial fluid, and arterial or venous blood during basal conditions (Fig. 5, bottom left). During hemodialysis, both interstitial fluid and blood concentrations were different from muscle, whereas they were not different from each other. This was caused by a fall in interstitial (P < 0.001 vs. basal), arterial (P < 0.001), and venous (P < 0.001) concentrations during hemodialysis, whereas intracellular muscle concentration was maintained.

For alanine, there were significant differences between concentrations at all sites during the basal period, except between arterial and venous blood (Fig. 5, top right). During hemodi-
analysis, alanine decreased in arterial blood ($P = 0.006$ vs. basal), venous blood ($P = 0.016$), and interstitial fluid ($P < 0.001$), making them all different from intracellular muscle concentration ($P < 0.001$) but not different from each other. In contrast to the response in the other compartment, muscle intracellular concentration of alanine actually increased during hemodialysis ($P < 0.001$). A similar response was observed with the HPLC data, except the response was statistically significant only when measured by means of tracer methodology.

It was not possible to do repeated-measures ANOVA in the case of lysine because of a lack of values caused by problems analyzing some of the samples. If $t$-tests were used for each site, significant drops were found in the arterial blood ($n = 9$), venous blood ($n = 9$), and interstitial ($n = 6$) concentrations, but no change in intracellular concentrations ($n = 6$; Fig. 5, bottom right).

Concentration ratios between blood, interstitial and intracellular fluid. Because the interstitial amino acid concentrations mirrored the changes in blood, the basal arterial-to-interstitial concentration ratios for phenylalanine ($0.88 \pm 0.07$), leucine ($1.11 \pm 0.23$), and alanine ($0.64 \pm 0.05$) did not change during hemodialysis ($0.82 \pm 0.04, 1.15 \pm 0.30, \text{and } 0.66 \pm 0.04$, respectively, NS). In contrast, the basal arterial-to-intracellular concentration ratios for phenylalanine ($0.80 \pm 0.03$), leucine ($0.93 \pm 0.02$), and alanine ($0.33 \pm 0.02$) all decreased during hemodialysis ($0.62 \pm 0.01, 0.68 \pm 0.02, 0.17 \pm 0.01$, respectively, $P < 0.00001$ vs. basal). The basal interstitial-to-intracellular concentration ratios for phenylalanine ($0.98 \pm 0.08$), leucine ($1.00 \pm 0.10$), and alanine ($0.54 \pm 0.06$) also decreased during hemodialysis ($0.78 \pm 0.04, 0.76 \pm 0.07, 0.27 \pm 0.02$, respectively, $P < 0.05$ vs. basal). In the case of lysine, the arterial-to-intracellular concentration ratio changed from $1.73 \pm 0.39$ during the basal state to $1.04 \pm 0.18$ during hemodialysis ($n = 6$, $P = 0.052$), whereas the corre-
sponding changes for the interstitial-to-intracellular ratio was from 0.99 to 0.81 and for the arterial-to-interstitial ratio from 0.99 to 1.04 during hemodialysis (no statistical analysis performed because of missing data).

**Amino acid kinetics.** Amino acid kinetics were calculated for two specific intervals: in the basal period before start of dialysis and from 30 to 120 min after start of hemodialysis (Table 1). There was a steady state in both concentration (Fig. 5).

**Table 1. Amino acid kinetics during basal period and from 30 to 120 min after start of hemodialysis**

<table>
<thead>
<tr>
<th>Period</th>
<th>Phenylalanine</th>
<th>Leucine</th>
<th>Alanine</th>
<th>Lysine</th>
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<tr>
<td>F_in</td>
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<td>372±33</td>
<td>1,217±116</td>
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<td>782±103*</td>
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<td>834±109*</td>
<td>1,099±109*</td>
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<td>127±19</td>
<td>134±19</td>
</tr>
<tr>
<td>Ox (+Met)</td>
<td>Basal</td>
<td>30±13</td>
<td>557±121</td>
<td>12±5</td>
</tr>
<tr>
<td></td>
<td>Hemo</td>
<td>26±8</td>
<td>466±128</td>
<td>646±136</td>
</tr>
</tbody>
</table>

Results are means ± SE in nmol/min⁻¹·100 ml leg⁻¹; n = 12 for phenylalanine, leucine, and alanine; n = 9 for lysine. For explanations, see MATERIALS AND METHODS and Fig. 2. Hemo, hemodialysis; F_in, rate at which the amino acid enters the leg via the artery; F_out, rate at which the amino acid leaves the leg via the vein; shunt, rate at which the amino acid is “shunted” from artery to vein without uptake into muscle; F_MA, rate of net amino acid movement from artery to muscle; F_VM, rate of net amino acid movement from muscle to vein; PS, rate of amino acid incorporation into protein; PB, rate of intracellular release of the amino acid from protein breakdown; Ox, rate of amino acid oxidation (leucine or alanine); Met, rate of metabolic conversion of alanine; De novo S, rate of de novo synthesis of alanine. *P < 0.05 vs. basal.
4) and enrichment (12) of the amino acids during the two periods, respectively.

During hemodialysis, inflow of amino acids to leg muscle decreased by ~30% for phenylalanine, 35% for leucine, 50% for alanine, and 42% for lysine (P < 0.001 vs. basal for each amino acid, respectively; Table 1). A greater percentage of the reduced delivery was taken up by muscle during hemodialysis. The shunting from artery to vein was reduced by ~40% for phenylalanine (P = 0.004), ~45% for leucine (P = 0.001), ~50% for alanine (P < 0.001), and 40% for lysine (P = 0.001), whereas the decrease in the rate of transport into the muscle cell was reduced by only ~20% for phenylalanine (P = 0.039) and leucine (P = 0.039) and by ~30% for alanine (P = 0.010). For lysine, the decrease in the rate of transport into the muscle cell did not reach statistical significance (P = 0.083).

The rates of phenylalanine, alanine, and lysine incorporation into protein decreased by ~50–75% during hemodialysis (P < 0.001 for each, respectively; Table 1). The rate of utilization of intracellular leucine, which includes utilization for both protein synthesis and oxidation, decreased from 159 ± 23 nmol·min⁻¹·100 ml leg⁻¹ during basal to 75 ± 14 nmol·min⁻¹·100 ml leg⁻¹ during hemodialysis (P < 0.001). The rate of incorporation of leucine into protein was decreased by ~50% during hemodialysis (P < 0.001), and leucine oxidation was a smaller part of leucine utilization, although the reduction during hemodialysis did not reach statistical significance vs. basal value (P = 0.176).

No change in the rate of intracellular release from protein breakdown as a result of hemodialysis was found for phenylalanine (P = 0.089) or leucine (P = 0.179), but for lysine a significant drop was observed (P = 0.001). The total rate of appearance of alanine into the intracellular compartment from protein breakdown and de novo synthesis was 848 ± 145 nmol·min⁻¹·100 ml leg⁻¹ during basal and did not change during hemodialysis (780 ± 147 nmol·min⁻¹·100 ml leg⁻¹, P = 0.428). Further calculations showed that there was no change in the rate of alanine release from protein breakdown (P = 0.085), and there was no change in the de novo synthesis of alanine (P = 0.59).

Finally, the rates of transport of amino acids from the muscle cell to the vein did not change significantly for any of the amino acids (Table 1), but in total, the outflow of amino acids from leg muscle decreased by ~30–40% (phenylalanine, P = 0.003; leucine, P = 0.001; alanine and lysine, P < 0.001) as a result of decreased shunt from arterial inflow.

**DISCUSSION**

The principal finding of this study was that the interstitial amino acid concentration decreased in parallel with the blood concentrations during hemodialysis, whereas the intracellular concentration of amino acids in muscle did not change. The EAA concentrations in the intracellular pool were maintained by coordinated responses of the factors governing appearance and disappearance into and out of the pool. Taken together with the previous reported response to hyperaminoacidemia (5, 7), it appears that maintenance of intracellular EAA concentrations is a metabolic priority, even at the expense of maintenance of the rate of muscle protein synthesis.

**Intracellular and interstitial EAA concentrations during hypoaminoacidemia.** The findings of this study are consistent with the previous observations that the intracellular concentrations of EAA in muscle are maintained relatively constant under a variety of conditions. A relatively stable intracellular concentration of phenylalanine has been found both during infusion of amino acids (5, 7) and after oral intake of amino acids (25). The present study extends the previous observations by demonstrating that intracellular amino acid concentrations are maintained when there is a decrease in blood amino acid levels.

In contrast to the intracellular concentrations, the interstitial concentrations of amino acids dropped during hypoaminoacidemia. The ratios between interstitial and intracellular concentrations dropped accordingly, whereas the ratios between arterial and interstitial concentrations were maintained. This is consistent with the process of diffusion governing the movement of amino acids between plasma and interstitial fluid, whereas the transport between the interstitial and the intracellular fluid is by specific transporters that are subject to regulation. The relation of the interstitial fluid as an intermediary between the intracellular fluid and blood is reflected by the fact that the interstitial amino acid concentrations were higher than the plasma concentrations. This has also been found by others (11, 16) and probably reflects the release of amino acids from the muscle.

Löfberg et al. (13) also observed a drop in plasma amino acid concentration during hemodialysis in humans, whereas only minor changes could be observed in intracellular amino acid concentration. In that study, no measurement of interstitial concentrations was done. The present study shows that the interstitial concentrations mirror the changes in plasma. Maggs et al. (15) found a drop in both plasma and muscle interstitial alanine concentrations during a hyperinsulinemic euglycemic clamp, whereas the intracellular concentration was not measured.

**Regulation of intracellular EAA concentrations.** A decrease in fractional synthetic rate of muscle protein without corresponding decreases in intracellular concentration of total EAA has been found in rats fed protein-deficient or protein-free diets (10). This was interpreted as amino acids being of low importance for the regulation of protein synthesis. The results of the present study suggest that the intracellular concentration of EAA is regulated and that a maintained concentration is made possible through changes in rates of transmembrane transport and de novo appearance and disappearance of EAA into and out of the intracellular free EAA pool and that changes in synthesis may occur in order to maintain the intracellular amino acid concentration constant.

Löfberg et al. (13) found a decreased protein synthesis during hemodialysis in humans, even though only small changes in intracellular amino acid concentrations could be found. In the current study, we have used a three-pool model to quantify muscle amino acid transmembrane transport, as well as amino acid appearance or disappearance into the free amino acid pool in the muscle cell (1, 3). We found that individual intracellular EAA concentrations were maintained by coordinated changes in muscle protein synthesis, inward and outward amino acid transport, and, when applicable, intracellular oxidation. The effectiveness of this type of regulation is consistent with the theory of metabolic control of branched pathways developed by Newsholme and Crabtree (19, 20). They quantified the extent to which greater regulatory sensitivity can be achieved when several processes are regulated compared with
a single process, since small changes in the rates of all the processes can occur more rapidly than if regulation was entirely dependent on large changes in one factor.

It appears that the principal response to the hypoaminoacidemia was a reduction in the rate of protein synthesis. It seems that protein synthesis is regulated by an extracellular rather than an intracellular signal. This is because muscle protein synthesis dropped in accord with the reduction in plasma EAA concentrations even though intracellular EAA concentration was maintained. This was also suggested by Bohé et al. (6), who found a closer relationship between muscle protein synthesis and the concentration of plasma EAA than intracellular EAA, after infusion of different doses of an amino acid mixture into healthy human volunteers. A similar, but less tight, relationship was also found between protein synthesis and plasma NEAA concentration (6). There may be a membrane-associated EAA sensor different from the amino acid transporters, or an intracellular sensor, near the cell membrane. In any case, the regulation seems to be a feed-forward mechanism whereby changes in protein synthesis precede the intracellular amino acid concentration changes. We can be sure that responses were due to changes in amino acid concentrations rather than hemodialysis, since replacement of amino acids during the hemodialysis restored the basal rate of muscle protein synthesis, as we have previously reported (12).

It was somewhat surprising that protein breakdown did not increase in response to the fall in plasma amino acid concentration. Previous in vitro studies (18) have shown that a decrease in free amino acid concentration causes acceleration in the rate of breakdown. Furthermore, we (30) had previously proposed that changes in amino acid availability linked the rates of muscle protein synthesis and breakdown. Thus, in a wide variety of circumstances, changes in muscle protein synthesis are accompanied by a corresponding change in breakdown so that, whereas the magnitude of such a change in breakdown might differ from the amount of change in synthesis, the two processes consistently changed in the same direction (30). For example, stimulation in synthesis will deplete the intercellular amino acid pool, thereby increasing breakdown sufficiently to restore basal amino acid concentrations. According to this rationale, one might have expected breakdown to increase when amino acid availability was decreased by hemodialysis. However, the decrease in synthesis apparently signaled by the change in extracellular amino acid concentrations, coupled with inward transport, effectively maintained the intracellular EAA concentration at the basal level. Therefore, there was no stimulus for muscle protein breakdown to increase. Thus, when considered in the context of the constant concentrations of EAA intracellularly, the absence of a change in breakdown was to be expected. Therefore, the rate of protein breakdown does not appear to be regulated by extracellular amino acid concentration; rather, we suggest that protein breakdown is regulated by the intracellular availability of amino acids. On the other hand, it may be that protein breakdown is regulated by slower-acting mechanisms than is the case for protein synthesis, such that prolonged depletion of plasma amino acids would have eventually resulted in accelerated breakdown.

The reduction in shunting of amino acids from artery to vein was greater than the drop in inward transport. For both phenylalanine and leucine, the inward transport was ~39% of the total inflow to the leg during basal state, whereas ~61% was shunted directly from artery to vein without being taken up into the muscle cell. During hemodialysis, the inward transport was 46–48% of the inflow, whereas the shunt was ~52–53%. For alanine, the inward transport was a smaller amount of the total inflow (15% during basal and 19% during dialysis) compared with phenylalanine and leucine, whereas the shunt was a greater part (85% during basal and 81% during dialysis). Thus it can be concluded that the muscle became more effective during hemodialysis, since a higher percentage of the available amino acids was taken up.

We used tracers with different transport mechanisms in this study. The results are comparable for all of the tracers (even though similar changes in protein synthesis could be expected for phenylalanine, leucine, and alanine, since the calculation of utilization of the last two for synthesis is based on the phenylalanine tracer calculations).

**Differences between EAA and non-EAA concentrations.** Muscle intracellular alanine concentration significantly increased during hemodialysis despite the fall in plasma concentration (Fig. 5, top right). Our interpretation is that the regulation of NEAA intracellular concentration is not as precise as for the EAA. However, on the basis of the present data we cannot conclude that it is only intracellular EAA that is regulated, because the HPLC data showed maintained concentrations also of the NEAA (Fig. 3). NEAA are of less importance than EAA for stimulation of protein synthesis (6, 8), but it may be that total NEAA concentration is of importance, e.g., for osmoregulation.

**Methodological considerations.** Muscle contains a high branched-chain amino acid aminotransferase activity leading to rapid conversion of leucine to \(\alpha\)-ketoisocaproate (KIC). It may therefore be that some of the \(^{14}C\)leucine used to determine relative recovery of interstitial leucine was converted to KIC and later appeared as \(^{14}C\)KIC in the dialysate. Estimations show that this phenomenon, at maximum, caused a 6–7.5% error in the determination of interstitial leucine concentration (27), which would not change our conclusion. Furthermore, there is no reason to believe that the proportion of \(^{14}C\)KIC in the dialysate would change from the basal to the hemodialysis situation. In addition, the recoveries of phenylalanine and leucine were the same, suggesting that the magnitude of error due to leucine being converted to KIC was even less than the estimated maximum. An alternative way of calculating interstitial concentrations of leucine is to assume that the relative recovery of leucine is similar to the relative recovery of phenylalanine. This is a valid assumption, since the molecular weight of phenylalanine is 165.29 compared with 131.17 for leucine. Thus one can expect that their relative recovery is very similar. If we use the measured relative recovery for phenylalanine in our calculations of interstitial leucine, the results remain unchanged, since the relative recovery for phenylalanine and leucine were similar.

The intracellular muscle concentrations of phenylalanine, leucine, alanine, and lysine were corrected for the relative contribution of the intra- and extracellular water (28). The wet weight-to-dry weight ratios of the biopsies did not change during the study; therefore, corrections for changes in wet/dry muscle ratios were not necessary. For each individual biopsy time point, the mean water content of the biopsies was between 82 ± 0.4 and 83 ± 1.5%.
The estimates of the parameter values in the three-pool tracer model depends on the assumption that phenylalanine is not metabolized in muscle. We analyzed some of the blood samples from the present study to determine the extent of conversion of phenylalanine into tyrosine across the leg. There was no increase in enrichment of tyrosine arising from labeled phenylalanine across the muscle. The amount of labeled tyrosine arising from labeled phenylalanine was calculated as follows: (tyrosine concentration–tyrosine enrichment)Arterial/(phenylalanine concentration–phenylalanine enrichment)Arterial – (phenylalanine concentration–phenylalanine enrichment)Venous.

The results showed that the conversion amounted to an average of 3 ± 5% during the whole study, or 5 ± 4% during the hemodialysis period. This was not different from zero. This is in accord with previous reported findings that phenylalanine hydroxylase activity is low in muscle (26), whereas the conversion mainly occurs in kidney and liver in humans (17, 24).

The changes in the electrolyte concentrations in the plasma compartment may also have influenced the concentration in the interstitial compartment and thus the amino acid transporters. However, whereas the changes were significant because of the consistency in the direction of changes, the magnitude of changes were unlikely to be of physiological significance.

Physiological significance. The rationale of a metabolic hierarchy whereby the intracellular amino acid pool is maintained at the expense of muscle protein synthesis is not readily evident. However, the response is consistent with other stressful states in which the muscle protein serves as a reservoir so that amino acid availability can be maintained for other essential tissues and organs. For example, net muscle loss enables maintenance of normal plasma amino acids in both severe trauma (2) and fasting (9), thereby maintaining availability for precursors, such as wound healing, and tissues, such as heart and skin, that have physiological priorities. In this light, it is significant that there were no significant changes in the outward flux of any individual amino acids despite the major reductions in inward flux. Thus maintenance of the intracellular amino acid pool may be necessary to maintain the outward flux of amino acids, thereby enabling muscle protein to effectively serve its role as the endogenous reservoir of amino acids.

In conclusion, it is clear that intracellular EAA concentrations are regulated at a constant level when plasma amino acids are acutely reduced as a consequence of hemodialysis. This was accomplished principally by a reduction in the rate of muscle protein synthesis and a corresponding decrease in the rate of inward transport. Interstitial amino acid concentrations reflected changes in plasma rather than intracellular concentrations. Finally, not only were intracellular EAA maintained during reduction of plasma amino acids caused by hemodialysis, but intracellular NEAA concentrations were also maintained.

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