End-Stage Renal Disease Patients Lose a Substantial Amount of Amino Acids during Hemodialysis

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ABSTRACT

Background: Poor nutritional status is frequently observed in end-stage renal disease patients and associated with adverse clinical outcomes and increased mortality. Loss of amino acids (AAs) during hemodialysis (HD) may contribute to protein malnutrition in these patients.

Objective: We aimed to assess the extent of AA loss during HD in end-stage renal disease patients consuming their habitual diet.

Methods: Ten anuric chronic HD patients (mean ± SD age: 67.9 ± 19.3 y, BMI: 23.2 ± 3.5 kg/m²), undergoing HD 3 times per week, were selected to participate in this study. Spent dialysate was collected continuously and plasma samples were obtained directly before and after a single HD session in each participant. AA profiles in spent dialysate and in pre-HD and post-HD plasma were measured through ultra-performance liquid chromatography to determine AA concentrations and, as such, net loss of AAs. In addition, dietary intake before and throughout HD was assessed using a 24-h food recall questionnaire during HD. Paired-sample t tests were conducted to compare pre-HD and post-HD plasma AA concentrations.

Results: During an HD session, 11.95 ± 0.69 g AAs were lost via the dialysate, of which 8.26 ± 0.46 g were nonessential AAs, 3.69 ± 0.31 g were essential AAs, and 1.64 ± 0.17 g were branched-chain AAs. As a consequence, plasma total and essential AA concentrations declined significantly from 2.88 ± 0.15 and 0.80 ± 0.05 mmol/L to 2.27 ± 0.11 and 0.66 ± 0.05 mmol/L, respectively (P < 0.05). AA profiles of pre-HD plasma and spent dialysate were similar. Moreover, AA concentrations in pre-HD plasma and spent dialysate were strongly correlated (Spearman’s ρ = 0.92, P < 0.001).

Conclusions: During a single HD session, ~12 g AAs are lost into the dialysate, causing a significant decline in plasma AA concentrations. AA loss during HD can contribute substantially to protein malnutrition in end-stage renal disease patients. This study was registered at the Netherlands Trial Registry (NTR7101). J Nutr 2020;150:1160–1166.

Keywords: protein, muscle wasting, nutrient loss, kidney disease, chronic hemodialysis patients

Introduction

Patients with end-stage renal disease fail to adequately remove metabolic waste products and excess fluids from the body (1). To prevent lethal consequences of waste product accumulation, hemodialysis (HD) is employed to replace 10–15% of renal clearance capacity (2). However, patients undergoing chronic hemodialysis (CHD) treatment typically develop impairments in physical function due to a decline in lean tissue mass, cardiorespiratory capacity, and muscle strength (3–5). Though muscle and strength loss can be part of the normal aging process, the progressive loss of skeletal muscle mass is remarkably accelerated in CHD patients (6, 7). Skeletal muscle wasting in CHD patients can be attributed to various factors, including inflammation, malnutrition, and nutrient loss during each HD session (8–10).

Amino acids (AAs) are among the nutrients lost in the dialysate during HD and are of key importance for muscle maintenance (10, 11). Previous work from our laboratory (12–15), as well as that from many others (16–21), has shown that skeletal muscle protein turnover is highly responsive to postprandial increases in plasma AA concentrations. In both healthy and clinical populations the postprandial rise in plasma AA concentrations stimulates muscle protein synthesis rates and inhibits protein breakdown, allowing net muscle protein accretion (14, 22). In CHD patients, muscle protein synthesis as well as breakdown rates are stimulated during HD (23, 24). Previous studies have shown that loss of AAs during
HD causes a decline in plasma AA concentrations in fasted patients (11, 25–29). Moreover, HD induces a negative net forearm AA balance in fasting patients, which may be indicative of muscle proteolysis (24).

In contrast to clinical practice in North America, most CHD patients in Europe are allowed to eat and drink during their HD treatment (30). There is an ongoing debate on the implementation of dietary protein intake during HD to counterbalance the HD-induced decline in plasma AA concentrations in routine care, as some nephrologists cite concerns regarding patient safety and increased staff burden. Moreover, it remains to be established whether habitual food intake before and during HD increases the subsequent loss of AAs in the dialysate. Previous estimates may, therefore, not accurately reflect AA loss in CHD patients consuming their habitual diet during HD.

Therefore, we selected 10 CHD patients to participate in a study in which we obtained blood samples and spent dialysate during HD to assess the selective AA loss in the dialysate. Plasma and dialysate AA concentrations were measured to calculate individual AA extraction rates and to evaluate the relationship between basal plasma AA concentrations, food intake, and AA extraction during HD. This study provides insights into the AA extraction and nutritional requirements of CHD patients consuming their habitual diet during HD.

**Methods**

**Subjects**

Ten patients with a urine production below 100 mL/d, undergoing HD 3 times per week with high-flux membranes for at least 6 mo, were recruited through the outpatient population visiting the HD department of Maastricht University Medical Centre+, Maastricht, The Netherlands. Patients with an active infection, cognitive disorder, or missed HD session in the last month prior to the study period were excluded. Patients’ characteristics are presented in Table 1. Patients were informed of the nature of the experimental procedures prior to providing written informed consent. The current study was approved by the Medical Ethical Committee of the Maastricht University Medical Centre+ and registered at the Netherlands Trial Registry (NTR7101). The applied study design complies with the standards outlined in the most recent version of the Helsinki Declaration.

**Study design**

A single test day per patient was scheduled during the patients’ second or third weekly HD session. Before HD, patients’ handgrip strength and body composition were measured using a mechanical dynamometer (Jamar) and a body composition monitor (Fresenius Medical Care), as described before (31). Directly before and after a 4-h HD session, blood was sampled from the arterial side of the arteriovenous shunt for analysis of plasma AA concentrations. Throughout HD, spent dialysate was continuously collected at a rate of 1.00 L/h in a container using a reversed injection pump (Alaris GW). Every 30 min the collection container was replaced with a new one. After homogenization of the contents of the filled container, a sample of the collected dialysate was obtained.

**Hemodialysis treatment**

Patients’ prescribed blood (300–400 mL/min) and dialysate flow rates (500–600 mL/min) were used during HD. The desired ultrafiltration volume (mean 1.75 ± 0.71 L) was determined by the treating nephrologist. HD sessions were performed with high-flux polysulfone (n = 7; FX-100, Fresenius Medical Care) and polynephron (n = 3; ELISO-17H, Nipro Medical Corporation) membranes, with surface areas of 2.2 and 1.7 m², respectively. Dialysate composition used was equal for all HD sessions and contained sodium (138 mM), potassium (2.00 mM), calcium (1.50 mM), magnesium (0.50 mM), chloride (109 mM), bicarbonate (32.0 mM), and glucose (1.00 g/L).

**Food intake**

Patients were encouraged to consume their habitual diet before and during the test day. Habitual food intake during HD consisted mainly of homemade sandwiches, cookies, coffee, and tea. During the 4th h of the HD session, dietary intake records of the participants were acquired through a 24-h food recall questionnaire. One researcher, who had received training by a licensed dietician, carefully instructed patients on how to perform the food recall questionnaire. All ingested foods and beverages were reported in household measurements or specified as portion sizes. Subsequently, energy and macronutrient intakes were calculated using free available software from the Dutch Nutrition Centre (mijn.voedingscentrum.nl) based upon product specifications provided by food suppliers and the Dutch Food Consumption Database 2016 (NEVO; RIVM) (32). Reported food intake was calculated for the HD session and the 24-h period.

**Plasma AA concentrations**

Blood samples were collected in EDTA-containing tubes and centrifuged at 3500 × g at 4 °C for 10 min to obtain plasma. Aliquots of plasma were frozen in liquid nitrogen and stored in a freezer at −80°C until further analysis. For determination of plasma AA concentrations, 50 μL of blood plasma was deproteinized using 100 μL of 10% S-sulfosalicylic acid (SSA) with 30 μL of the metabolomics AA mix MSK-A2 internal standard (Cambridge Isotope Laboratories). Subsequently, 50 μL of ultra-pure demineralized water was added and this solution was centrifuged at 14,000 × g at 4°C for 15 min. After centrifugation, 10 μL of the supernatant was added to 70 μL borate reaction buffer (Waters). In addition, 20 μL of AccQ-Tag derivatizing reagent solution (Waters) was added, after which the mixture was heated to 55°C for 10 min. AA concentrations were measured using free available software from the Dutch Nutrition Centre (mijn.voedingscentrum.nl) based upon product specifications provided by food suppliers and the Dutch Food Consumption Database 2016 (NEVO; RIVM) (32). Reported food intake was calculated for the HD session and the 24-h period.
FIGURE 1  AA concentrations in (A) pre- and post-HD plasma and (B) spent dialysate of CHD patients. Plasma concentrations of 22 AAs are expressed as μmol/L. Values represent means ± SEMs, n = 10. *Post-HD plasma AA concentrations are significantly different from pre-HD plasma AA concentrations, P < 0.05. AA, amino acid; CHD, chronic hemodialysis; HD, hemodialysis.

profiles in the derivative were determined by ultra-performance liquid chromatography mass spectrometry (UPLC-MS; Acquity UPLC-H-class with QDa detector; Waters) as described previously (33).

Dialysate AA concentrations
Spent dialysate samples were collected in sterile tubes, immediately frozen in liquid nitrogen, and stored in a freezer at −80°C until further analysis. Collected dialysate was concentrated 5 times through freeze-drying 25 mL of the sample and dissolving the dried product in 5.0 mL 0.1 M hydrogen chloride. After homogenization, 50 μL of the concentrated sample was deproteinized using 100 μL of 10% SSA with 50 μM of the metabolomics amino acid mix MSK-A2 internal standard and processed in the same manner as plasma samples. Subsequently, AA profiles were determined through UPLC-MS.

Calculations
AA loss in the dialysate (g) was calculated by multiplying the mean total amino acid (TAA) concentration of spent dialysate (grams per liter) with spent dialysate and ultrafiltration volume (liters). Essential amino acid (EAA) values are the sums of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine values. Nonessential amino acid (NEAA) values equal the sums of alanine, arginine, asparagine, aspartic acid, beta alanine, cystine, glutamic acid, glutamine, glycine, proline, serine, tryptophan, and tyrosine values. Branched-chain amino acid (BCAA) values are the totals of leucine, isoleucine, and valine values.

Statistical analysis
All data are expressed as means ± SEM unless indicated otherwise. Time-dependent variables (i.e., TAA, EAA, and individual AA loss per 30 min) were analyzed by a 1-factor repeated-measures ANOVA. If a statistically significant time effect was found, post hoc paired-samples t tests were performed to locate the effects. Pre-HD and post-HD plasma AA concentrations were compared using paired-samples t tests. Correlations between dialysate AA concentrations and pre-HD plasma AA concentrations and dietary intake were assessed through determining the parametric Pearson’s or the nonparametric Spearman’s rank correlation coefficients for normally and nonnormally distributed data, respectively. Statistical significance was set at P < 0.05. All analyses were performed using SPSS statistics software (version 24.0; IBM Corp.).

Results
Plasma AA concentrations
Pre-HD plasma TAA, NEAA, and EAA concentrations averaged 2.88 ± 0.15, 2.08 ± 0.11, and 0.80 ± 0.05 mmol/L, respectively. Post-HD plasma TAA, NEAA, and EAA concentrations were

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significantly reduced to 2.27 ± 0.11, 1.62 ± 0.07, and 0.66 ± 0.05 mmol/L, respectively (P < 0.05). Pre-HD and post-HD plasma BCAA concentrations were 0.35 ± 0.03 and 0.30 ± 0.03 mmol/L, respectively (P = 0.11). Whereas most individual AA concentrations decreased during HD, we observed a significant increase in plasma tryptophan concentrations (Figure 1A; P = 0.003).

Spent dialysate AA concentrations
In the spent dialysate, the AAs with the highest and lowest average concentrations were glutamine and aspartic acid, respectively (Figure 1B). Spent dialysate TAA concentrations averaged 0.73 ± 0.03 mmol/L and did not differ between the 30-min sampling periods throughout the HD session (P = 0.94). Spent dialysate volume per HD session averaged 128 ± 5.05 L. TAA, NEAA, EAA, and BCAA losses during a single HD session are depicted in Figure 2. AA concentrations in spent dialysate were strongly correlated with pre-HD plasma AA concentrations (Figure 3; Spearman’s ρ = 0.92, P < 0.001).

Dietary intake prior to and during HD
Reported 24-h dietary protein and energy intakes averaged 1.03 ± 0.13 g/kg and 28.3 ± 2.9 kcal/kg, respectively (Table 2). All included patients consumed food and beverages during HD. Patients ingested 0.33 ± 0.05 g protein/kg and 8.9 ± 1.0 kcal/kg during a single HD session. Protein intake during HD was not associated with an attenuated decline in plasma AA concentrations over the HD session (P = 0.22). Protein intake was positively correlated with the incremental AUC of spent dialysate BCAA concentrations (Figure 4A; Pearson’s r = 0.64, P = 0.045). Furthermore, the correlation of protein intake with the incremental AUC of spent dialysate TAA concentrations nearly reached statistical significance (Figure 4B; Pearson’s r = 0.62, P = 0.055).

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Plasma TAA concentrations after HD were ∼tions throughout HD are depicted in concentrations of most AAs during HD, which resulted in from the circulation (11). We observed a decline in plasma harmful substances, HD also extracts small-sized nutrients of AAs during HD results in a significant decline in circulating plasma AA concentrations.

HD is a life-saving treatment for end-stage renal disease patients with inadequate residual renal function (34). Besides ingestion of a typical meal (containing 20–25 g protein). The loss of AAs during HD results in a significant decline in circulating plasma AA concentrations.

In conclusion, 8–15 g of AAs are extracted from the circulation during a single HD session. In the current study, the habitual dietary protein intake of Dutch CHD patients during HD did not fully compensate for this loss, resulting in a significant decline in circulating plasma AA concentrations. This observed AA extraction contributes significantly to protein malnutrition in CHD patients and emphasizes the need to develop effective and individualized nutritional strategies to improve nutritional status in patients frequently undergoing HD.

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The author's responsibilities were as follows—FKH, JSJS, JPK, and LJvC: designed the research; FKH: conducted the experimental trials with assistance from NJHB and JSJS; JMXvK: performed the amino acid analysis; FKH and JSJS: wrote the manuscript and had primary responsibility for final content; and all authors: were responsible for the study design and decision to publish and read and approved the final manuscript.

References


