ORIGINAL RESEARCH

Differences in Amino Acid Loss Between High-Efficiency Hemodialysis and Postdilution and Predilution Hemodiafiltration Using High Convection Volume Exchange—A New Metabolic Scenario? A Pilot Study

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Objective: The objective of the study was to quantify the loss of total amino acids (TAAs), nonessential amino acids, essential amino acids, and branched chain amino acids (BCAAs) produced by high-efficiency hemodialysis (HEHD), postdilution hemodiafiltration (HDFpost), and predilution hemodiafiltration (HDFpre) using high ultrafiltration volumes; and to define the specific AA losses registered in HEHD, HDFpost, and HDFpre; to identify a potential metabolic and nutritional decline into protein energy wasting; to compare AA analysis of arterial blood samples taken from healthy controls and patients with end-stage renal disease undergoing hemodialysis.

Design and Methods: Identical dialysis monitors, membranes, and dialysate/infusate were used to homogenize extracorporeal body influence. Ten patients were recruited and randomized to receive treatment with HEHD, HDFpost, and HDFpre it was used on-line dialytic water methodologies (OL); patients' AA arterial concentrations were measured at the start and on completion of dialysis; TAA from the dialyzer filter was calculated, and baseline levels were subsequently compared with findings obtained 1 year later. Finally, the results obtained were compared with the data from a study of 8 healthy volunteers conducted using bioimpedance analysis and laboratory blood tests to assess nutritional status.

Results: A higher convective dose results in a higher weekly loss of TAA, nonessential AAs, essential AAs, and BCAAs (HEHD: 15.7 g; HDFpost-OL: 16.1 g; HDFpre-OL: 16.3 g, $P < .01$). After 12 months, the same hemodialys patients showed a reduced body and water intracellular mass and reduced phase angle. Arterial concentrations of TAAs and BCAAs were lower than those detected in healthy subjects ($P < .01$).

Conclusion: The study shows that the AA losses in dialytic liquid are greater after high exchange volume HDF techniques, especially HDFpre. The AA losses are not metabolically compensated, so these increase the derangements of predialytic arterial plasma AA levels. Both AA losses and arterial AA perturbations further worsened body composition already after 12 months of additional dialysis.

Introduction

IN THE RECENT years, significant technological advances have been made in the field, thus promoting an enhanced depurative efficiency through the use of high-volume diffusive and convective dialysis membranes.[1] The use in hemodialysis monitors of new and increasingly precise

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feedback systems regulating the application of convective therapies has promoted the establishing of a strict equilibrium between ultrapure plasmatic water and reinfusion solutions aimed at maintaining an appropriate hydroelectric balance in patients undergoing dialysis.[2] Accordingly, personalization of dialysis treatment based on clinical and metabolic status of the patient is becomingly an increasingly feasible option. Additional technological progress is represented by developments in the manufacturing of online (OL) dialysate/infusate using ultrapure water, thanks to the increasingly diffuse use of biosmosis essential for OL water production.[3] These new systems facilitate the high precision ultrafiltration/substitution of high infusion volumes up to 1.2-fold the dry weight of the patient.[4] However, throughout the world, hemodiafiltration techniques are seldom used; in Europe, they account for less than 10-15% of all dialysis methods.[5,6] In the context of this percentage, predilution hemodiafiltration (HDFpre) accounts for less than 3-5%, with the largest share being represented by postdilution hemodiafiltration (HDFpost). Indeed, the efficacy of combined convection-diffusion technologies such as HDFpost derives from a series of parameters correlating the ratio of ultrafiltration rate/plasmatic water flow (mL/minute; filtration fraction [FF]) with a reduced incidence of cardiovascular death. Indeed, a series of studies have been conducted to establish overall reinfusion rates for ultrafiltration plasmatic water. The Spanish Hemodiafiltraciòn on-line study[7,8] and the Turkish HDF OL-HDF[9] study determined total reinfusion volumes in HDFpost as being in the range of 16-20 L/session. In addition, the European Dialysis study group yielded a more precise determination of effective convection rate (ECR). In HDFpost, FF may be achieved at volumes ranging from 20% to 30% and in HDFpre, from 20% to 37.7%.[5] The use of 40 L/session in HDFpre has recently been reported.[10] The use of high convection rates promotes the elimination of middle molecules and medium–high-molecular-weight uremic toxins and the removal of plasma albumin.[11] The clinical benefits of HDFpre and HDFpost technologies versus high-efficiency hemodialysis (HEHD) have been well described elsewhere and include increased cardiovascular stability during dialysis sessions and a lower incidence of cardiovascular events[12]. However, in the present study, we hypothesize that HDFpre and HDFpost may induce a higher loss of amino acids (AAs) compared with diffusive hemodialysis methodologies. In fact, to the best of our knowledge, few studies conducted to date have identified both the class and quantity of AAs lost during HD and HDFpost,[13,14] and no studies have addressed this issue in the context of HDFpre. AA loss is certainly not a trivial matter, particularly when dealing with elderly patients or patients featuring an inadequate protein/calorie intake, in which an excessive loss of AA might facilitate the onset of the protein energy wasting as a combination of malnutrition and systemic inflammation.[15] Protein energy wasting is characterized by a high prevalence in both patients with end-stage renal disease patients (11-25%)[16,17] and those undergoing chronic hemodialysis (30-75%)[18] but is also a negative prognostic indicator for patient survival, physical function, and quality of life.

Therefore, the main aim of this observational prospective study was to quantify AA losses registered using the 3 types of dialysis methods investigated. To this regard, metabolic compensation of the body subsequent to loss of AAs in dialysis fluid was determined by measuring plasma AA concentrations during both predialytic and postdialytic sessions. The second aim of the study was to investigate whether over time AA changes may result in metabolic and nutritional decline in patients subjected to these methods of dialysis. Finally, a subanalysis was performed to compare plasma AA concentrations measured in arterial blood between healthy controls[19,20] and long-term dialysis patients.

Methods

Population

Ten patients undergoing chronic hemodialysis with a treatment duration exceeding 6 months were recruited to the study. All patients were Caucasian males. The inclusion criteria included the following: patients were required to be in a metabolic steady state, aged between 18 and 80 years, free from acute or chronic inflammatory disease, malignant cancers, and/or autoimmune diseases over the previous 6 months, to not have undergone previous kidney transplant that had since failed, to not currently be taking steroid and/or immunosuppressant treatment, and to be devoid of heart failure and conditions associated with acute of chronic respiratory deficit and liver cirrhosis. The mean age of the 10 patients recruited was 70.4 ± 9.5 years (54-80 years), and the mean dialysis age was 77.6 ± 37.7 months. Dry weight of the patients at the start of the study was 72.7 ± 12.3 kg (47-87.4). Data obtained from this sample of patients were compared, and statistical differences were evaluated with the findings of a study conducted on a group of 8 healthy volunteers.[19,20]

Dry weight and target weight were calculated on the basis of both intradialytic and extradialytic clinical assessment and on total body water values determined by means of bioimpedance analysis.

All patients were informed of the aims of the study and signed an informed consent form relating to their participation in the study. Ethical approval for the study was obtained from the Ethics Committee of the Public Health Corporation.

Study Design

Patients were randomized to the following clearance techniques: HEHD, HDFpost, and HDFpre. The sequences of methods selected were as follows: in 3 patients, HEHD-HDFpost-HDFpre; in 2 patients, HEHD-HDFpre-HDFpost; and in 5 patients, HDFpost-HDFpre-HEHD.

For each dialysis method (HEHD, HDFpost, HDFpre), over the week preceding testing patients underwent 3 sessions using the same method. The test was then conducted during the subsequent fourth session, thus corresponding to the longest interdialytic gap. This regimen was adhered to for all 3 methods applied to avoid carry-over effects from the previous methods. The study was conducted between January 2017 and February 2018.

Hemodialysis Procedures

In all patients, the same dialysis monitor and the same type of membranes (nanotechnology low- and high-flux polysulfone) were used, with the following characteristics: in HEHD membranes, ultrafiltration coefficient of 18 mL/minute/mm Hg/hour and a total dialysis surface area of 1.8 m^2 were used; in HDFpost and HDFpre membranes, ultrafiltration coefficient of 59 mL/minute/mm Hg/hour and a total dialysis surface area of 1.8 m^2 were used; blood flow (Qb): 300 ml/minute with vascular access established by means of arteriovenous fistula in the verified absence of recirculation; dialysate flow (Qd): 500 mL/minute; session duration 240 minutes. With regard to HDFpost and HDFpre methods, the ultrafiltration target was to apply reinfusion rates aimed at achieving maximum dialysis efficiency: at least 30-40% dry weight in HDFpost and 60% dry weight in HDFpre. The study duration was 21 days, inclusive of the 3 dialysis sessions performed before testing, and the 14-day gap between the first and last tests. The following variables were monitored: dialysis efficiency (equilibrated Kt/V, eqKt/V) and equilibrated protein catabolic rate + urea reduction rate. Dialysis solution or infusate was acetate free, with the following mandatory concentrations: sodium 138-142 mmol/L, potassium 2-3 mmol/L, $HCO3^-$, 30-35 mEq/L, calcium 1.5 mg/dL, glucose 5.55 mmol/L. All methods provided for the online production of ultrapure fluid periodically subjected to rigorous microbiological testing (colony-forming units = 0/mL) and calculation of endotoxin load (Limulus Amebocyte Lysate Test < 0.001 EU/mL).

Body Composition

Body composition was determined using bioimpedance vector analysis (BIVA). Hydration status, lean mass, and fat mass on reaching dry weight were calculated using Renal EFG BIVA™ Technology (EFG Diagnostics, Belfast, UK) with alternated microcurrent, safe for use in patients with active devices, and application of 2 high sensitivity electrodes (Bivatrodes, Akern s.r.l.).[21,22] Electrical resistance of fluids and cell capacitance were determined. Body mass index was also measured on achievement of dry weight.

Biohumoral Variables

Blood chemistry tests were used to measure the erythrocyte count, Hb, lymphocytes, platelets, C3, total and Low Density Lipoprotein cholesterol, creatinine, total protein, albumin, presession and postsession sodium and potassium, calcium, phosphates, C-reactive protein, pH and presession and postsession bicarbonate; presession and postsession blood urea nitrogen. Blood chemistry tests relating to nutritional status and assessment of bioimpedance tests were carried out at the start of the study and after 12 months treatment.

Amino Acid Kinetics

To avoid interference with plasma AA concentrations, the patients were not allowed to eat over a 6-hour period before or during the dialysis session on blood and dialysate test days. To ensure the highest possible degree of validity of results, accurate methods were used in the sampling and collection of plasma samples to be used in AA concentration tests: before and during the dialysis session 10 mL of whole blood were collected in 2 heparinized test tubes and stored at room temperature (to avoid thermal hemolysis). Plasma was separated within 2 hours of collection by centrifuging at 3,000 rpm for 10 minutes. The plasma obtained was frozen in 2 mL cryogenic test tubes at a temperature of −20°C. Dialysate exiting the outlet filter was sampled by means of the continuous spilling technique[23,24] to allow the quantification of lost AAs, thank to the contribution of a high precision volumetric pump used in drug administration (Agilia®, Fresenius Kabi) located at the dialysate outlet and featuring a constant rate of aspiration over the 4 hours of treatment. The flow velocity of dialysate sampled corresponded to 1% of total dialysate flow. The liquid collected was then mixed to render the solution homogenous and 4 mL sampled and stored in a freezer in two 2 mL test tubes. Within 2 days of collection, the samples were transferred in dry ice to the laboratory where they were stored. These substrates were determined for each dialysis session in arterial blood and in dialysis fluid. Blood determinations were performed both before the start of dialysis treatment and at the end of the session. AAs in each blood sample were measured in triplicate. The mean of the 3 determinations was calculated. Concentrations of free AAs were measured using an AMINOQUANT II AA analyzer based on the HP 1090 high-performance liquid chromatography system with fully automated precolumn derivatization using both orthophthalaldehyde and 9-fluorophenyl-methyl-chloroformate reaction chemistries according to the manufacturer's protocol. The results were obtained by injecting 1 μL of the derivatized mixture and measuring absorbance simultaneously at 338 nm and 262 nm. The AA concentration was expressed in both micromol/L and in mg/dl and compared in our laboratory with standard values; data obtained in these studies were subsequently compared statistically using the same laboratory procedure with those of 8 healthy volunteers in whom AA arterial concentrations had been determined.[19,21] Twenty AAs were determined, although analysis was limited to total

AAs (TAAs), essential AAs (EAAs) including branched chain AAs (BCAAs), nonessential AAs (NEAAs), and the ratio of all classes of AA/TAAs.

Statistics

Statistical analysis was performed using the linear mixed models, in which patient identification was introduced as a random effect. This operation allowed us to take into account potential differences resulting solely from patient details and to eliminate them from the model, thereby increasing power and precision. All analyses were performed using software R ver. 3.4.1. The disconnection/connection difference, percentage loss between disconnection and connection, and the results of statistical tests are reported for all comparisons.

Results

Hydroelectric Regulation and Dialysis Adequacy

The findings of the study confirmed that the variables obtained were within the ranges defined by the study. The dialysis solutions used displayed feature characteristic of ultrapure waters with total absence of microbial growth and negative Limulus Amebocyte Lysate Test. The mean concentrations of dialysis fluids were sodium, 139.9 ± 1.2 mmol/L; potassium, 2.9 ± 0.3 mmol/L; $HCO3^-$, 32.5 ± 1.9 mEq/L; calcium, 1.5 mg/dL; and glucose, 5.55 mmol/L. No acetate-containing dialysates were used. A thrice-weekly dialysis regimen with session duration of 240 minutes was used. Interdialytic weight gain was well controlled in the long interdialytic gap in HEHD, HDFpost, and HDFpre, where the following values were detected: 2.8 ± 0.6 Kg, 3.0 ± 0.6 Kg, and 2.6 ± 0.8 Kg, respectively (P = not significant).

High ECRs corresponded to 30.1 ± 3.7% dry weight in HDFpost, while in HDFpre, a very high ECR of 65.1 ± 7.9% dry weight was obtained.[5] A stable hydroelectric status was maintained throughout the 3 dialysis methods; HEHD: Na connection/disconnection: 138.4 ± 1.7/141.2 ± 1.7 mmol/L; HDFpost: Na connection/disconnection: 139.5 ± 1.2/141.4 ± 1.0 mmol/L; HDFpre: Na connection/disconnection: 138.4 ± 2.7/141.0 ± 1.5 mmol/L (P = not significant.); plasma potassium: HEHD K connection/disconnection: 5.3 ± 0.8/3.9 ± 0.3 mmol/L; HDFpost: K connection/disconnection: 5.4 ± 1.0/3.9 ± 0.4 mmol/L; HDFpre: K connection/disconnection: 5.3 ± 0.8/4.0 ± 0.2 mmol/L (P = not significant); serum bicarbonate: HEHD: $HCO3^-$: connection/disconnection: 21.2 ± 1.7/25.1 ± 2.0 mEq/L; HDFpost: $HCO3^-$: connection/disconnection: 21.5 ± 2.1/25.1 ± 1.9 mEq/L; HDFpre: $HCO3^-$ connection/disconnection: 21.5 ± 1.8/25.3.0 ± 1.8 mEq/L (P = not significant).

Table 1 shows the blood chemistry values obtained at the start of the study: serum protein concentrations were in the normal range, with a modest state of systemic inflammation and hypocholesterolemia; a decrease in specific immunological capacity (lymphocytes) was observed. After 1 year, serum protein concentrations remained within normal range, with an additional, although not significant, increase in state of systemic inflammation and persistence of hypercholesterolemia. Complement component C3 remained within normal range, although values were significantly lower. Moreover, a low total lymphocyte count persisted.

Table 1. Blood Chemistry Data of All Patients at the Start of the Study and After 12 Months

	Start	Twelve Months
Studied patients	*10*	*10*
Total protein, g/dL	*6.7* ± 0.6	*6.6* ± 0.5
Albumin, g/dL	*3.9* ± 0.2	*3.8* ± 0.3
Blood urea nitrogen, mg/dL	*63* ± 10	*68* ± 9.7
Creatinine, mg/dL	*9.9* ± 1.6	*10.3* ± 1.8
Total cholesterol, mg/L	*128* ± 24	*131.2* ± 6.8
CRP, mg/dL*	*5.8* ± 2.4	*7.0* ± 6.3
C3, mg/dL†	*94.8* ± 9.4 ‡	*87.7* ± 9.5 ‡
Hb, g/dL	*12.3* ± 2.3	*11.8* + *0.7*
Lymphocytes, mm^3	*1135* ± 307	*1180* ± 539
Calcium, mg/dL	*9.3* ± 0.9	*8.9* ± 0.7
Phosphates, mg/dL	*5.7* ± 1.5	*5.3* ± 1.2

CRP normal values: < 5 mg/L.
*CRP, C-reactive protein.
†C3: normal values: 80-160 mg/dL.
‡P < .001.

Table 2 illustrates the main dialysis parameters. Mean reinfusion volumes in HDFpost and HDFpre of 29.8% dry weight and 64.5% dry weight, respectively, were detected. Values obtained for eqKt/V, urea reduction rate, and infusion volumes indicated the adequacy of dialysis performed.[25] It should be underlined that patients were not affected by thirst over the immediately postdialysis period.[26] Patients had a mean daily protein intake, calculated on the basis of eqPCR, of 0.98 ± 0.17 g/Kg/day at the start of the study and 0.99 ± 0.16 g/Kg/day after 1 year. Three of 10 patients were taking anticholesterol drugs: one, ezetemibe + simvastatin, one, omega-3 fatty acids, and the last one, omega-3 fatty acids + ezetimibe.

Body Composition Calculated Using Bioimpedance Analysis

At the start of the study, when compared with healthy volunteers, all patients manifested an altered distribution of body fluids resulting in a reduction of intracellular and an expansion of extracellular fluids. The total amount of body fluids was generally reduced. As a consequence, dialysis patients displayed a decrease in body cell mass with reduction in cell mass and marked reduction in muscle mass. Patients undergoing dialysis moreover presented with a reduced integrity of tissues as expressed by the phase angle. Twelve months after the start of the study, the

Table 2. Dialysis Adequacy, Solutes, and Infusion Volumes.

	HEHD	HDFpost	HDFpre
Studied patients	10	10	10
Average age, years		70.4 ± 9.5	
Dialysis vintage, months		77.6 ± 37.7	
Dry weight, Kg		72.7 ± 12.3	
Interdialytic weight gain (IWG), Kg	2.8 ± 0.6	3.1 ± 0.6	2.8 ± 0.5
Equilibrated Kt/V	1.37 ± 0.2	1.41 ± 0.1	1.38 ± 0.1
Equilibrated protein catabolic rate, g/Kg/day	0.93 ± 0.1	0.92 ± 0.1	0.89 ± 0.1
Urea reduction rate, %	72.5 ± 4.5	74.1 ± 2.6	73.5 ± 3.0
Dialytic sodium final concentration, mmol/L	139.9 ± 1.2	139.7 ± 1.3	139.7 ± 1.4
Dialytic potassium final concentration, mmol/L	2.9 ± 0.3	2.8 ± 0.4	2.9 ± 0.3
Dialytic bicarbonate final concentration, mEq/L	32.5 ± 1.9	32.4 ± 1.8	32.5 ± 1.9
Reinfusion total volume (Vuf), L	3.2 + 0.5*	21.7 ± 3.7*	46.9 ± 7.9*
Qd + Vuf + IWG L, liters per session	122.8 ± 0.7*	144.8 ± 4.1*	169.3 ± 7.7*

HDFpost, postdilution hemodiafiltration; HDFpre, predilution hemodiafiltration; HEHD, high-efficiency hemodialysis.
*$P < .001$; total reinfusion; in HEHD, the total reinfusion corresponds to the incremental interdialytic weight gain (IWG).

distribution of both body fluids and metabolically active cell mass and the reduction of tissue integrity had worsened. Table 3 reports values relating to body compartments detected using bioimpedance analysis compared with the normal range reported for the most ethnically comparable population to our population over the age of 60 years.[27]

Plasma AA Concentrations Before Dialysis Sessions

The study showed how patients displayed marked alterations of the circulating class of AA before dialysis sessions compared with healthy controls (Table 4). Indeed, patients showed a significant reduction in TAAs ($P < .001$) as a result of the low levels of nonessential AAs, whereas similar levels of EAAs (leucine, isoleucine, valine threonine, lysine, methionine, phenylalanine, tryptophan) were observed in controls and renal patients. Thus, in the patients studied, the EAA/TAA ratio was found to be significantly higher than in normal subjects ($P = .0015$). Moreover, in the context of EAA, concentrations of BCAA (leucine, isoleucine, valine) were similar between patients and controls, owing to a significantly higher BCAA/TAA ratio in patients ($P < .001$) as a result of reduced TAA.

AA Losses in Dialysis Fluid

The study revealed a loss of AA in dialysis fluid for each treatment modality. The results indicated that the total amounts of AAs lost per session differed for the 3 hemodialysis methods. When considering AA in terms of concentrations (mg/dL) and thrice-weekly loss (Fig. 1), the overall loss of AA during dialysis was significantly higher in HDFpre than in HDFpost and HEHD. In turn, AA loss in HDFpost was higher than in HEHD. A similar loss of EAA was registered for both HDF techniques, although this was significantly higher compared with HEHD. A significantly higher loss of BCAA was observed in HDFpre than in HDFpost, with the latter registering higher losses than those detected for HEHD. To summarize, HDFpre

Table 3. Body Composition Calculated Using Bioimpedance Analysis at the Start of the Study and After 12 Months in All Patients.

	Normal Range Considered	Start of the Study	After 12 Months	*P*
Total body water, %	*56.5* ± 4.7	53.5 ± 6.9	55.2 ± 7.0	.24
Body cell mass, %	*48.4* ± 4.8	43.9 ± 5.4	41.1 ± 4.9	.04
Extracellular water, %	*45.9* ± 3.9	54.9 ± 5.0	57.5 ± 4.6	.05
Intracellular water, %	*54.1* ± 3.9	45.0 ± 5.0	42.5 ± 4.8	.04
Muscle mass, %	*43.6* ± 6.3	38.3 ± 5.6	37.7 ± 5.5	.20
Fat free mass, %	*66.1* ± 17.3	68.0 ± 2.6	70.3 ± 8.8	.23
Fat mass, %	*33.9* ± 17.3	32.0 ± 8.4	29.7 ± 8.8	.24
Phase angle, (°)	*5.8* ± 0.5	4.4 ± 0.7	4.0 ± 0.6	.03
Dry weight BMI	*>25*	25.6 ± 3.3	24.9 ± 3.4	.06

BMI, body mass index.
The first column illustrates the normal ranges for healthy individuals over the age of 60 years.

Table 4. Differences in Arterial AA Concentrations (mg/dL) Between Healthy Subjects and All Dialysis Patients.

	Eight Healthy Subjects (n = 8)	Ten Dialysis Patients (n = 10) (Average of Methods Used)	*P*
TAA	33.1 ± 0.8	25.8 ± 4.3	<.01
EAA	8.9 ± 0.2	8.6 ± 1.9	.61
NEAA	24.4 ± 0.6	17.4 ± 3.2	<.001
BCAA	3.5 ± 0.2	3.4 ± 0.7	.66

BCAAs, branched chain amino acids; EAAs, essential amino acids; NEAAs, nonessential amino acids; TAAs, total amino acids.

elicited a higher loss of TAAs, BCAAs, and NEAAs compared with HDFpost. In turn, HDFpost produced a higher loss of all classes of AA considered compared with HEHD.

Plasma AAs Concentrations After Dialysis Sessions and 12 Months After the Start of the Study

The results of the study revealed a reduction in plasma AA concentrations after all types of dialysis sessions. However, a significantly higher reduction of plasma AA concentrations was registered for methods using higher plasmatic water exchange volumes compared with the HEHD diffusive technique (Table 5). Moreover, the HDFpre method elicited a higher decrease in plasma BCAAs than HDFpost. Twelve months after the start of the study, samples collected immediately before dialysis sessions revealed significant reductions in plasma concentrations of EAAs (−49%) and BCAAs (−18%) compared with the baseline values. Plasma concentrations of NEAAs and TAAs after 12 months revealed no significant variations (Table 6).

To provide a soundly based metabolic assessment of the ratio between total AA and the different classes of EAAs, NEAAs, and BCAAs, these data have been illustrated in Table 7. Although EAAs are not lacking in dialysis patients, EAA/TAA, NEAA/TAA, and BCAA/TAA plasma ratios remain proportional at both the start and end of sessions. These ratios are likewise proportionally maintained in dialysis fluid outlet from the filter.

Discussion

The present study confirmed our hypothesis whereby at the current state of the art, the use of high exchange volume techniques such as HDFpost, and particularly HDFpre, results in a considerably higher loss of AA in dialysis fluid compared with losses observed with currently applied extracorporeal high diffusive methods. This is the first report to highlight the magnitude of loss of AA in dialysis fluid during HDFpre. The study also yielded findings in support of our second hypothesis according to which, over time, subjects undergoing dialysis are subjected to a general worsening of metabolic status, including body composition and specific cell immunity. Furthermore, the study demonstrated how, in the period immediately subsequent to the dialysis, session plasma AA concentrations were reduced compared with the levels observed during the predialysis period, thus indicating the absence and inefficacy in dialysis patients of a metabolic compensation mechanism geared at replacing AAs lost in dialysis

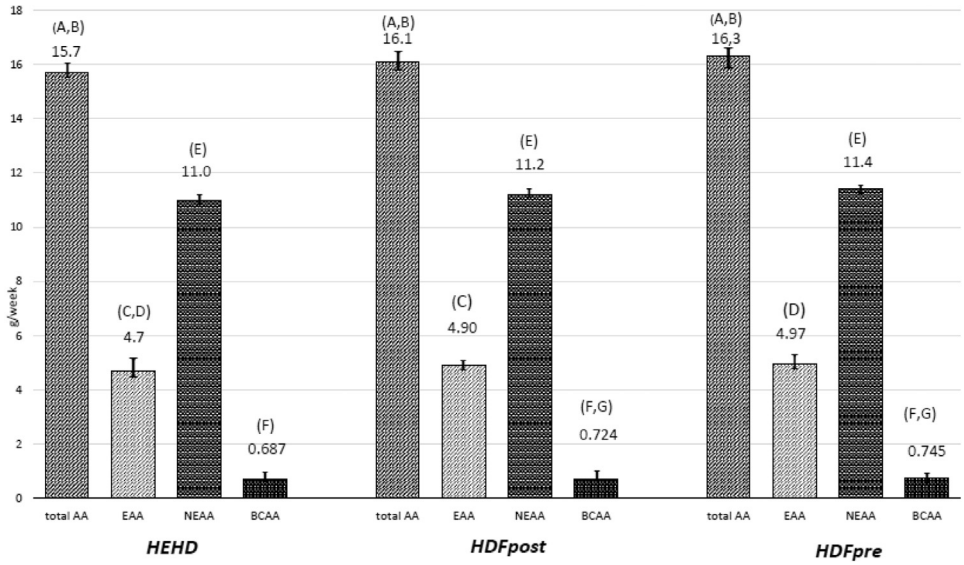

Figure 1. Differences in loss (±standard deviation) of different classes of AAs in the 3 treatment groups. AA losses per session were normalized by total reinfusion volumes. (A) HEHD versus HDFpre and HDFpost: $P < .01$; (B) HDFpost versus HDFpre: $P = .05$; (C) HEHD versus HDFpost: $P = .03$; (D) HEHD versus HDFpre: $P < .01$; (E) HEHD versus HDFpost and HDFpre: $P < .01$; (F) HEHD versus HDFpost and HDFpre: $P < .01$; (G) HDFpost versus HDFpre: $P = .04$. HDFpost, postdilution hemodiafiltration; HDFpre, predilution hemodiafiltration.

Table 5. Average Differences of AA Plasma Levels Between Predialysis and Postdialysis Bicarbonate Hemodialysis (BD), HDF Online in Postdilution (HDFpost), and HDF Online in Predilution (HDFpre).

Amino Acid Plasma Levels, mg/dL	Average Levels Immediately Before All 3 Dialysis Treatments at the Start of the Study		HEHD		HDFpost		HDFpre	
	Average ± SD	%	Average ± SD	%	Average ± SD	%	Average ± SD	%
Total AA								
Start	25.8 ± 4.3	−33.5	26.1 ± 19.2	−26.4[A]	26.6 ± 3.7	−37.9[A]	25.5 ± 3.7	−36.0[A]
End	17.3 ± 3.3		19.2 ± 4.5		16.5 ± 2.1		16.3 ± 1.9	
NEAA								
Start	17.4 ± 3.2	−33.9	18.4 ± 1.3	−33.9[A]	18.8 ± 1.5	−38.1[A]	17.4 ± 1.2	−39.3[A]
End	11.5 ± 2.2		12.1 ± 0.9		11.0 ± 0.8		10.7 ± 0.8	
EEA								
Start	8.6 ± 1.9	−32.6	8.1 ± 1.7	−27.1[A]	8.0 + 1.7	−35.0[A]	8.3 ± 1.6	−37.3[A]
End	5.8 ± 1.4		5.9 ± 0.9		5.2 + 0.9		5.2 ± 0.9	
BCAA								
Start	3.4 ± 0.7	−32.4	3.3 ± 0.7	−24.2[A]	3.2 + 0.8	−31.2[A,B]	3.5 ± 0.6	−37.1[A,B]
End	2.3 ± 0.5		2.5 ± 0.7		2.2 ± 0.3		2.2 ± 0.4	

BCAAs, branched chain amino acids; HDFpost, postdilution hemodiafiltration; HDFpre, predilution hemodiafiltration; HEHD, high-efficiency hemodialysis.

AA values at the start of dialysis were normalized considering hemodilution before treatment

The near left-hand column shows plasma AA levels recorded before the start of the treatment.

[A,B]BD versus HDFpost and HDFpre and HDFpost versus HDFpre. $P < .001$.

fluid. Finally, subanalysis conducted on arterial plasma concentrations before dialysis sessions showed a marked alteration of AA concentrations in subjects undergoing hemodialysis compared with healthy individuals.

Dialysis Techniques and AA Losses

Based on a thrice-weekly dialysis regimen, the loss of AA in dialysis fluid amounted to approximately 15-16g, although this figure rose significantly when using high convection volume exchange techniques versus diffusive methods. Yearly AA loss in the dialysis fluid of patients on thrice-weekly hemodialysis is estimated to be in the region of 800 g using the HEHD diffusive technique, 837 g in HDFpost, and 850g in HDFpre. The study highlighted a marked homogeneity of AA concentrations in the dialysis fluid of all types of treatment applied, as demonstrated by the low values of standard deviation detected. The latter was likely due to the technique used and to the degree of homogeneity of all parameters applied in this study with the aim of avoiding methodological bias, which were seen to be associated in other studies[13,14] with inevitable interferences deriving from the heterogeneity of methods and treatments. Comparable parameters were applied to all 3 methods used in the present study: eqKt/V,[25] the same type of membrane, duration of treatment, the same Qb and Qd, use of ultrapure dialysis fluid, the same monitors, materials, and extracorporeal circuit devices. Finally,

Table 6. Arterial Predialysis AA Concentrations of All Patients Studied at the Start of the Study and After 12 Months

	Average of All 3 Dialysis Treatments (Start of the Study)	Average of All 3 Dialysis Treatments (After 12 Months)
Total AA, mg/dL	26.0 ± 4.5	28.3 ± 5.3
NEAA, mg/dL	17.4 ± 3.2*	21.2 ± 6.3*
EEA, mg/dL	8.6 ± 1.9	4.4 ± 1.1
BCAA, mg/dL	3.4 ± 0.7†	2.8 ± 0.7†

BCAAs, branched chain amino acids.

*$P < .001$.

†$P = .018$.

Table 7. Ratio of the 3 Classes of Amino Acids Versus Total Amino Acids in Different Samples

Ratio	Sampling	HEHD	HDFpost	HDFpre
EAA/TAA	Start	0.31 ± 0.05	0.30 ± 0.04	0.32 ± 0.04
	End	0.31 ± 0.04	0.31 ± 0.04	0.32 ± 0.04
	Dialysis fluid	0.30 ± 4.3	0.31 ± 0.05	0.31 ± 0.04
NEAA/TAA	Start	0.69 ± 0.05	0.70 ± 0.04	0.68 ± 0.04
	End	0.69 ± 0.04	0.69 ± 0.04	0.68 ± 0.04
	Dialysis fluid	0.70 ± 0.04	0.71 ± 0.05	0.68 ± 0.03
BCAA/TAA	Start	0.14 ± 0.03	0.13 ± 0.02	0.15 ± 0.02
	End	0.15 ± 0.02	0.14 ± 0.01	0.15 ± 0.02
	Dialysis fluid	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.02

BCAA, branched chain amino acids; EAA, essential amino acid; HDFpost, postdilution hemodiafiltration; HDFpre, predilution hemodiafiltration; HEHD, high-efficiency hemodialysis; NEAA, nonessential amino acid; TAA, total amino acid.

the precision of this method in determining mass AA transfer using the technique of continuous spilling of dialysis fluid throughout the entire dialysis session, and taking into account the exact amount of dialysis fluid exiting the filter outlet, should be underlined. The justification underlying our choice of method based on convective adequacy is of similar importance; using the HDFpost method, FF applied was more than 8% higher than levels reported in literature and more than 58% higher using HDFpre.[5,10] These percentages are higher than those recently reported in literature,[5,26] thereby allowing us to achieve significantly higher exchange volumes using online HDFpost and HDFpre compared with the previously indicated parameters, obtaining for HDFpost a plasmatic water exchange of 30.6% dry weight and 66.7% for HDFpre, thus exceeding significantly the mean 8.3% FF dose used in HDFpost[7-9] and particularly the 58.5% FF in HDFpre.[5,10]

Plasma AAs and Body Composition

The study demonstrated how patients with end-stage renal disease were characterized by marked alterations of plasma AA availability and decline in body composition. This metabolic profile was found to have worsened further after an additional 12 months of dialysis.

At the start of the study, patients showed a reduction in circulating TAA levels because of low levels of NEAAs and unimpaired circulating EAA and BCAA levels, similar to those observed in healthy controls. Consequently, this plasma AA profile is the opposite of that observed in patients with Chronic Kidney Disease (CKD) on conservative therapy in whom the main characteristics are low plasma BCAA, mainly Valine and high NEAA concentrations.[28]

Thus, the contributions of EAAs and BCAAs to circulating total AAs in the patients studied are significantly higher than in healthy individuals. At first glance, this finding may paradoxically suggest a better nutritional status of patients compared with healthy subjects.

To explain this phenomenon, we postulate that normal EAA and BCAA levels are not the result of increased patient intakes of high-quality protein but rather to an increased muscle release of these AAs. Several factors support this hypothesis. First, in the predialysis session, patients presented with metabolic acidosis associated with decompensated acidemia.[29] Intracellular acidosis is responsible for increased muscle protein breakdown. Second, patients had a systemic chronic inflammation causing muscle protein catabolism. Third, patients were characterized by a chronic lower protein intake: the difficulty of achieving recommended optimum intake of protein and calories in patients undergoing hemodialysis is widely acknowledged.[30] Fourth, during each treatment session, patients' loss of AAs was not immediately compensated by body metabolism. Indeed, at the end of the dialysis session, plasma AA profile had worsened compared with presession values. Finally, hemodialysis per se causes an acute increase in muscle protein catabolism,[31] while at the same time, a decrease in muscle protein synthesis.[32] This increased muscle protein breakdown may also be associated with high muscle protein synthesis, although the rate of catabolism exceeds the rate of protein synthesis: marked muscle hypercatabolism invariably ensues. Indeed, during each hemodialysis session, arteriovenous AA balance becomes increasingly negative compared with presession values.[31] The dialysis procedure likely induces inflammation, thus contributing to exalted muscle protein degradation.

Briefly, normal EAA and BCAA concentrations in patients on chronic dialysis may reflect a marked AA release from muscle resulting in long duration of muscle wasting[32,33] and is not able to maintain a good nutritional status and stable metabolic balance. The finding of an increased muscle hypercatabolism in our patients was supported by the fact that they were found to be affected by sarcopenia. Moreover, increased contributions of EAAs and BCAAs to TAAs are similar to those detected in cachectic cancer patients,[34,35] in whom increased EAA and BCAA/TAA ratios are associated with a decrease in psoas muscle volume.[36] On the other hand, acidosis and cancer share an increased ubiquitin-proteasome proteolytic pathway.[37] With respect to NEAAs, it is plausible that the reduced plasma levels observed may be because of an overconsumption of these substrates by the body. Acidosis-induced muscle hypercatabolism represents an important factor for high AA consumption, as catabolic processes necessitate the production of large amounts of cell energy. AAs, including NEAAs, are implicated in the formation of energy in cells as they are the only substrates used in the tricarboxylic chain acid cycle. Muscle biopsy carried out in subjects with chronic kidney disease found increased transaminase activities in the tricarboxylic cycle.[38]

The overconsumption of NEAAs by the body may also occur during dialysis sessions to decrease whole-body protein catabolism, thereby suggesting a central utilization of AA released from skeletal mass.[31] This may explain the discrepancy between deterioration of body tissue and maintenance of normal circulating albumin found in this study, although it fails to explain the low blood lymphocyte count observed. We hypothesize that the lack of one or more AAs of specific importance in lymphocyte proliferation, differentiation, and maturation may be involved. The preservation of circulating albumin was enhanced by the absence of insulin resistance, because of the fact that patients with coexistent insulin resistance or diabetes were excluded from the study. The finding of preserved albumin is of particular importance because of the fact that proteins are lost in dialysis fluid.

Twelve Months After the Start of the Study

The results of this study demonstrate how 12 months after the start of the study, plasma EAA and BCAA

concentrations were significantly reduced compared with the levels observed at the outset. This indicates a progressive imbalance over time between AA consumption and exogenous dietary intake. Indeed, patients' protein intakes both at the start of the study and 1 year later were lower than recommended. This, however, did not match the loss of AA, including EAA, and muscle protein synthesis maintained during dialysis. In the long run, this phenomenon may have caused an impoverishment of EAA arterial levels. It may also be possible that increased amounts of EAAs could have been consumed in the formation of NEAAs, thus contributing further to impoverishment of circulating levels. Likely, reduced EAA availability in arterial blood may have resulted in an increased body catabolic/anabolic activity ratio leading to increased loss of metabolically active tissues, as highlighted 12 months after the start of the study. Indeed, 1 year into the study, the CKD5D population studied displayed a worsening of intracellular dehydration, hypervolemia, and cell mass wasting.

Conclusion

The present study demonstrates how CKD5D patients are affected by dramatic alterations of plasma AA availability and deteriorated body composition. These phenomena occur in all extracorporeal sessions with efficient high diffusive and convective power especially convective HDFpre. The perturbations of AA metabolism provide a mechanistic plausibility for a further worsening of patient-deteriorated body composition already after an additional 12 months of dialysis. Patient metabolism cannot compensate the AA losses which consequently increase the observed derangements of predialysis arterial AA levels.

Practical Application

It is necessary to clarify that we do not intend to limit the purifying effectiveness of all the most modern extracorporeal therapies. But it is necessary to compensate AA loss possibly by avoiding intravenous administration during the extracorporeal session and only in HEHD and HDFpost, as result of a greater loss by the increase in the blood-dialysate diffusion gradient. During HDFpre, the AAs would all be lost with the ultrafiltered liquid by the dialyzer membranes. Therefore, a mixture of AAs needs to be administered orally few hours after the hypercatabolic effect of dialysis and/or few hours before dialysis. The minimum recommended dose is about 4 g/day. The mixture should be consist of all EAAs and BCAAs to optimize protein synthesis, and specific mixtures tailored to the metabolism of patients undergoing dialysis should be used because there are huge variations in the concentration of each AA.

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References

1. Potier J, Bowry S, Canaud B. Clinical performance assessment of CorDiax filters in hemodialysis and hemodiafiltration. *Contrib Nephrol.* 2017;189:237-245.

2. Ronco C. Hemodiafiltration: technical and clinical issues. *Blood Purif.* 2015;(40 Suppl 1):2-11.

3. Bolasco P, Contu A, Meloni P, Vacca D, Murtas S. The evolution of technological strategies in the prevention of dialysis water pollution: sixteen years' experience. *Blood Purif.* 2012;34:238-245.

4. Bolasco P, Altieri P, Andrulli S, et al. Convection versus diffusion in dialysis: an Italian prospective multicentre study. *Nephrol Dial Transpl.* 2003;18(Suppl 7):vii50-vii54. discussion vii59-62.

5. Tattersall JE, Ward RA, On Behalf of the EUDIAL Group. Online hemodiafiltration: definition, dose quantification and safety revisited. *Nephrol Dial Transpl.* 2013;22:542-550.

6. ERA-EDTA Registry Annual Report 2015. Academic Medical Center, Department of Medical Informatics, Amsterdam, the Netherlands, 2017 Disclaimer ERA-EDTA, its Registry Committee, AMC and its department of Medical Informatics disclaim.

7. Farrington K, Davenport A. The ESHOL study: hemodiafiltration improves survival—but how? *Kidney Int.* 2013;83:979-981.

8. Maduell F, Varas J, Ramos S, et al. Hemodiafiltration reduces all-cause and cardiovascular mortality in incident hemodialysis patients: a Propensity-MatcHEHD cohort study. *Am J Nephrol.* 2017;46:288-297.

9. Ok E, Asci G, Toz H, et al., On behalf of the 'Turkish Online Haemodiafiltration Study. Mortality and cardiovascular events in on line Haemodiafiltration (OL-HDF) compared with high-flux dialysis: results from the Turkish OL-HDF Study. *Nephrol Dial Transpl.* 2013;28:192-202.

10. Masakane I, Kikuchi K, Kawanishi H. Evidence for the clinical Advantages of Predilution on- line hemodiafiltration. *Contrib Nephrol.* 2017;189:17-23.

11. van Gelder MK, Abrahams AC, Joles JA, Kaysen GA, Gerritsen KGF. Albumin handling in different hemodialysis modalities. *Nephrol Dial Transpl.* 2018;33:906-913.

12. Locatelli F, Altieri P, Andrulli S, et al. Hemofiltration and hemodiafiltration reduce intradialytic hypotension in END STAGE RENAL DISEASE. *J Am Soc Nephrol.* 2010;21:1798-1807.

13. Iktzler TA, Flakoli PJ, Parker RA, Hakim M. Amino acid and albumin losses during hemodialysis. *Kidney Int.* 1994;46:830-837.

14. Gil HW, Yang JO, Lee EY, Lee EM, Choi JS, Hong SY. The effect of dialysis membrane flux on amino acid loss in hemodialysis patients. *J Korean Med Sci.* 2007;22:598-603.

15. Rodríguez Ayala E, Divino Filho JC, Alvestrand A, et al. Inflammation contributes to low plasma amino acid concentrations in patients with chronic kidney disease. *Am J Clin Nutr.* 2005;82:342-349.

16. Cuppari L, Meireles MS, Ramos CI, Kamimura MA. Subjective global assessment for the diagnosis of protein-energy wasting in non-dialysis-dependent chronic kidney disease patients. *J Ren Nutr.* 2014;24:385-389.

17. Kovesdy CP, Kopple JD, Kalantar-Zadeh K. Management of protein-energy wasting in non- dialysis-dependent chronic kidney disease: reconciling low protein intake with nutritional therapy. *Am J Clin Nutr.* 2013;97:1163-1177.

18. Garibotto G, Fiorini F, Sala MR, et al. Amino acid loss with polyethersulfone. *Contrib Nephrol.* 2003;138:59-67.

19. Aquilani R, La Rovere MT, Corbellini D, et al. Plasma amino acid Abnormalities in chronic heart failure. Mechanisms, potential Risks and targets in human Myocardium metabolism. *Nutrients.* 2017;9.

20. Jha V, Jairam A, Sharma MC, Sakhuja V, Piccoli A, Parthasarathy S. Body composition analysis with bioelectric impedance in adult Indians with END STAGE RENAL DISEASE: comparison with healthy population. *Kidney Int.* 2006;69:1649-1653.

21. Kyle UG, Bosaeus I, De Lorenzo AD, et al. Bioelectrical impedance analysis-part II: utilization in clinical practice. *Clin Nutr.* 2004;23:1430-1453.

22. Santoro A, Ferramosca E, Mancini E, et al. Reverse mid-dilution: new way to remove small and middle molecules as well as phosphate with

high intrafilter convective clearance. *Nephrol Dial Transpl*. 2007;22:2000-2005.

23. Bolasco P, Galfré A, Caria S, Scotto P, Murtas S. Phosphate nutritional intake control between patient undergoing conventional thrice weekly and infrequent hemodialysis. *Int J Clin Nutr.* 2017;5:18-23.

24. Daugirdas JT, Greene T, Depner TA, Levin NW, Chertow GM. Modeled urea distribution volume and mortality in the HEMO Study. *Clin J Am Soc Nephrol*. 2011;6:1129-1138.

25. Canaud B, Barbieri C, Marcelli D, et al. Optimal convection volume for improving patient outcomes in an international incident dialysis cohort treated with online hemodiafiltration. *Kidney Int*. 2015;88:1108-1116.

26. Atilano-Carsi X, Bajo MA, Del Peso G, Sánchez S y, Selgas R. Vectores de impedancia bioeléctrica de referencia para la población española. *Nutr Hosp.* 2015;31:1336-1344.

27. Cano NJ, Fouque D, Leverve XM. Application of branched-chain amino acids in human pathological states: renal failure. *J Nutr.* 2006;136:299S-307S.

28. Garibotto G, Russo R, Sofia A, et al. Skeletal muscle protein synthesis and degradation in patients with chronic renal failure. *Kidney Int*. 1994;45:1432-1439.

29. Zoccali C, Mallamaci F. Moderator's view: low-protein diet in chronic kidney disease: effectiveness, efficacy and precision nutritional treatments in nephrology. *Nephrol Dial Transplant*. 2018;33:387-391.

30. Raj DS, Welbourne T, Dominic EA, Waters D, Wolfe R, Ferrando A. Glutamine kinetics and protein turnover in end-stage renal disease. *Am J Physiol Endocrinol Metab.* 2005;288:E37-E46.

31. Pérez-Torres A, González Garcia ME, San José-Valiente B, et al. Protein-energy wasting syndrome in advanced chronic kidney disease: prevalence and specific clinical characteristics. *Nefrologia*. 2018;38:141-151.

32. Löfberg E, Essén P, McNurlan M, et al. Effect of hemodialysis on protein synthesis. *Clin Nephrol*. 2000;54:284-294.

33. Norton JA, Gorschboth CM, Wesley RA, Burt ME, Brennan MF. Fasting plasma amino acid levels in cancer patients. *Cancer.* 1985;56:1181-1186.

34. Ahmadnia Z, Joukar F, Hasavari F, Roushan ZA, Khalili M. Dietary Patterns and Risk of Breast cancer in Women in Guilan Province, Iran. *Asian Pac J Cancer Prev.* 2016;17:2035-2040.

35. Kitagawa M, Haji S, Amagai T. High serum essential amino acids as a predictor of skeletal muscle depletion in patients with cachexia and advanced gastrointestinal cancers. *Nutr Clin Pract*. 2017;32:645-651.

36. Lecker SH, Goldberg AL, Mitch WE. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. *J Am Soc Nephrol*. 2006;17:1807-1819.

37. Pastoris O, Aquilani R, Foppa P, et al. Altered muscle energy metabolism in post-absorptive patients with chronic renal failure. *Scand J Urol Nephrol*. 1997;31:281-287.

38. Pastoris O, Aquilani R, Foppa P, Bovio G, Segagni S, Baiardi P, et al. Altered muscle energy metabolism in post-absorptive patients with chronic renal failure. *Scand J Urol Nephrol*. 1997;31(3):281-287.