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Amino acid and albumin losses during hemodialysis

T. ALP IKIZLER, PAUL J. FLAKOLL, ROBERT A. PARKER, and RAYMOND M. HAKIM

Department of Medicine, Division of Nephrology, Department of Surgery and Department of Preventive Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Amino acid and albumin losses during hemodialysis. Protein and calorie malnutrition are prevalent in chronic hemodialysis (HD) patients and has been linked to increased mortality and morbidity in this patient population. Concern has been raised that the open pore structure of high flux membranes may induce the loss of more amino acids (AA) compared to low flux membranes. To address this issue, we prospectively analyzed pre- and post-HD plasma AA profiles with three different membranes in nine patients. Simultaneously, we measured dialysate AA losses during HD. The membranes studied were: cellulosic (cuprophane-CU), low flux polymethylmethacrylate (LF-PMMA), and high flux polysulfone (HF-PS) during their first use. Our results show that pre-HD plasma AA profiles were abnormal compared to controls and decreased significantly during HD with all dialyzers. The use of HF-PS membranes resulted in significantly more AA losses into the dialysate when compared to LF-PMMA membranes (mean ± SD; 8.0 ± 2.8 g/dialysis for HF-PS, 6.1 ± 1.5 g/dialysis for LF-PMMA, $p < 0.05$, and 7.2 ± 2.6 g/dialysis for CU membranes, P = NS). When adjusted for surface area and blood flow, AA losses were not different between any of the dialyzers. We also measured dialysate AA losses during the sixth reuse of the HF-PS membrane. Losses of total AA increased by 50% during the sixth reuse of HF-PS membrane compared to its first use. In addition, albumin was detected in the dialysate during the sixth reuse of HF-PS membrane. We therefore measured albumin losses in all patients dialyzed with HF-PS membranes as a function of reuse. Albumin losses increased significantly beyond 15 reuses. Average albumin losses were 1.5 ± 1.3 g/dialysis below the 15th reuse, but increased to 9.3 ± 5.5 g/dialysis during the 20th reuse. We conclude that the abnormal plasma AA profile in HD patients is further exacerbated with hemodialysis for most of the individual amino acids, and that dialysate AA losses are modulated by membrane characteristics and reuse. Further, HF-PS membranes with reuse numbers over 15 lose substantial amounts of albumin in the dialysate.

Protein and calorie malnutrition are estimated to be present in approximately 20% of patients on dialysis [1–3]. There is increasing evidence linking poor nutritional status to increased mortality and morbidity in this patient population [4–6]. Several factors have been identified which may contribute to this problem. Among these are anorexia and decreased nutrient intake [7, 8], hormonal derangements, such as insulin resistance, increased glucagon sensitivity and excess parathyroid hormone levels [9–12], intake of multiple medications [13], metabolic acidosis [14, 15], and frequent hospitalizations [16].

Dialysis-related factors may also have an important impact on malnutrition [17, 18]. Hemodialysis with certain types of membranes have been shown to increase protein catabolism [19, 20]. Gutierrez et al have reported the increased breakdown of skeletal muscle protein during sham dialysis with bio*in*compatible cellulosic membranes in healthy subjects [21]. This effect was not seen during sham dialysis with biocompatible membranes.

Losses of amino acids during hemodialysis may also contribute to malnutrition [22, 23]. Although amino acid losses during dialysis have been reported previously, these studies have been performed using conventional cellulosic membranes [24–26]. Losses with membranes with higher pore sizes, the so called high-flux membranes, have not been published [22]. In addition, the effect of biocompatibility and the effect of reuse of these membranes on losses of amino acids have not been reported previously. Such studies are important with the increasing prevalence of reuse, which is estimated to be practiced in more than 80% of dialysis units, particularly in dialysis units using high-flux membranes. The reprocessing of such membranes for reuse may potentially affect their pore sizes and change the magnitude or distribution of amino acid losses in the dialysate.

In this study, we measured the impact of three different hemodialysis membranes on plasma amino acid concentrations and simultaneously measured the amino acid losses in the dialysate with same membranes. We also assessed the effect of reuse on these variables in high flux dialyzers.

Methods

Patient characteristics

Nine male patients participated in the study which was conducted at the Veterans Administration Medical Center (VAMC) in Nashville. The mean age of the patients was 61 ± 10.8 years and the mean time on hemodialysis was 29 ± 14.9 months. Three of the patients were insulin-dependent diabetics. All patients were dialyzed with high flux polysulfone membranes prior to entering the study. Urea kinetic modelling and protein catabolic rates (PCR) of the patients were calculated every three months (Mistebar® Computer Cons., Woodridge, Illinois, USA). The KT/V and PCR results of the nine patients participating in the study were 1.49 ± 0.09 and 0.95 ± 0.11 g/kg/day, respectively, and they had a mean serum albumin level of 3.71 ± 0.30 g/dl. All patients had a small meal approximately one hour prior to each dialysis treatment which was similar in composition. The average food composition of their meal was 15.2 ± 6.8 grams of protein and 365 ± 162 calories.

Membrane characteristics

The characteristics of the membranes used in the study are shown in Table 1. The low flux cellulosic (CU) membrane and the

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Table 1. Membrane characteristics of the study dialyzers

Membrane	Cuprophane (CU)	Polymethylmethacrylate (PMMA)	Polysulfone (PS)
Manufacturer	Terumo T150	Toray B2 1.5H	Fresenius F80
Surface area m^2	1.5	1.5	1.8
Pore size distr. (Å)	15–35	15–20	60–80
UFR *ml/hr/mm Hg*	5.1	5	55
Qb *ml/min*	300	300	400
Qd *ml/min*	500	500	500

Abbreviations are: Qb, blood flow, Qd, dialysate flow.

low flux polymethylmethacrylate (PMMA) membrane had similar surface areas of 1.5 square meters (m^2), whereas the high flux polysulfone (PS) membrane had a surface area of 1.8 m^2. The CU membrane used in this study had a pore size ranging between 15 and 35 angstroms (Å) which is higher than the conventional cuprophane membranes. The PMMA membrane had a pore size between 15 to 20 Å while PS had a pore size ranging from 60 to 80 Å.

Clinical Protocol

All patients were dialyzed consecutively with each of the three study dialyzers during the same week. All hemodialysis procedures were performed with a volumetric control device (Fresenius 2008 E). Blood flow rates were 300 ml/min for the low flux CU and PMMA membranes and 400 ml/min for high flux PS. Dialysate flow rates were 500 ml/min for all treatments and dialysis time was 240 minutes per session. The dialysate used during the study was identical for all treatments and contained sodium (139 mEq/liter), potassium (2 or 3 mEq/liter according to the patient's needs), calcium (2.5 mEq/liter), and bicarbonate (39 mEq/liter).

The dialysis unit has been practicing reuse for the past 10 years using an automated reuse machine (Seratronics DRS 4), with bleach and formaldehyde. The concentration of the bleach in contact with the dialyzer was 0.27%. The contact time of the dialyzer with the bleach was five minutes for the PS membranes. Formaldehyde exposure was for 2.5 minutes at a final dilution of 1.5%. Dialyzers were incubated in 1.5% formaldehyde for at least 24 hours at 38°C.

Plasma amino acids

Plasma amino acid concentrations were measured during the first use of all three study dialyzers. Measured parameters included: pre- and post-dialysis (15 min following termination of dialysis) blood samples from arterial blood lines for amino acid (AA) profiles and blood chemistries at each dialysis sessions. For comparison, control plasma samples were obtained from six healthy individuals, both fasting and one hour following ingestion of breakfast containing approximately 15 grams of protein. Plasma samples were separated from blood within 15 minutes of collection and all specimens were stored at temperatures −20°C immediately, until specific assays were performed.

Dialysate amino acid losses

Dialysate amino acid losses were initially measured during the first use of all three study dialyzers, and subsequently, during the sixth reuse of PS membrane. We did not carry out measurements of the other two membranes beyond their first use. Blood flow, ultrafiltration rate (and surface area) were similar during the first and sixth use of this dialyzer. Mean ultrafiltration rate for these patients was 665 ± 87.5 ml/hr.

The volumetric dialysis machines (Fresenius 2008 E) were specially equipped with a dialysate sampling port which allowed continuous collection of efferent dialysate fluid throughout dialysis at a constant rate identical in volume to the total volume of ultrafiltrate. Typical collection volumes were 2 to 3 liters per dialysis session. Aliquots of dialysate were stored at −20°C.

Assays

Blood chemistries (BUN, creatinine, glucose, sodium, potassium, chloride, and total CO_2) were performed using standard laboratory techniques. Plasma and dialysate amino acid profiles were measured by reverse-phase high pressure liquid chromatography (HPLC) using a modified version of the methods of Bidlingmeyer, Cohen and Tarvin [27] and Heinrikson and Meredith [28] in which amino acids are derivatized with phenylisothiocyanate. The phenylthiocarbamoyl amino acids are separated over a 65 minute gradient and detected at 254 nm. Glutamine concentrations were determined enzymatically [29].

Microalbumin was detected in dialysate samples collected during the sixth reuse of high flux PS. We therefore collected dialysate aliquots from additional patients during their subsequent clinical dialysis using the high flux PS membrane. Microalbumin assays were done using Beckmann Array Analyzer with specific immunoprecipitation method.

Statistical analysis

Repeated measures analysis of variance was used to assess overall differences between the three types of membranes studied. When these were statistically significant, we used paired *t*-tests to assess differences between pairs of membranes; in the absence of a significant overall effect, these were considered artifacts of the repeated significance tests. Paired *t*-tests were also used to assess changes within a group when only two measurements were being considered (such as, effect of dialysis on plasma amino profiles with a single membrane, or differences in effect with reuse of a membrane.) Unpaired *t*-tests were used to compare results between groups (such as, between the control and the pre-dialysis group).

Possible correlations between the pre-dialysis concentrations of amino acids, their concentrations in the dialysate and their clearances were assessed using a random effects model (with the subject as random factor) and after adjusting for the molecular weight of the amino acid and the membrane used. Standard (Pearson) correlations are also reported.

Results

Blood chemistries

Pre-dialysis serum concentrations of BUN, creatinine, sodium, potassium, chloride, and tCO_2 were not different between patients for any of the dialyzers. Of note, pre-dialysis tCO_2 levels (23 ± 4 mmol/liter) were in the normal range (23 to 30 mmol/liter) in our study patients and increased to 27 ± 4 mmol/liter post-dialysis. Hemodialysis treatment resulted in the expected changes in routine blood chemistries and there were no significant differences in post-hemodialysis levels between the three membranes.

Table 2. Pre- and post-dialysis plasma amino acid levels with different dialyzers and controls (μmol/liter; $N = 9$)

Membrane		CU		PMMA		PS	
Amino acid	Controls	Pre	Post	Pre	Post	Pre	Post
1M-histidine[a]	6.3 ± 10.5	19 ± 20	9 ± 11[b]	19 ± 18	15 ± 25	20 ± 30	9 ± 14
3M-histidine[a]	2.2 ± 0.9	27 ± 14	10 ± 7[b]	30 ± 15	13 ± 10[b]	29 ± 14	10 ± 6[a]
Alanine[a]	643.0 ± 112.2	288 ± 101	231 ± 68	228 ± 115	192 ± 61	230 ± 97	169 ± 66
Arginine	117.1 ± 17.2	97 ± 36	78 ± 22[b]	95 ± 41	67 ± 22	103 ± 32	65 ± 23[ab]
Asparagine[a]	114.1 ± 49.7	58 ± 21	52 ± 12	59 ± 19	50 ± 14	61 ± 20	48 ± 11[ab]
Aspartate	5.2 ± 1.5	4.0 ± 2.1	2.3 ± 0.9[b]	3.7 ± 0.9	2.0 ± 0.7[b]	4.7 ± 3.9	1.9 ± 0.8[a]
Citrulline[a]	36.2 ± 18.4	115 ± 47	59 ± 21[b]	113 ± 42	53 ± 15[b]	111 ± 46	48 ± 19[a]
Glutamate[a]	21.2 ± 7.5	142 ± 53	100 ± 43[b]	138 ± 41	89 ± 51[b]	134 ± 46	94 ± 53[a]
Glycine	296.3 ± 95.6	257 ± 103	215 ± 67	245 ± 66	190 ± 80[b]	261 ± 127	205 ± 86[a]
Glutamine	526.8 ± 65	495 ± 38	427 ± 38[b]	484 ± 63	378 ± 37[b]	525 ± 93	366 ± 56[a]
Histidine	89.3 ± 46.0	56 ± 15	44 ± 9[b]	58 ± 20	47 ± 15	60 ± 18	41 ± 12[ab]
Hydroxyproline[a]	14.4 ± 4.5	34 ± 15	20 ± 9[b]	34 ± 23	16 ± 11[b]	31 ± 20	18 ± 11
Isoleucine	55.3 ± 20.0	61 ± 15	60 ± 16	68 ± 15	52 ± 21[bc]	75 ± 21	55 ± 21[ab]
Leucine	113.3 ± 30.8	100 ± 22	103 ± 24	110 ± 27	92 ± 33	125 ± 32	92 ± 29[ab]
Lysine	124.7 ± 46.0	139 ± 45	114 ± 32[b]	146 ± 46	110 ± 40	155 ± 48	107 ± 36[ab]
Methionine	27.9 ± 6.6	26 ± 10	22 ± 6	27 ± 16	21 ± 8	27 ± 11	20 ± 8[a]
Ornithine[a]	40.8 ± 10.1	61 ± 22	37 ± 14[b]	67 ± 11	40 ± 13[b]	66 ± 19	37 ± 11[a]
Phenylalanine[a]	45.8 ± 9.8	61 ± 13	63 ± 7	63 ± 17	58 ± 12	68 ± 16	55 ± 10[ab]
Proline[a]	485.8 ± 122.9	297 ± 150	230 ± 103[b]	276 ± 98	202 ± 80[b]	284 ± 116	190 ± 87[a]
Serine[a]	159.2 ± 44.7	75 ± 16	63 ± 13[b]	75 ± 22	52 ± 26[b]	78 ± 27	59 ± 20[a]
Taurine[a]	76.0 ± 13.3	53 ± 24	34 ± 12[b]	41 ± 16	30 ± 12[b]	45 ± 21	36 ± 27[a]
Threonine[a]	163.5 ± 64.0	96 ± 38	80 ± 26[b]	103 ± 47	77 ± 31[b]	109 ± 54	78 ± 37[ab]
Tryptophan[a]	57.1 ± 12.1	22 ± 8	25 ± 6	21 ± 7	22 ± 7	22 ± 10	23 ± 8
Tyrosine[a]	44.1 ± 16.4	38 ± 15	36 ± 10	37 ± 17	32 ± 12	42 ± 15	31 ± 12[ab]
Valine	186.1 ± 64.2	179 ± 48	138 ± 34[b]	196 ± 51	135 ± 47[b]	201 ± 56	128 ± 43[ab]
Total[a]	3452 ± 332	2797 ± 598	2252 ± 448[b]	2737 ± 514	2034 ± 457[b]	2864 ± 687	1982 ± 531[ab]
BCAA	354.8 ± 114.5	340 ± 83	301 ± 72	375 ± 88	279 ± 100[b]	400 ± 102	274 ± 91[ab]
Ess	980.2 ± 259.8	836 ± 204	727 ± 152[b]	888 ± 215	679 ± 206[b]	944 ± 239	662 ± 204[ab]
Non-Ess[a]	2472 ± 292	1960 ± 415	1525 ± 312[b]	1849 ± 308	1354 ± 274[b]	1921 ± 468	1320 ± 334[a]

Values are given as mean ± SD.
[a] Control and average Pre-dialysis groups significantly different; $P < 0.05$.
[b] $P < 0.05$ vs. Pre.
[c] $P < 0.05$ vs. CU-Post.

Plasma amino acid profiles

The concentration of individual amino acids as well as the concentration of groups of amino acids (essential, non-essential, and branched chain) in plasma before and after dialysis for each of the membrane, and with non-fasting controls are shown in Table 2. Pre-hemodialysis plasma amino acid levels were not significantly different between the three dialysis groups for any individual amino acid, or for any of the groups of amino acids. However, compared to normal non-fasting controls, pre-dialysis plasma amino acid levels showed notable difference. Specifically, plasma levels of threonine, tryptophan, alanine, proline, serine, asparagine, and taurine were significantly reduced, while plasma levels of phenylalanine, glutamate, citrulline, ornithine, and methylated histidines were significantly elevated compared to non-fasting controls ($P < 0.05$). When grouped together, total and non-essential amino acid concentrations were also significantly lower than the non-fasting controls ($P < 0.01$ for total and non-essential amino acids).

Table 3 shows the change in individual amino acids, as well as groups of amino acids during hemodialysis with each membrane. For each of the membrane, the reduction in plasma amino acid levels for essential ($P < 0.05$ in each membrane), non-essential ($P < 0.001$ in each membrane) and total amino acids ($P < 0.001$ in each membrane) were statistically significant (Fig. 1). Of note is that the decrease in BCAA levels were statistically significant for PMMA and PS membranes ($P < 0.01$), but not for CU membranes and the magnitude of the decrease in total, branched-chain, and essential amino acid levels were significantly higher for the PS membrane compared to CU membrane ($P < 0.05$).

Figure 2 (A and B) shows the pre- and post-hemodialysis plasma levels versus controls for the essential and non-essential amino acids, respectively. As can be seen, the majority of the individual amino acid concentrations were low pre-dialysis (compared to controls), and their concentrations were further reduced with dialytic therapy. Plasma concentrations of a few individual amino acids, such as methylated histidines, citrulline, hydroxyproline and glutamate, which were higher than the controls pre-dialysis were normalized during hemodialysis.

Dialysate amino acid losses

Amino acid losses in the dialysate for all three membranes are shown in Table 4. Total amino acid losses as well as essential and non-essential groups were significantly higher with the high flux PS in comparison to low flux PMMA (8.0 ± 2.8 g/dialysis vs. 6.1 ± 1.5 g/dialysis, $P < 0.05$). Although more amino acids were lost with CU membrane (7.1 ± 2.6 g/dialysis) in comparison to PMMA membrane, this difference was statistically significant only for non-essential amino acids.

When the results were normalized for the surface area of dialyzers, the losses for CU, PMMA, and PS were 4.8 ± 1.7, 4.1

Table 3. Effect of different dialysis membranes on difference of post to pre-HD plasma amino acid concentrations (μmol/liter; $N = 9$)

Amino acid	CU[a]	PMMA[a]	PS[a]
1Methylhistidine	−9.2 ± 9.4[c]	−3.6 ± 10.6	−11.0 ± 15.7
3Methylhistidine	−17.8 ± 7.8[c]	−17.3 ± 6.0[c]	−19.5 ± 8.7[c]
Alanine	−56.4 ± 84.4	−36.3 ± 97.4	−60.7 ± 82.3
Arginine[b]	−18.8 ± 19.3[c]	−28.4 ± 38.6	−38.9 ± 23.2[cd]
Asparagine	−6.6 ± 10.4	−8.7 ± 15.8	−13.8 ± 13.3[cd]
Aspartate	−1.7 ± 1.9[c]	−1.7 ± 1.1[c]	−2.8 ± 3.8
Citrulline	−55.9 ± 30.3[c]	−59.8 ± 43.8[c]	−63.0 ± 33.5[c]
Glutamine	−67.7 ± 31.8[c]	−106 ± 69.4[c]	−159 ± 115.7[c]
Glutamate	−41.8 ± 35.6[c]	−48.5 ± 43.7[c]	−39.4 ± 38.6[c]
Glycine	−41.8 ± 57.1	−54.4 ± 56.5[c]	−56.3 ± 64.7[c]
Histidine	−12.0 ± 10.1[c]	−10.8 ± 18.6	−19.7 ± 11.0[cd]
Hydroxyproline	−14.3 ± 7.5[c]	−17.7 ± 21.1[c]	−13.6 ± 10.4[c]
Isoleucine[b]	−0.8 ± 11.3	−16.2 ± 15.4[cd]	−19.4 ± 21.3[cd]
Leucine[b]	3.1 ± 16.5	−18.3 ± 27.0	−33.0 ± 38.6[cd]
Lysine[b]	−24.6 ± 27.0[c]	−36.1 ± 48.1	−48.1 ± 35.0[cd]
Methionine	−3.5 ± 6.6	−5.8 ± 13.2	−7.0 ± 6.9[c]
Ornithine	−23.2 ± 17.1[c]	−27.5 ± 10.2[c]	−28.4 ± 11.8[c]
Phenylalanine[b]	1.8 ± 10.2	−5.5 ± 13.1	−13.0 ± 15.0[cd]
Proline	−66.2 ± 71.9[c]	−74.6 ± 67.5[c]	−94.0 ± 90.1[c]
Serine	−12.1 ± 7.4[c]	−22.4 ± 20.1[c]	−19.6 ± 13.1[c]
Taurine	−19.1 ± 18.1[c]	−10.9 ± 13.3[c]	−8.4 ± 23.6
Threonine	−15.4 ± 19.8[c]	−26.5 ± 30.3[c]	−30.1 ± 24.6[cd]
Tryptophan	2.7 ± 3.8	0.8 ± 3.7	1.2 ± 5.7
Tyrosine[b]	−1.8 ± 8.0	−5.0 ± 12.1	−11.4 ± 12.3[cd]
Valine	−41.1 ± 33.5[c]	−61.7 ± 32.8[c]	−73.4 ± 50.5[cd]
Total[b]	−544 ± 288.5[c]	−703 ± 435.4[c]	−882 ± 508.4[cd]
BCAA[b]	−38.9 ± 54.4	−96.1 ± 71.0[c]	−126 ± 104.1[cd]
Essential[b]	−109 ± 125.5[c]	−209 ± 216.7[c]	−281 ± 194.5[cd]
Non-essential	−436 ± 179.8[c]	−495 ± 271.3[c]	−601 ± 334.3[c]

Values are given as mean ± SD.

[a] Negative values indicate decrements of concentrations at the end of dialysis.

[b] Change with dialysis significantly different between the three groups (repeated measures analysis of variance, $P < 0.05$).

[c] Change with dialysis significantly different from zero (paired t-test, $P < 0.05$).

[d] Change with dialysis significantly different from change in cuprophane group (paired t-test, $P < 0.05$).

± 1.0, and 4.4 ± 1.6 grams/dialysis session, respectively. These values were not statistically different from each other ($P > 0.2$ for repeated measures analysis of variance; $P > 0.4$ for comparison of PS membrane with PMMA membrane). In addition, since the blood flow used for PS dialysis was 400 ml/min, resulting in higher (approximately 15%) clearances for small molecules, the losses with the PS membrane, normalized for both surface area and blood flow, was not statistically different from the other membranes.

Amino acid lost in the dialysate was significantly associated with the pre-dialysis concentration in plasma ($P < 0.001$) after adjustment for molecular weight and subject, as described earlier. For each membrane, there was a slight negative association between molecular weight and clearance rate (r value ranging from −0.14 to −0.24, $P < 0.05$ in each membrane) and a strong negative association between molecular weight and dialysate amino acid losses (r value ranging from −0.36 to −0.45, $P < 0.0001$ for each membrane).

Effect of reuse on high flux PS

Amino acid losses. With the sixth reuse of PS, total amino acid losses increased to 12.2 ± 4.4 grams per dialysis session (Table 4). Although this represented a 50% increase in amino acid losses, it did not reach statistical significance ($P = 0.065$), because of the wide variations in individual values. This increase in total amino acid losses was predominantly due (but not limited) to a statistically significant increase in losses of non-essential amino acids. Specifically, 18 amino acids (Table 4) showed increased losses during the sixth reuse of PS in comparison to the first use of PS membrane ($P < 0.05$, sign test, two-sided).

Albumin losses. After detecting microalbumin in several dialysate samples taken during the sixth reuse of PS membrane in our initial study population, we collected dialysate aliquots from all patients during their clinical dialysis with reused high flux PS membranes. However, no further blood specimens were collected during these procedures.

Albumin losses in the dialysate increased steadily with increasing reuse number. The average concentrations of albumin in the dialysate for ranges of reuse numbers are shown in Figure 3. As can be seen, below the 10th reuse ($N = 21$), the quantity of albumin lost is 0.98 ± 0.91 grams per dialysis session (range 0 to 18 grams), increasing to 1.94 ± 1.54 grams (range 0.3 to 8.6 grams) for dialyzers with 10 to 14 reuses ($N = 33$). The average loss of albumin for dialyzers with 15 to 19 reuses ($N = 36$) was 4.3 ± 3.29 grams (range 0.5 to 9.4 g), 9.28 ± 5.50 g/treatment (range 1.1 to 20.8 g) for dialyzers with 20 to 24 reuses ($N = 24$) and 10.78 ± 7.87 g/treatment (range 1.1 to 25.6 g) for dialyzers with over 24 reuses ($N = 11$), a sixfold increase over albumin losses before the 15th reuse.

Discussion

Multiple factors may contribute to the abnormal amino acid profile seen in chronic renal failure [9]. These include inadequate nutritional intake [19], uremic disturbances in amino acid metabolism [30], loss or fibrosis of renal tissue [31], metabolic acidosis [14] and hormonal derangements [32]. Some of these factors, such as metabolic acidosis, are partially corrected with dialytic therapy, but others, such as decreased intake or hormonal disturbances, may persist or worsen after initiation of dialysis. Decreased, metabolically active, renal tissue is potentially another cause of specific abnormalities seen in pre-dialysis plasma amino acid profiles [31]. Increases in phenylalanine and citrulline and decreases in arginine and serine levels, as seen in our patients, have been attributed to reduction of their renal metabolism resulting from a decrease in metabolically active renal tissue [9]. On the other hand, decreased plasma levels of several essential amino acids such as histidine, threonine, and tryptophan observed in our patient population, which were generally 30 to 40% less than the normal controls, may reflect decreased food intake of these amino acids. The differences in amino acid profiles between normal controls and dialysis patients are even more pronounced for many specific amino acids when compared to post-hemodialysis plasma levels (Fig. 2A for essential and Fig. 2B for non-essential amino acids).

Increased attention has been paid to the plasma levels of leucine, isoleucine, and valine, the so-called branched-chain amino acids (BCAA) because of their importance in skeletal muscle energy metabolism, and because leucine has been shown to enhance protein synthesis *in vitro* [33]. Although plasma BCAA levels have been reported previously to be lower in chronic renal failure patients [34], pre-dialysis BCAA levels in our study were

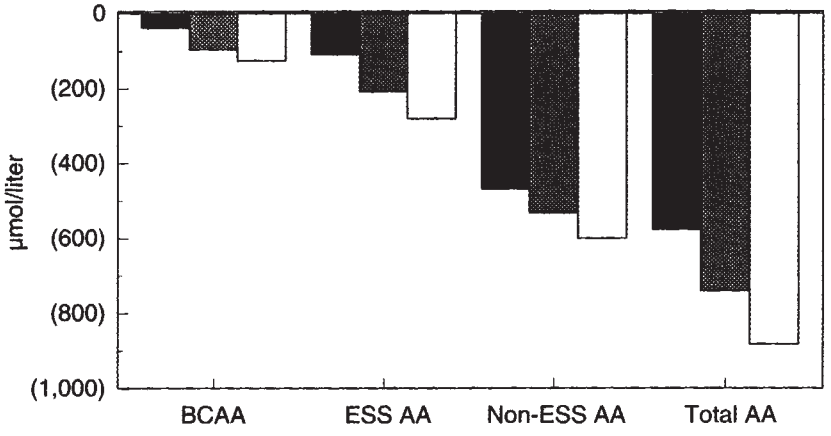

Fig. 1. *Decrease (Post-Pre) in plasma branched chain (BCAA), essential (ESSAA), non-essential (Non-ESSAA) and total (TotalAA) amino acid levels during hemodialysis with different membranes.* Symbols are: (■) CU; (▨) PMMA-LF; (□) PS-HF. All changes are statistically significant ($P < 0.05$), except BCAA during CU dialysis. The magnitude of change with PS-HF is significantly higher in comparison to CU for total, branched chain, and essential amino acids ($P < 0.05$).

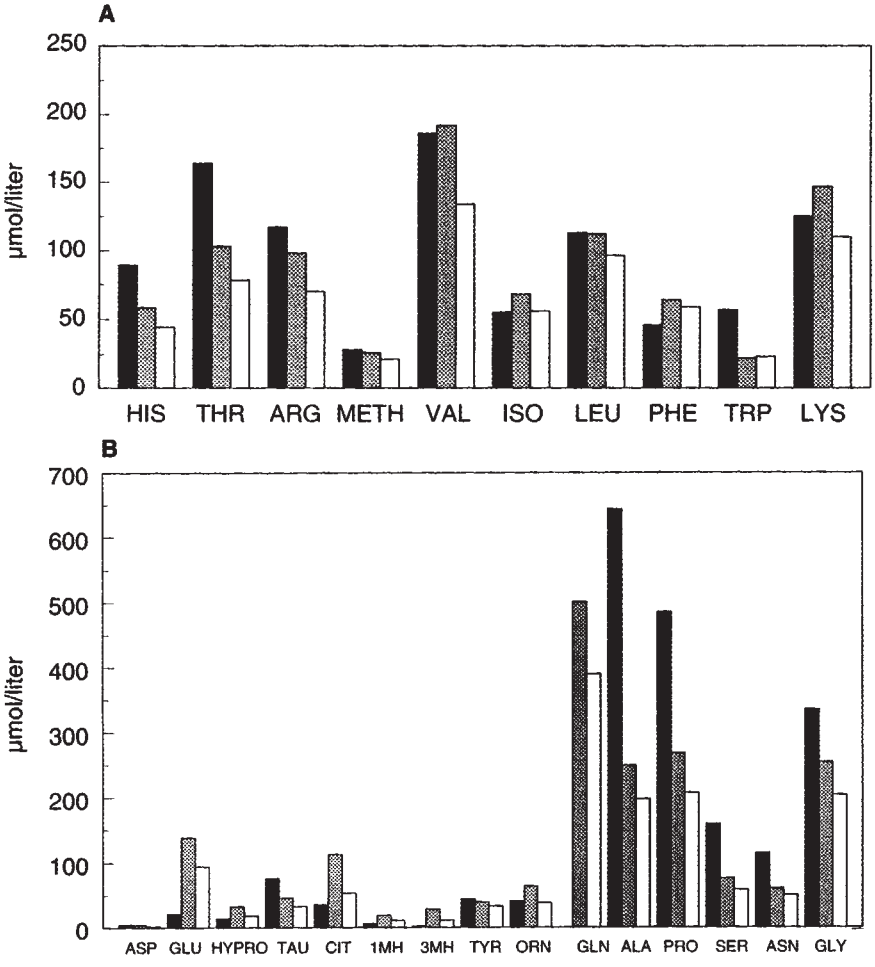

Fig. 2. *(A). Pre-hemodialysis (▨), post-hemodialysis (□), and non-fasting healthy control (■) concentrations of individual plasma* essential *amino acids.* **(B).** Pre-hemodialysis (▨), post-hemodialysis (□), and non-fasting healthy control (■) concentrations of individual plasma *non-essential* amino acids.

within the normal range. This is possibly due to the normalization of metabolic acidosis in our patient population since metabolic acidosis has been implicated in increased degradation of these amino acids [35].

Hemodialysis resulted in a significant decrease in plasma levels of individual and total plasma amino acid levels with each of the three membranes used. However, this reduction of the plasma amino acid pool does not account for the total amino acid losses (in the dialysate) during hemodialysis. Assuming an average weight of 75 kg, a volume of distribution of 0.25 liter/kg, an average intradialytic change of 750 μmol/liter, and an average molecular weight of 170 μg/μmol, the decrease in the plasma pool of amino acids accounts for less than 20% (1.5 to 2.2 g) of the total loss in the dialysate. This discrepancy suggests an enhanced

Table 4. Amino acid losses in the dialysate with different dialyzers (μmol/liter; $N = 9$)

Amino acid	CU	PMMA	PS-1st use	PS-6th reuse
1-Methylhistidine	1.7 ± 1.2	1.0 ± 0.9	1.7 ± 1.9	NM
3-Methylhistidine	2.1 ± 1.4	1.3 ± 1.4	2.1 ± 1.1	NM
Alanine	49 ± 24[a]	32 ± 13	44 ± 24	84 ± 48
Arginine	16 ± 7[a]	12 ± 5	18 ± 8	22 ± 17
Asparagine	11 ± 7	7.7 ± 4.2	12 ± 6[a]	27 ± 11[c]
Aspartate	1.0 ± 0.3[a]	0.6 ± 0.2	0.8 ± 0.5	4.6 ± 2.7[c]
Citrulline	11 ± 5[a]	8.0 ± 5.0	11 ± 8	12 ± 9
Glutamate	25 ± 10[a]	18 ± 4	27 ± 11[a]	23 ± 23
Glycine	49 ± 30	39 ± 18	53 ± 33	84 ± 58
Glutamine	70 ± 10	82 ± 12	82 ± 12	92 ± 49
Histidine	7.2 ± 3.6	6.4 ± 2.7	9.6 ± 4.3[ab]	13.2 ± 6.2
Hydroxyproline	4.1 ± 2.2	3.2 ± 1.6	4.6 ± 3.2	9.8 ± 7.5
Isoleucine	12 ± 6	11 ± 4	16 ± 7[a]	15 ± 9
Leucine	21 ± 8	19 ± 7	27 ± 9[a]	34 ± 12
Lysine	20 ± 10	19 ± 7	27 ± 13[a]	18 ± 12
Methionine	4.7 ± 3.9[a]	2.6 ± 2.4	4.3 ± 3.1[a]	22 ± 14[c]
Ornithine	7.8 ± 3.9	8.2 ± 3.1	10.4 ± 4.7[b]	7.6 ± 4.1
Phenylalanine	12 ± 6	10 ± 5	14 ± 5[a]	12 ± 6
Proline	52 ± 26[a]	38 ± 16	52 ± 27	156 ± 54[c]
Serine	19 ± 17	14 ± 9	19 ± 12[a]	44 ± 50
Taurine	9.0 ± 4.4	7.3 ± 3.3	8.9 ± 3.4	37 ± 20[c]
Threonine	13 ± 11	6.8 ± 5.9	15 ± 14[a]	18 ± 11
Tryptophan	3.2 ± 1.4	2.6 ± 0.9	3.2 ± 1.1	7.3 ± 2.7[c]
Tyrosine	6.8 ± 3.9	5.2 ± 3.0	7.9 ± 3.4[a]	9.6 ± 4.0
Valine	31 ± 15	27 ± 11	36 ± 16[a]	52 ± 12
Total	457 ± 178	382 ± 102	504 ± 190[a]	804 ± 302
BCAA	63 ± 28	57 ± 22	79 ± 32[a]	102 ± 29
Essential	139 ± 62	117 ± 42	169 ± 73[a]	214 ± 65
Non-essential	318 ± 118[a]	265 ± 63	335 ± 120[a]	590 ± 246[c]
Total *g/dialysis*	7.2 ± 2.6	6.1 ± 1.5	8.0 ± 2.8	12.2 ± 4.4

Values are given as mean ± SD.
[a] $P < 0.05$ vs. PMMA.
[b] $P < 0.05$ vs. CU.
[c] $P < 0.05$ vs. PS 1st use.

appearance of new amino acids during dialysis, most likely from other tissue stores such as in skeletal muscle [36, 37].

Our study also revealed that hemodialysis with high flux PS membrane results in more amino acid losses per dialysis session (8.0 ± 2.8 g) in comparison to hemodialysis with low flux PMMA membrane (6.1 ± 1.5 g), but not in comparison to CU membrane (7.2 ± 2.6 g). Wolfson, Jones and Kopple have earlier reported amino acid losses of 8.2 grams per dialysis session, using conventional cellulosic membranes [26], a value comparable to our results. Our study extends this finding to high flux membranes with larger pore sizes; although, as discussed earlier, these losses may not be intrinsic to this membrane, but more related to the larger surface area and higher blood flows used in clinical dialysis with the PS membranes, nevertheless, the amino acid losses during conventional clinical dialysis with such membranes are substantially higher.

A notable feature of the plasma amino acid concentrations during dialysis was the fact that there was no significant decrease in intradialytic plasma BCAA levels with CU dialysis, in contrast to PMMA and PS dialysis. Since losses of BCAA to the dialysate were similar between these two different types of membranes, the lack of significant intradialytic change in plasma BCAA levels with CU suggests an increased appearance of BCAA during dialysis with cellulosic membrane. This suggestion is further supported by the observation that the change in plasma levels of total amino acids was significantly less with CU membrane than PS membrane, in spite of the similar losses to the dialysate. As was previously demonstrated in healthy subjects during sham dialysis [21], the complement activating properties of cellulosic membranes [38], may be a potential explanation for this increased release of amino acids in patients dialyzed with CU membranes.

Losses of amino acids in the dialysate were further exacerbated when the high flux PS membrane was reused. Our results show that at the sixth reuse of high flux PS, an average of 12.2 ± 4.4 grams of total amino acids are lost to the dialysate during each dialysis session, a 50% increase over the value seen during the first use. Increased diffusive and convective losses of amino acids due to increased porosity of the membranes are likely as chemicals used for reprocessing the dialyzer, specifically bleach, tend to enlarge their pores. Indeed, another clinically important evidence of increased pore size with increasing reuse is the detection of albumin in the dialysate during the sixth reuse of PS which was not present during the first use of the same membranes. Thus after the sixth reuse, albumin is detected in the dialysate during almost every dialysis session. Although the losses are small before 15 reuses, averaging 1.5 grams per dialysis session, after the 15th reuse, albumin losses increase exponentially and average up to 10 grams at the 25th reuse. These findings are consistent with recent published results from Diaz et al [39] and Graeber et al [40], who reported increased clearance of β2-microglobulin and increased protein losses with increasing reuses of high flux membranes. Using polydisperse dextran, preliminary studies of reused polysulfone dialyzers indicate the presence of larger pore sizes in the reused dialyzers compared to new dialyzers (Unpublished data). This chronic loss of albumin may result in decreased serum albumin concentrations and possible changes in lipid profiles in these patients. Moreover, these albumin losses may counteract the possible beneficial nutritional effect of higher doses of dialysis [41], and provide an explanation for the results of a recent report which suggested that serum albumin levels in chronic hemodialysis patients are not improved with increasing dialysis doses [42]. Since the dialysis dose is often increased by changing patients to high flux dialyzers [43], albumin losses at high reuse numbers with these dialyzers may counteract the expected increase in plasma albumin levels. Indeed, after discontinuing reuse of HF-PS membranes beyond 15, the concentration of serum albumin in the patients increased by an average of 0.22 ± 0.19 g/dl over three months.

In conclusion, our results reveal that hemodialysis patients display several abnormalities in plasma amino acid profiles. Specifically, most of the essential amino acid levels are decreased while some of the non-essential amino acid levels are increased. Although the etiology of these abnormalities is likely to be multifactorial [15, 44, 45], our findings suggest that the hemodialysis membrane itself has a major impact on these observations either by increasing catabolism and/or from losses into the dialysate. In addition, high flux PS membrane allows more amino acid losses compared to the low flux PMMA membrane. With the sixth reuse of the PS, amino acid losses increase 50%. More importantly, increased number of reuses of the high flux PS membrane leads to leakage of albumin into the dialysate. After the 15th reuse, a mean of 6.8 grams of albumin is lost per dialysis procedure.

The clinical consequences and importance of this increased amino acid losses and the adverse effect of reuse procedure on

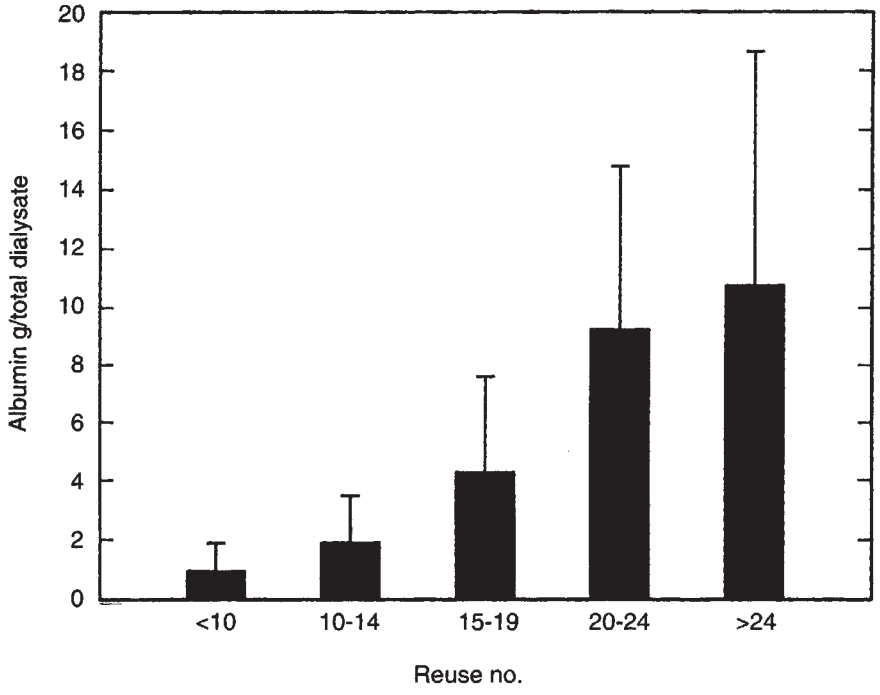

Fig. 3. *Dialysate albumin losses per dialysis session with increasing reuse numbers for high flux PS membranes.*

amino acid and albumin losses with high flux PS membranes need to be more clearly defined by long term clinical studies. Although the total loss of 6 to 8 grams of amino acids per dialysis session represent a small fraction (8 to 12%) of the patients' daily protein intake, nevertheless, this is a loss that these patients can ill afford. In addition, these data demonstrate an important link between the dialysis membrane and protein catabolism.

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Reprint requests to Raymond M. Hakim, M.D., Ph.D., Vanderbilt University Medical Center, 1161 21st Ave. S. & Garland, Division of Nephrology, S-3307 MCN, Nashville, Tennessee 37232-2372, USA.

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