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Effect of in vivo contact between blood and dialysis membranes on protein catabolism in humans

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Effect of in vivo contact between blood and dialysis membranes on protein catabolism in humans. To investigate whether the contact between blood and dialysis membranes might induce muscle protein degradation, the exchange of free amino acids across leg tissues was measured by catheterization technique in three groups of healthy subjects before and after a 150 minute sham-hemodialysis procedure (SHDP), that is, in vivo passage of blood (100 ml/min) through a dialyzer but with no circulating dialysate. Dialyzers with either regenerated cellulose membrane (group CU, $N = 10$ and group CU-IND, $N = 6$) or polyacrylonitrile membrane (group AN, $N = 8$) were used In group CU-IND indomethacin was administered before (100 mg) and at the end (50 mg) of SHDP. Leg blood flow was measured by venous occlusion plethysmography. In group CU net leg release of tyrosine and phenylalanine increased from 3.4 ± 0.8 and 3.6 ± 0.8 nmol/min/100 g tissue, respectively, before SHDP to 7.8 ± 1.8 and 8.3 ± 1.8 nmol/min/100 g tissue, respectively, at 345 minutes after the start of SHDP ($P < 0.01$). The total release of all measured amino acids increased from 148 ± 31 to 309 ± 50 nmol/min/100 g tissue ($P < 0.01$). The results indicate that interaction between blood and regenerated cellulose membranes leads to accelerated net protein breakdown. In group CU-IND no change in leg amino acid release was observed following SHDP, suggesting that the increased net protein catabolism is mediated by prostaglandins. Sham hemodialysis using AN membranes did not result in increased amino acid efflux from leg tissues, implying that the protein catabolic effect of blood-membrane contact depends on the biochemical properties of dialyser.

Several reports have documented that malnutrition is frequently present in patients undergoing maintenance hemodialysis therapy (HD) [1–3]. It is generally accepted that suboptimal nutritional status is associated with increased morbidity and may contribute to poor rehabilitation and quality of life [4]. Low nutritional intake, because of anorexia and vomiting, is a major contributing factor to malnutrition in many hemodialysis patients. However, it has long been recognized that dietary requirements of protein are higher in hemodialysis patients than in normal subjects and non-dialyzed uremics [5]. Healthy subjects have a daily minimum protein requirement of 0.6 g/kg [6], and non-dialyzed uremic patients may be in nitrogen balance on 0.6 g/kg/day of high quality protein [7] or less if the diet is supplemented with essential amino acids [8] or their keto acids [9]. However, signs of malnutrition have been observed in substantial numbers of apparently well rehabilitated hemodialysis patients, who had a daily protein intake of about 1 g/kg body wt/day [1].

There is now evidence that the hemodialysis procedure *per se* is a strong catabolic stimulus [10]. This may be partly explained by the loss of free amino acids into the dialysis fluid, a loss reported to amount to 5 to 8 g per dialysis, about one third of which consists of essential amino acids [11–14]. In addition 4 to 5 g of peptide bound amino acids are also lost per dialysis [11, 12]. Thus the total amount of amino acids lost is about 9 to 13 g per dialysis [11–13]. However, the loss of free and bound amino acids is not sufficient to fully account for the increased protein requirements in hemodialysis patients compared to non-uremic individuals and non-dialyzed uremic patients. Consequently, the possibility must be considered that additional factors not related to the dialytic removal of amino acids are involved.

Interaction between blood and artificial membranes in a dialyzer induces biological effects such as stimulation of the complement system, aggregation of granulocytes, transient leucopenia and release of granulocytic enzymes, clotting and thrombocyte activation [15]. It has been proposed that several side-effects of hemodialysis might be attributed to the increased release of cytokines from monocytes during dialysis, induced by complement activation, endotoxins or acetate in the dialysis fluid [16]. It has later been demonstrated that hemodialysis leads to increased monokine production [17]. Also, it has been shown that interleukin-1 (IL-1) levels are increased in hemodialysis patients in the interdialytic period and that hemodialysis leads to further increases in circulating IL-1 levels [18]. Among the established effects of monokines is the induction of protein catabolism which seems to take place by way of a local release of prostaglandin E_2 in skeletal muscle, which in turn stimulates lysosomal protein degradation. This mechanism has been proposed for the increase in muscle protein breakdown observed in trauma and sepsis [19, 20].

To investigate whether the blood-membrane interaction might induce increased muscle protein catabolism, we studied the exchange of free amino acids across the leg in 10 fasting healthy subjects before and after a sham-hemodialysis procedure, that is, in vivo passage of blood, but with no circulating dialysate, through a dialyzer with regenerated cellulose membrane. Our results indicate that interaction between blood and

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regenerated cellulose membranes leads to accelerated net protein breakdown. In six additional subjects to whom indomethacin was administered before and at the end of the sham hemodialysis no increase in leg amino acid release was observed, suggesting that the net increase in leg protein catabolism is mediated by prostaglandins. Results of several studies have shown that hemodialysis using a dialyzer of polyacrylonitrile membranes leads to less pronounced complement activation and leukopenia as compared with regenerated cellulose membranes [21]. Therefore sham hemodialysis was performed using polyacrylonitrile membranes. In this series no increase in leg amino acid release was observed, implying that the effect of contact between blood and dialysis membrane also on protein catabolism depends on membrane material.

Methods

Subjects

Three groups of healthy volunteers were studied. Ten subjects (group CU), four females and six males, with a mean age of 30 years (range 21 to 40 years) and a mean body weight corresponding to 103 ± 3% of ideal body weight (based upon medium framed individuals, Metropolitan Life Insurance Tables, 1959) underwent sham hemodialysis using regenerated cellulose membrane (Cuprophan®). Six subjects (group CU-IND), two females and four males, with a mean age of 28 years (range 21 to 36 years) and with a body weight corresponding to 109 ± 4% of ideal body weight, were studied with sham hemodialysis using Cuprophan® membrane, after administration of indomethacin. In eight subjects (group AN), seven males and one female, with a mean age of 29 years (range 21 to 39 years) and with a body weight corresponding to 109 ± 3% of ideal body weight, sham hemodialysis was performed using a membrane of polyacrylonitrile (AN69®). The nature, purpose and potential risks of the study were carefully explained to all subjects before they consented to participate in the study. The study protocol was approved by the Ethics Committee of the Karolinska Institute at Huddinge Hospital.

Protocol

All subjects were asked not to take any drugs or contraceptive pills for two weeks prior to the study. The subjects adhered to their ordinary diet until the evening before the study when they began an overnight fast. In all subjects polyethylene catheters were inserted into a femoral artery and ipsilateral femoral vein, for the amino acid determinations. One hour after the insertion of these catheters blood samples were taken to determine the basal plasma concentrations of amino acids. Thereafter sham hemodialysis (vide infra) was started (0 min). In all subjects blood samples were taken after 150 and 345 minutes, and in five subjects of group CU also after 540 minutes. On each occasion four arterial and femoral venous blood samples were taken for amino acid determination. At each time point the arterial-femoral venous differences were calculated as the mean difference between the four arterial and venous plasma samples. Leg blood flow was measured by venous occlusion plethysmography immediately after blood sampling at each time point. Plasma flow (Q_p) was derived from the subjects blood flow (Q_b) and hematocrit (Htc) using the formula $Q_p = Q_b \times (100 - Htc)/100$. Leg amino acid balance was calculated by multiplying the arterio-femoral venous concentration difference for individual amino acids multiplied by leg plasma flow. The six subjects of group CU-IND were given indomethacin by rectal administration at −60 minutes (100 mg) and immediately after the sham hemodialysis procedure at 150 min (50 mg).

Blood-membrane contact

Access for blood circulation through the dialyzer was obtained by catheterization (at −60 min) of the contralateral femoral vein with a single lumen catheter and a cubital vein with a dialysis needle. In groups CU and CU-IND sham hemodialysis was performed using a plate dialyzer with regenerated cellulose (Cuprophan®) membranes (Gambro Lundia 11.5 μ; 1.0 m^2) and in group AN Acrylonitrile-Sodium Methallyl Sulfonate (AN69) membrane (Filtral 12; 1.15 m^2). After rinsing the blood and dialysate compartments of the dialyzer with one liter of saline each, another liter of saline was passed through the blood compartment and filtration of saline through the membrane was achieved by using a negative transmembrane pressure of 440 mm Hg. During the sham hemodialysis procedure blood was circulated through the dialyzer at a rate of 100 ml/min for 150 minutes. The venous blood line was heated to 37°C, thereby preventing the cooling of blood. To exclude the possibility of ultrafiltration or diffusion of amino acids through the dialysis membrane, no dialysate was circulated through the dialyzer and the dialysate ports were clamped. Continuous monitoring of blood flow, venous and arterial pressure, etc., was obtained with a Gambro AK 10 monitor. Before blood circulation through the dialyzer was started a single bolus dose of heparin (100 IE/kg body wt) was administered intravenously.

Analytical methods

Blood samples for amino acid analysis were kept on ice. Plasma proteins were precipitated with 4% sulphosalicylic acid. Samples were then centrifuged at + 8°C and the supernatants were kept frozen (−80°C) until analysis. Plasma concentrations of individual amino acids were measured by ion exchange chromatography using an automatic amino acid analyzer (LKB 4460). Asparagine and glutamate were not well separated by the chromatography and are not reported.

Presentation of data

All data are presented as means ± SEM. Nonparametric statistics were used. The Kruskal-Wallis followed by the Mann-Whitney U test were used to test basal values in the three groups. The Friedman two-way analysis of ranks and the Wilcoxon matched-pairs signed-ranks test were applied to test changes from basal values [22]. A two-tailed P value <0.05 was considered statistically significant.

Results

Leg plasma flow

Data on plasma flow are given in Table 1. The plasma flow did not differ significantly between the three groups. Following sham dialysis plasma flow increased significantly in groups CU and CU-IND. At 345 minutes, plasma flow was on average 75% higher in group CU ($P < 0.02$) and 48% higher in group CU-IND ($P < 0.05$) compared with basal values. In the five subjects of

Table 1. Leg plasma flow in the basal state and at 150 and 345 min after the start of sham hemodialysis

Group	Basal mean ± SEM	150 min mean ± SEM	345 min mean ± SEM
CU	0.75 ± 0.15	0.81 ± 0.14	1.31 ± 0.13[b]
CU-IND	0.54 ± 0.09	0.73 ± 0.06[a]	0.80 ± 0.10[a]
AN	0.74 ± 0.17	0.50 ± 0.04	0.70 ± 0.10

Data are expressed as ml/min/100 g tissue.
[a] $P < 0.05$ compared with basal
[b] $P < 0.02$ compared with basal

group CU in whom plasma flow was measured at 540 minutes, a tendency to return toward basal levels was observed. There was no significant change in plasma flow in the AN-group.

Plasma amino acid concentrations

The arterial concentrations of individual amino acids in plasma are given in Table 2. In the basal state lower plasma concentration levels of several amino acid were observed in the CU group than in the CU and AN groups. The sum of all measured amino acids was significantly lower in the CU group than in the CU-IND and AN groups ($P < 0.05$). Individual amino acid levels and total amino acid concentration did not change from basal values in any of the groups during the 345 to 540 minute observation period.

Arterio-venous amino acid differences

In the basal state the arterio-venous amino acid differences tended to be larger in the CU-IND group than in the CU group, but the difference reached statistical significance only for phenylalanine and histidine (Table 3). However, calculated for all measured amino acids the difference was not significant ($P = 0.11$). The arterio-venous difference for histidine was larger in the AN-group than in the CU-group. Following sham hemodialysis the arterio-venous differences of all individual amino acids tended to increase in CU-group. Calculated for all measured amino acids the arterio-venous concentration difference was -198 ± 20 μmol/liter in the basal state and -247 ± 27 μmol/liter at 345 minutes ($P < 0.01$). In group CU-IND, arterio-venous concentration differences tended to be smaller at 345 minutes compared with basal values, but this change did not reach statistical significance. The arterio-venous differences did not change significantly in AN-group.

Leg amino acid exchange

Data on leg amino acid exchange in the three study groups are given in Table 4. In group CU net release of leg amino acids remained constant during the 150 minutes of the sham-HD procedure, but increased markedly during the following hours. Compared with basal release the total efflux of all measured amino acids approximately doubled at 345 minutes ($P < 0.01$, Fig. 1). Significant increases were also observed for threonine, alanine, leucine, tyrosine, phenylalanine, lysine and histidine ($P < 0.01$ to 0.05). In the five subjects of group CU who were followed for 540 minutes after the start of sham hemodialysis the total release of all measured amino acids reached its maximum at 345 minutes (212 ± 63 nmol/min/100 g tissue, $P < 0.05$ vs. basal). Thereafter release tended to decrease, but the efflux was still higher at 540 minutes (173 ± 81 nmol/min/100 g tissue,) than before the start of sham hemodialysis (88 ± 30 nmol/min/100 g tissue), although the difference did not reach statistical significance. No significant changes in the release of any individual amino acid or the sum of all measured amino acids were observed at 150 or 345 minutes in CU-IND and AN-groups.

Table 2. Arterial amino acid concentrations in the basal state and at 150 and 345 minutes after the start of sham hemodialysis

	Group	Basal mean ± SEM	150 min mean ± SEM	345 min mean ± SEM
ASP	CU	7 ± 1	7 ± 1	7 ± 1
	CU-IND	6 ± 1	5 ± 1	5 ± 1
	AN	5 ± 0.4[a]	5 ± 1	5 ± 1
THR	CU	95 ± 9	92 ± 6	88 ± 6
	CU-IND	143 ± 11[a]	133 ± 9	120 ± 8
	AN	128 ± 14	130 ± 14	124 ± 11
SER	CU	95 ± 7	96 ± 8	97 ± 7
	CU-IND	147 ± 12[a]	145 ± 9	135 ± 12
	AN	116 ± 13	122 ± 11	117 ± 10
GLN	CU	598 ± 47	573 ± 57	537 ± 63
	CU-IND	828 ± 119	801 ± 108	788 ± 103
	AN	635 ± 32	637 ± 32	633 ± 35
GLY	CU	156 ± 12	155 ± 13	150 ± 11
	CU-IND	251 ± 26[a]	233 ± 21	245 ± 26
	AN	235 ± 21[a]	230 ± 22	234 ± 15
ALA	CU	167 ± 13	158 ± 13	150 ± 12
	CU-IND	235 ± 14[a]	234 ± 16	219 ± 15
	AN	222 ± 16[a]	226 ± 17	206 ± 15
VAL	CU	181 ± 15	195 ± 15	204 ± 16
	CU-IND	253 ± 19[a]	253 ± 21	257 ± 19
	AN	218 ± 13	221 ± 15	223 ± 11
ILE	CU	45 ± 5	48 ± 5	50 ± 5
	CU-IND	74 ± 7[a]	70 ± 6	71 ± 6
	CONTROL	55 ± 5	58 ± 5	60 ± 5
LEU	CU	93 ± 10	113 ± 11	122 ± 11
	CU-IND	140 ± 12[a]	147 ± 14	154 ± 12
	AN	57 ± 6	57 ± 4	62 ± 2
TYR	CU	41 ± 4	41 ± 3	42 ± 3
	CU-IND	63 ± 3[a]	53 ± 4	54 ± 3
	AN	54 ± 3[a]	52 ± 3	50 ± 3
PHE	CU	40 ± 3	45 ± 3	46 ± 3
	CU-IND	53 ± 2[a]	58 ± 4	59 ± 3
	AN	51 ± 4	53 ± 3	54 ± 1
LYS	CU	143 ± 12	158 ± 11	155 ± 10
	CU-IND	184 ± 4[a]	206 ± 8	197 ± 5
	AN	174 ± 10[a]	194 ± 10	182 ± 12
HIS	CU	64 ± 5	67 ± 4	67 ± 4
	CU-IND	79 ± 3	82 ± 3	77 ± 2
	AN	84 ± 5[a]	92 ± 7	92 ± 3
ARG	CU	65 ± 5	66 ± 5	65 ± 6
	CU-IND	93 ± 14	92 ± 9	89 ± 7
	AN	77 ± 5	80 ± 4	82 ± 5
Σ AA	CU	1783 ± 116	1808 ± 121	1772 ± 111
	CU-IND	2516 ± 144[a]	2482 ± 124	2438 ± 148
	AN	2167 ± 98[a]	2226 ± 79	2193 ± 84

Data are expressed as μmol/liter.
[a] $P < 0.05$ compared with group CU

Discussion

The purpose of this study was to study the effect of in vivo contact between blood and regenerated cellulose dialysis membranes on protein metabolism by the measurement of the exchange of free amino acids across the leg. The leg amino acid exchange was measured in normal subjects before and after sham hemodialysis, that is, in vivo passage of blood through a

Table 3. Arterio-femoral venous amino acid differences in the basal state and at 150 and 345 minutes after the start of sham hemodialysis

	Group	Basal mean ± SEM	150 min mean ± SEM	345 min mean ± SEM
ASP	CU	0.4 ± 1.0	0.7 ± 1.0	1.0 ± 0.0
	CU-IND	1.2 ± 0.5	0.7 ± 0.5	0.0 ± 0.5
	AN	0.7 ± 0.1	0.7 ± 0.3	0.3 ± 0.4
THR	CU	−13.0 ± 2.0	−9.0 ± 3.0	−15.0 ± 2.0
	CU-IND	−19.0 ± 2.0	−14.0 ± 3.0	−15.0 ± 4.0
	AN	−13.7 ± 2.0	−12.5 ± 2.4	−16.1 ± 3.5
SER	CU	5.0 ± 3.0	5.0 ± 3.0	3.0 ± 1.0
	CU-IND	3.0 ± 2.0	−1.0 ± 6.0	1.0 ± 2.0
	AN	1.6 ± 2.3	−31.1 ± 35.0	0.9 ± 2.4
GLN	CU	−75.0 ± 12.0	−78.0 ± 15.0	−86.0 ± 11.0
	CU-IND	−115.0 ± 27.0	−110.0 ± 32.0	−98.0 ± 25.0
	AN	−63.0 ± 13.0	−82.0 ± 18.0	−72.0 ± 14.0
GLY	CU	−15.0 ± 3.0	−13.0 ± 3.0	−18.0 ± 2.0
	CU-IND	−25.0 ± 4.0	−39.0 ± 16.0	−24.0 ± 5.0
	AN	−18.8 ± 2.7	−18.5 ± 5.0	−22.0 ± 6.3
ALA	CU	−56.0 ± 4.0	−61.0 ± 5.0	−63.0 ± 7.0
	CU-IND	−86.0 ± 8.0	−72.0 ± 12.0	−64.0 ± 14.0
	AN	−55.5 ± 13.0	−72.3 ± 18.0	−57.0 ± 17.0
VAL	CU	−4.0 ± 2.0	2.0 ± 4.0	−8.0 ± 2.0
	CU-IND	−8.0 ± 3.0	−8.0 ± 6.0	−5.0 ± 4.0
	AN	−7.1 ± 3.1	−1.8 ± 2.0	−7.3 ± 3.3
ILE	CU	−3.0 ± 2.0	−3.0 ± 2.0	−4.0 ± 1.0
	CU-IND	−4.0 ± 1.0	−5.0 ± 2.0	−4.0 ± 1.0
	AN	−4.8 ± 1.5	−2.6 ± 0.7	−3.7 ± 1.8
LEU	CU	−5.0 ± 2.0	−3.0 ± 2.0	−9.0 ± 2.0
	CU-IND	−9.0 ± 1.0	−9.0 ± 5.0	−8.0 ± 1.0
	AN	−9.6 ± 3.2	−3.1 ± 2.0	−6.4 ± 3.3
TYR	CU	−4.0 ± 1.0	−4.0 ± 2.0	−7.0 ± 1.0
	CU-IND	−7.0 ± 0.5	−6.0 ± 2.0	−5.0 ± 1.0
	AN	−5.8 ± 0.7	−4.9 ± 1.0	−5.9 ± 1.4
PHE	CU	−5.0 ± 1.0	−5.0 ± 2.0	−7.0 ± 1.0
	CU-IND	−8.0 ± 1.0[a]	−6.0 ± 2.0	−6.0 ± 1.0
	AN	−5.4 ± 1.3	−5.0 ± 1.9	−6.4 ± 1.8
LYS	CU	−12.0 ± 3.0	−11.0 ± 3.0	−17.0 ± 3.0
	CU-IND	−17.0 ± 4.0	−15.0 ± 8.0	−14.0 ± 5.0
	AN	−11.3 ± 4.3	−9.6 ± 2.8	−16.3 ± 3.6
HIS	CU	−3.0 ± 2.0	−3.0 ± 2.0	−6.0 ± 1.0
	CU-IND	−8.0 ± 1.0[a]	−6.0 ± 2.0	−7.0 ± 2.0
	AN	−8.9 ± 0.7[a]	−4.5 ± 1.1	−7.0 ± 2.5
ARG	CU	−7.0 ± 3.0	−7.0 ± 2.0	−11.0 ± 2.0
	CU-IND	−8.0 ± 5.0	−11.0 ± 4.0	−14.0 ± 1.0
	AN	−7.3 ± 1.5	−8.1 ± 3.1	−9.9 ± 1.7
Σ AA	CU	−198.0 ± 20.0	−174.0 ± 11.0	−247.0 ± 27.0[b]
	CU-IND	−307.0 ± 37.0	−297.0 ± 71.0	−258.0 ± 56.0
	AN	−209.0 ± 40.0	−255.0 ± 41.0	−228.0 ± 53.0

Data are expressed as μmol/liter.
[a] $P < 0.05$ compared with CU
[b] $P < 0.01$ compared wtih basal

Table 4. Leg exchange of amino acids in the basal state and at 150 and 345 min after the start of sham hemodialysis

	Group	Basal mean ± SEM	150 min mean ± SEM	345 min mean ± SEM
ASP	CU	0.3 ± 0.3	0.6 ± 0.5	0.6 ± 0.6
	CU-IND	0.5 ± 0.2	0.8 ± 0.4	0.0 ± 0.3
	AN	0.6 ± 0.3	0.3 ± 0.2	0.2 ± 0.3
THR	CU	−11.2 ± 3.0	−7.7 ± 2.5	−17.4 ± 3.3[a]
	CU-IND	−10.5 ± 2.2	−10.9 ± 2.4	−11.1 ± 3.5
	AN	−9.1 ± 1.7	−5.7 ± 0.9	−10.1 ± 2.2
SER	CU	3.5 ± 1.9	3.2 ± 1.6	4.2 ± 1.5
	CU-IND	0.8 ± 1.0	−2.1 ± 4.5	1.4 ± 1.7
	AN	1.7 ± 1.9	−15.2 ± 18.0	0.4 ± 1.7
GLN	CU	−52.0 ± 13.0	−62.0 ± 18.0	−117.0 ± 19.0
	CU-IND	−69.0 ± 25.0	−92.0 ± 25.0	−74.0 ± 21.0
	AN	−41.3 ± 11.8	−37.3 ± 6.2	−45.1 ± 8.3
GLY	CU	−12.3 ± 4.6	−11.8 ± 3.9	−21.1 ± 3.6
	CU-IND	−13.8 ± 3.6	−38.5 ± 15.1	−17.6 ± 3.2
	AN	−11.6 ± 1.9	−8.5 ± 2.1	−12.9 ± 3.2
ALA	CU	−42.3 ± 9.1	−51.2 ± 11.5	−76.4 ± 12.0[b]
	CU-IND	−45.5 ± 8.9	−52.2 ± 9.2	−45.2 ± 6.0
	AN	−32.7 ± 7.3	−32.6 ± 6.2	−33.0 ± 8.0
VAL	CU	−3.0 ± 1.6	−1.2 ± 4.4	−10.7 ± 4.3
	CU-IND	−4.6 ± 1.4	−6.6 ± 4.6	−3.2 ± 3.2
	AN	−3.7 ± 2.5	−0.5 ± 1.0	−4.4 ± 2.1
ILE	CU	−2.3 ± 0.6	−2.8 ± 0.7	−5.1 ± 1.4
	CU-IND	−2.1 ± 0.7	−4.0 ± 1.8	−3.0 ± 1.1
	AN	−2.5 ± 0.9	−1.2 ± 0.3	−2.3 ± 1.2
LEU	CU	−4.0 ± 0.9	−3.3 ± 1.2	−12.0 ± 3.3[b]
	CU-IND	−4.7 ± 1.0	−7.1 ± 3.5	−5.5 ± 0.8
	AN	−5.8 ± 1.7	−1.2 ± 0.7	−3.9 ± 2.2
TYR	CU	−3.4 ± 0.8	−4.3 ± 1.3	−7.8 ± 1.8[b]
	CU-IND	−3.5 ± 0.6	−4.7 ± 1.1	−3.8 ± 0.5
	AN	−4.3 ± 1.0	−2.4 ± 0.6	−4.0 ± 1.1
PHE	CU	−3.6 ± 0.8	−4.4 ± 1.3	−8.3 ± 1.8[c]
	CU-IND	−4.6 ± 1.0	−4.9 ± 1.5	−4.1 ± 0.5
	AN	−3.4 ± 0.8	−2.2 ± 0.7	−3.8 ± 1.0
LYS	CU	−10.4 ± 3.0	−10.1 ± 3.5	−21.8 ± 5.0[a]
	CU-IND	−8.4 ± 1.8	−12.0 ± 5.9	−9.6 ± 2.9
	AN	−7.9 ± 2.7	−4.1 ± 0.9	−10.0 ± 2.0
HIS	CU	−1.5 ± 0.6	−3.6 ± 1.7	−7.4 ± 1.6[c]
	CU-IND	−4.5 ± 0.9	−4.3 ± 1.3	−4.9 ± 1.5
	AN	−6.3 ± 1.4	−2.1 ± 0.6	−4.4 ± 1.7
ARG	CU	−6.3 ± 2.6	−5.9 ± 1.3	−10.3 ± 2.8
	CU-IND	−5.3 ± 2.9	−9.3 ± 2.9	−10.6 ± 2.4
	AN	−5.7 ± 1.4	−3.5 ± 1.2	−6.3 ± 1.2
Σ AA	CU	−148.0 ± 31.0	−152.0 ± 41.0	−309.0 ± 50.0[c]
	CU-IND	−171.0 ± 45.0	−215.0 ± 55.0	−188.0 ± 37.0
	AN	−133.0 ± 31.0	−116.0 ± 6.7	−140.0 ± 28.0

Data are expressed as nmol/min/100 g tissue.
[a] $P < 0.05$ compared with basal
[b] $P < 0.02$ compared with basal
[c] $P < 0.01$ compared with basal

dialyzer for 150 minutes, but with no circulating dialysate. The sham-HD procedure was designed to establish a continuous in vivo contact between blood and dialysis membranes, resembling that occurring during a clinical hemodialysis treatment, and to avoid the loss of amino acids from the blood by dialysis. Following sham-HD a significant increase in net release of amino acids from the leg was observed in the CU group. At 345 minutes after the start of sham-HD the total release of all measured amino acids was approximately doubled compared with the basal state (Fig 1). Similar increases in efflux were observed for most individual amino acids.

It should be emphasized that the precision of tissue balance calculations is critically dependent on the accuracy of the measurements of amino acid concentrations and blood flow. In group CU the enhanced release of amino acids at 345 minutes was associated with marked increases in leg blood flow. An overestimation of leg blood flow could thus have led to an erroneous conclusion of increased leg amino acid release. However, several reasons render this possibility unlikely. First, the values of resting blood flow observed in the present study agree with previously reported measurements of blood flow using occlusion plethysmography [23]. Second, the increase in leg blood flow of approximately 75% ($P < 0.01$) is out of proportion to the methodological error involved in the estimation of leg blood flow by occlusion plethysmography. In our study the coefficient of variation for the determinations of leg blood flow, based on repeated measurements at each time point

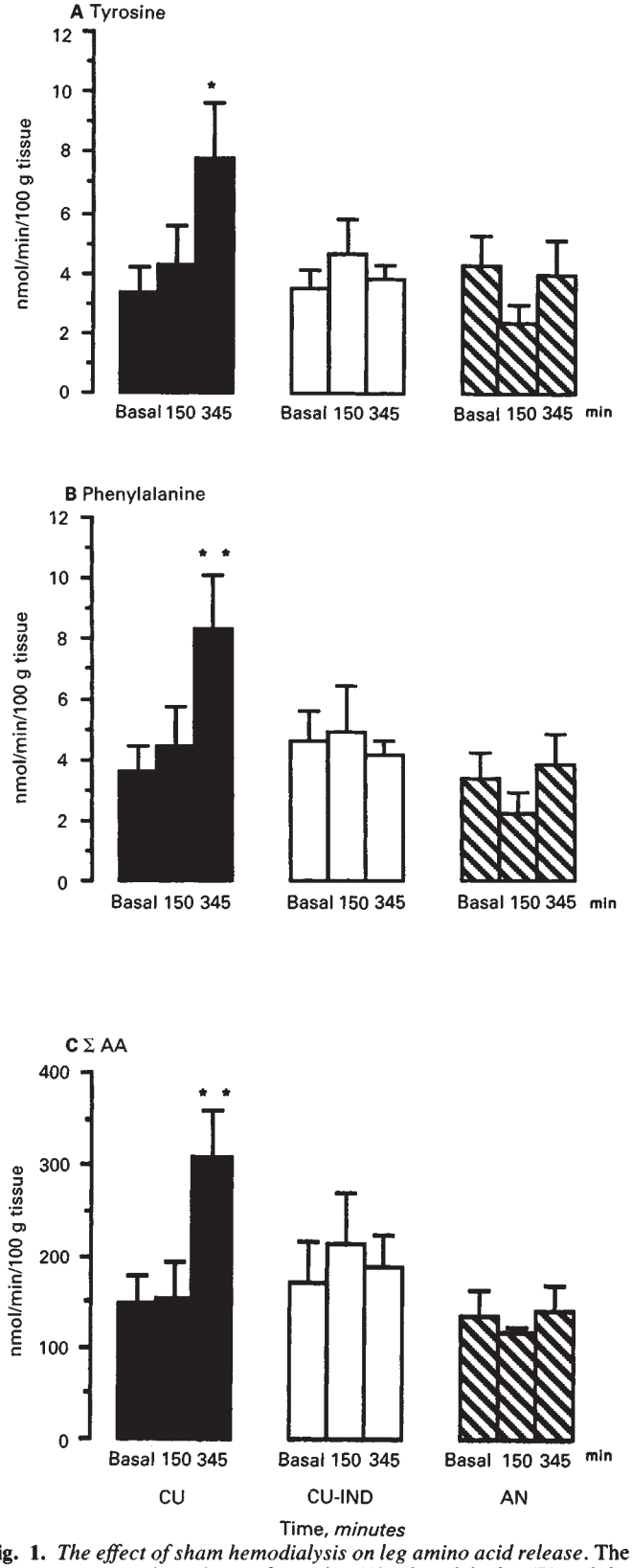

Fig. 1. *The effect of sham hemodialysis on leg amino acid release.* The bars represent net leg release of tyrosine (**A**), phenylalanine (**B**) and the sum of all measured amino acids Σ AA (**C**) before (basal) and at 150 and 345 min after the start of the sham hemodialysis procedure in the CU (filled bars), CU-IND (open bars) and AN groups (striped bars). * and ** denote $P < 0.02$ and $P < 0.01$, respectively, compared with basal.

was 13.6 ± 2.2%. Third, in the CU group a tendency to increased arterio-femoral venous differences, that is, release, at 345 minutes as compared with basal values was observed for all individual amino acids. Calculated for all measured amino acids the increase was statistically significant ($P < 0.005$). Fourth, in group CU-IND leg blood flow was approximately 50% higher at 345 minutes compared to the basal level. However, in this group there was a tendency to decreased rather than increased arterio-femoral venous amino acid concentration differences, resulting in unchanged efflux of amino acids from the leg. The present results therefore allow the conclusion that in vivo contact between blood and regenerated cellulose membranes in a dialyzer leads to increased release of amino acids from peripheral tissues.

In the CU group the leg release of tyrosine and phenylalanine increased by approximately 130% ($P < 0.02$). These amino acids are neither synthesized nor degraded in muscle tissue [24]. Although both skin and bone marrow actively metabolize amino acids and protein, amino acid exchange in the leg mainly represents exchange across muscle. An increased net release of these amino acids therefore directly reflects either a decreased rate of protein synthesis or an increased rate of muscle protein breakdown. An assumption implicit in this conclusion is that the intracellular free pools of these amino acids are unchanged. The intracellular free amino acid concentrations were not measured in this study. However, under the experimental conditions of sham-hemodialysis plasma amino acid concentrations remained at basal levels throughout the study, and it is therefore reasonable to assume that the intracellular free amino acid concentrations were not changed. The increased net release of tyrosine and phenylalanine following sham-HD therefore implies that blood-membrane interaction leads to an increase in protein breakdown or decrease in protein synthesis or a combination of both.

It is recognized that blood-membrane interaction in a dialyzer induces several biological effects, such as stimulation of the complement system, aggregation of granulocytes, transient leucopenia and release of granulocytic enzymes, clotting and thrombocyte activation [15]. These signs of bioincompatibility are especially prominent when using dialyzers with regenerated cellulose membranes. It has been demonstrated that interaction between blood and such membranes induces release of elastase and other granulocytic enzymes [25–28]. However, since catabolic granulocytic enzymes, such as elastase, are rapidly inactivated in plasma [27], it is doubtful that they can have a generalized effect on protein turnover. More probably, blood-membrane interaction may cause the release of other factors which directly or indirectly induce catabolism of muscle protein. One such factor is IL-1 (so called endogenous pyrogen), which, together with other monokines, is synthesized and released by monocytes after stimulation by exogenous pyrogens or by complement activation through C5a [29]. According to the IL-1 hypothesis the increased release of IL-1 from monocytes during hemodialysis may be induced by endotoxins directly [30] or via complement activation or acetate in the dialysis fluid [16]. Furthermore, recent observations have confirmed that the concentrations of IL-1 are elevated in dialysis patients before dialysis and that IL-1 levels increase further during dialysis with regenerated cellulose membranes [18, 31]. However, in the present study blood was circulated through a

dialyzer without concomitant circulation of dialysate, so endotoxins or acetate could not have contributed to monocyte activation.

Utilizing partially purified human IL-1, Baracos et al [19] demonstrated that IL-1 induces protein breakdown. However, in later experiments they were unable to induce a similar effect with recombinant IL-1 [20]. Further studies are required to identify the specific monokine(s) which stimulate muscle protein breakdown. Also, it has been appreciated that the assays showing raised circulating levels of IL-1 have been unspecific [32]. Similar assays were used for measuring this monokine in hemodialysis patients [18, 31]. It is therefore possible that other monokines may have been measured in addition to IL-1. Therefore, the alleged role of IL-1 with respect to protein breakdown can not with certainty be separated from that of other monokines. In this study the peak effect of blood-membrane contact on leg amino acid balance in group CU was observed 345 minutes after the start of the 150 minute sham hemodialysis, an observation of interest when discussing the potential role of IL-1 as a mediator of the protein breakdown associated with hemodialysis, since in human studies increased release of IL-1 has been observed three to four hours after activation of monocytes [33]. It has been shown in other clinical studies that the serum urea concentration rises faster in the first three to four hours after a regular hemodialysis treatment than in the remaining interdialytic period. However, it remains controversial whether this phenomenon reflects increased urea generation or redistribution of the urea [34].

Since it has been demonstrated that monokines induce increased muscle proteolysis through activation of PGE_2 [19], our observation that sham-HD did not increase amino acid release in the six subjects of group CU-IND to whom indomethacin was administered supports the suggestion that the catabolic effect of blood-membrane interaction may be mediated through stimulation of prostaglandins production by some monokine(s). An effect on leg release of amino acids by indomethacin through any other mechanism than prostaglandin inhibition is not likely, since results from in vitro studies indicate that indomethacin does not influence the release of amino acids from skeletal muscle in the absence of a catabolic stimulus [35].

Interestingly, sham-hemodialysis using AN membranes did not result in increased amino acid efflux from leg tissues, implying that the protein catabolic effect of blood-membrane contact depends on the biochemical properties of the dialyzer. Haeffner-Cavaillon et al [30] recently showed a close correlation between intradialytic increases in IL-1 production and C3a levels in a group of dialysis patients using regenerated cellulose membranes. In another group of patients in whom no complement activation occurred when dialyzed with AN membranes IL-1 remained at baseline levels. These results may indirectly support our hypothesis that the catabolism-triggering effect of contact between blood and CU membranes is mediated by IL-1 via complement activation.

Unexpectedly, we found that basal arterial concentrations of several individual amino acids were significantly lower in the CU-group compared with CU-IND and AN groups. However, the balance of amino acid across the leg tissues did not differ between the three groups at the basal state. Therefore it is not likely that the difference in basal arterial amino acid levels contributed to the different response in the three groups with respect to leg amino acid exchange during sham-HD. One may speculate whether hormonal mechanisms might have contributed to the increased leg release of amino acids. Theoretically, a decrease in circulating insulin levels would result in enhanced efflux of amino acids from muscle. Plasma insulin levels were not measured. However, under the conditions of sham-hemodialysis loss of insulin by dialysis can be excluded. Although some insulin may have adhered to the dialysis membranes early during the sham-hemodialysis procedure, this should in healthy subjects have resulted in an increased release of insulin from the β-cells. It is therefore not likely that hypoinsulinemia contributed to the enhanced leg amino acid release, which reached its maximum more than five hours after the start of the sham-hemodialysis. One may also speculate that the stress related to the extracorporeal circulation *per se* or prolonged fasting may have contributed to the increase amino acid release in the CU-group. However sham-hemodialysis using AN69 membrane did not result in increased amino acid release. Since identical protocol were followed in the two series of sham hemodialysis using regenerated cellulose and AN 69 membrane, respectively, it seems highly unlikely that other factor than blood-membrane interaction, such as time, recumbent position, the stress of extracorporeal circulation or heparin administration, appreciably contributed to the observed increase in leg amino acid release in association with sham-hemodialysis using regenerated cellulose membrane.

As commented above, leg blood flow increased markedly in both the CU and the CU-IND groups, although the increase tended to be larger in the former group. In contrast leg blood flow did not change during or following sham-hemodialysis using AN membranes. The mechanism(s) causing the increase in blood flow in the two regenerated cellulose groups is not evident. However, IL-1 has been shown to play a role in modulating vascular reactivity under various conditions. Thus, IL-1 inhibits vascular smooth muscle contractility in vitro [36]. Also infusion of IL-1 into rabbits have been shown to induce vasodilatation [37]. Furthermore, in healthy subjects marked increases in arm blood flow have been observed after stimulation of endogenous IL-1 activity by the administration of etiocholanolone [38]. One may therefore speculate whether IL-1 or some other factor, triggered by blood-membrane interaction, could have mediated the increase in blood flow. In the CU-IND group the increase in leg blood flow may have been attenuated by indomethacin.

Although the effect of blood-cuprophane interaction on protein metabolism was not directly determined by kinetic measurements of tracer substances, our results allow some quantitative estimation with respect to whole body protein balance. In the present study amino acids were measured in plasma. However, blood cellular elements, presumably erythrocytes, contribute to the net flux of amino acids from muscle. Study of amino acid exchange across the leg in humans has shown that the directions of net amino acid transport in blood cells and plasma are parallel, and that for most free amino acids the arterial-femoral venous differences in whole blood are at least as large as those in plasma [39]. Hence, there should be little risk that leg amino acid efflux will be overestimated if, in the following quantitative assessment, the arterial-femoral venous differences for whole blood amino acids are assumed to equal those observed for plasma amino acids. If it is further assumed

that the release of amino acids from leg muscle reflects that of the total body muscle mass, estimated to be 40% of the body weight, the efflux of tyrosine and phenylalanine from the total muscle mass at 345 minutes exceeded that in the basal state by 2400 and 2600 nmol/min, respectively. From the present data it can be assumed that amino acid release increased after 2.5 hours from the start of sham-HD and continued to increase for a further three and a half hours. It then returned towards basal level during the following three to four hours. The total amount of tyrosine and phenylalanine released from muscle in response to the sham-hemodialysis procedure can then be calculated to at least 500 and 550 μmol, respectively. By knowing the amino acid composition of skeletal muscle [40], these amounts can be calculated to correspond to a loss of 13 to 16 g of muscle protein. Although the changes in amino acid metabolism during a single sham-hemodialysis in healthy subjects do not necessarily reflect those occurring in uremic patients undergoing maintenance hemodialysis, it is not inconceivable that the stimulation of protein catabolism could be even higher during clinical dialysis than in our experiments, since most hemodialysis treatments are performed using higher blood flow, longer duration, and sometimes larger surface area as compared with the present experimental study (blood flow 100 ml/min/m^2 during 150 min). Rotellar et al [41] recently reported that dialysis for six hours using two parallel 2.5 m^2 hollow-fiber cellulose acetate dialyzers (blood flow 500 ml/min) resulted in a larger loss of urea into the dialysate than could be accounted for by urea clearance from body fluids. This discrepancy may be due to enhanced urea formation during dialysis corresponding to an additional protein breakdown of about 60 g. An increase of this magnitude would be in keeping with our results in normal subjects, considering that the surface area was larger, the blood flow higher, and the duration of the dialysis considerably longer in Rotellar's study. Increased catabolism at each dialysis treatment of 15 g of protein, that is, an amount approximately equal to the calculated increase in protein degradation in the present study may, in addition to the 10 to 15 g of free and peptide-bound amino acids lost by dialysis, be critical in many maintenance hemodialysis patients who tend to have a low protein intake. If patients can not meet the increased protein requirement by eating a protein-rich diet, malnutrition will develop, leading to increased morbidity and impaired quality of life.

In dialysis patients with suboptimal nutritional status it is of obvious importance to minimize catabolic stimuli associated with the dialytic procedure. In view of the present results, suggesting that polyacrylonitrile membranes do not induce increase in muscle protein breakdown, hemodialysis using membranes with less complement activating properties may seem attractive. However, one should be cautious in directly extrapolating data obtained under the experimental conditions of sham hemodialysis to hemodialysis treatment. In clinical dialysis non-complement dependent factors, such as acetate in the dialysate or contamination of the dialysis fluid by endotoxins, may lead to an increase in IL-1 production. The impact of such factors might be enhanced when dialysis is performed using high permeability membranes.

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