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Hemodialysis-associated protein catabolism with and without glucose in the dialysis fluid

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Hemodialysis-associated protein catabolism with and without glucose in the dialysis fluid. The effects of hemodialysis on protein and energy metabolism were studied in eight hemodialysis patients. The leg exchange of amino acids (AA) was measured during hemodialysis using a dialysis fluid with 10 mmol/liter glucose (GD) or without glucose (GFD). Arterial AA concentrations decreased by about 30% in GD and GFD. During dialysis, similar increases in the efflux of AA from leg tissues (mainly muscle) were observed in GD and GFD (basal 105 ± 104, vs. 71 ± 62 nmol/min/100 g tissue; dialysis 295 ± 46 vs. 289 ± 60 nmol/min/100 g tissue). The efflux of AA remained largely unchanged at one hour after the end of GD and GFD. Losses of AA to the dialysate were similar during GD (8.3 ± 0.9 g) and GFD (7.9 ± 0.4; NS). GFD resulted in a loss of 26 g of glucose whereas 30 of glucose was absorbed during GD. The amount of urea removed by dialysis and the post-dialysis increase in plasma urea were similar in GD and GFD. In conclusion, the addition of glucose to the dialysis fluid may help the energy balance, but it does not appear to reduce the negative effects of hemodialysis on protein metabolism.

Wasting and malnutrition are common in chronic hemodialysis patients [1–3]. Reduced intakes and increased requirements of nutrients due to the uremic state contribute to the development of malnutrition in these patients [4]. In addition, hemodialysis treatment has been reported to be a catabolic event manifested by increased urea appearance during dialysis [5, 6] and a negative nitrogen balance on "dialysis days" regardless of protein intake [7]. The presence of endotoxins in the dialysis fluid may elicit an inflammatory response with the release of cytokines (IL-1, TNF) which are known to stimulate proteolysis [8]. Blood contact with bioincompatible dialysis membranes has been reported to accelerate muscle protein breakdown in normal subjects [9, 10].

The loss of free and bound amino acids during dialysis, which amounts to about 6–13 g per dialysis, may constitute an additional catabolic factor [11–13]. Another potential cause of net protein catabolism may be the loss of 25 to 30 g glucose during hemodialysis with glucose-free dialysis fluid [6, 14], which elicits an increased splancnic glucose production to sustain blood glucose levels. During and after a meal, glucose may be generated from carbohydrates in the food and from glycogenolysis in the liver. However, in starvation, increased mobilization of amino acids from muscle stores is required in order to maintain glucose homeostasis (gluconeogenesis). Decreases in gluconeogenetic precursors, which cannot be accounted for by losses in the dialysate, such as of pyruvate and lactate [14] or alanine [15], have been taken as evidence of enhanced gluconeogenesis due to loss of glucose during dialysis [16]. Accordingly, one would expect that hemodialysis with glucose-containing dialysis fluid might have a positive effect on protein metabolism by decreasing gluconeogenesis and perhaps also by stimulating the secretion of insulin, which is known to induce a decrease in proteolysis [17].

Only a few studies have addressed the problem of whether hemodialysis with glucose in the dialysis fluid may result in reduced protein catabolism, compared to dialysis with glucose-free dialysis. Farrell and co-workers [5, 6] studied urea appearance repeatedly during hemodialyses with either glucose-free dialysis fluid or dialysis fluid containing glucose (11 mmol/liter or 26 mmol/liter) and found no difference whether or not the dialysis fluid contained glucose. Kopple et al [11] observed in patients studied after an overnight fast that the addition of glucose (25 mmol/liter) to the dialysis fluid resulted in a decreased dialytic loss of amino acids when compared to glucose-free dialysis, and they concluded that glucose in the dialysis fluid has a protein-sparing effect. However, no more than four dialysis sessions with glucose and three sessions with glucose were studied. Hence, the results have to be considered as preliminary. More recently, Ono, Sasaki and Waki [13] reported that in non-fasting patients the dialytic loss of amino acids was not significantly different with or without glucose in the dialysis fluid.

In patients who are in good clinical condition the moderate losses of glucose and amino acids during dialysis should easily be compensated for by adequate nutritional intake and especially by ingestion of food during dialysis. However, many dialysis patients suffer from anorexia, gastrointestinal or other disturbances, which may grossly impair or preclude oral intake of nutrients before and during dialysis. It is therefore important to know if it is worthwhile to use glucose-containing dialysis fluid in order to reduce protein catabolism and amino acid loss during hemodialysis in fasting patients, especially since earlier results are scanty and of doubtful significance.

To elucidate this question we have investigated the metabolic effects of hemodialysis with or without glucose in the dialysis fluid by measuring the exchange of amino acids across the leg, the dialytic losses of urea and amino acids and the dialytic loss or uptake of glucose. The studies were made in chronic hemodialysis patients after an overnight fast.

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Methods

Subjects

Eight male chronic uremic patients participated in the study. They ranged in age from 48 to 77 years (median 59 years) and had undergone maintenance hemodialysis for a median of 22.5 months (range 8 to 57 months). All patients were in good general and nutritional health (body mass index 22.5 ± 0.9, hematocrit 28 ± 0.7%, protein catabolic rate >1.0 g protein/kg body weight*day). None of them had a residual renal urea clearance >0.1 ml/min. The causes of renal failure included chronic glomerulonephritis in five patients, polycystic kidney disease in one and amyloidosis in two patients. All were dialyzed with a glucose-free dialysis fluid, thrice weekly for three to four hours, blood flow 250 to 400 ml/min and dialysate flow 500 ml/min (Kt/V >1.0). The Baxter CA® 170 g dialyzer was used in six patients and the Gambro GFS*Plus* 20 dialyzer in two patients. The nature, purpose and potential risks of the study were carefully explained to all of them before they consented to participate. The study protocol was approved by the Ethics Committee of the Karolinska Institute at Huddinge Hospital.

Procedure

Hemodialysis. Each patient was studied on two occasions with a one-week interval, in random order, using dialysis fluid with (GD) or without (GFD) glucose. The fluid contained sodium 142 mmol/liter, calcium 1.5 mmol/liter, magnesium 0.5 mmol/liter, bicarbonate 32 mmol/liter, potassium 1 or 2 mmol/liter, according to the patients needs, and either glucose 10 mmol/liter or no glucose. Apart from the presence or absence of glucose in the dialysis fluid, the procedure was identical on both occasions and in accordance with the patients previous hemodialyses. An arteriovenous fistula or Gortex graft was used as the blood access for hemodialysis. After a 12-hour overnight fast, polyethylene catheters for blood sampling were inserted into a femoral artery and ipsilateral femoral vein. After the insertion of the femoral catheters, the patients rested for 60 minutes before the hemodialysis was started.

Sampling and measurements. Plasma glucagon, plasma insulin, plasma glucose and serum urea were determined in samples from arterial blood, whereas samples for amino acid determinations were taken simultaneously from arterial and venous catheters. Samples for glucagon and insulin were taken pre-dialysis (basal), at the end of dialysis (end) and 60 minutes after dialysis (60 min post-dialysis). Samples for plasma amino acids and plasma glucose were taken in the basal state, at 60 minutes, 120 minutes, 180 minutes, at the end of dialysis (180 min, $N = 1$; 210 min, $N = 5$; 240 min, $N = 2$) and at 60 minutes post-dialysis. Serum urea was determined at the start, at the end of dialysis and at 10, 20, 30, 40 and 60 minutes post-dialysis. Leg blood flow was measured by venous occlusion plethysmography [18], immediately after blood sampling for determination of amino acids at each time-point. Dialysate samples were taken from a sampling port in the dialysate outflow tube. Two ml samples were withdrawn every 15 minutes throughout the dialysis. These samples were placed together in a jug packed with ice. Following dialysis and after appropriate mixing, a 10 ml sample was taken to determine the glucose, urea and the various amino acid concentrations. The uptake of glucose from the dialysis fluid or the loss of glucose into the dialysate was calculated as the difference between the initial concentration of glucose in the dialysis fluid and the total amount of glucose contained in the circulated dialysate during the hemodialysis. The total amount of dialysate circulated through the dialyzer during dialysis was obtained by examining the display on channel two (circulated dialysate + ultrafiltration) in the Filtration Control Monitor (FCM; Gambro, Lund, Sweden). The leg plasma flow (Qp) was derived from the subjects blood flow (Qb) and hematocrit (Hct), using the formula $Qp = Qb \times (100 - Hct)/100$. The leg amino acid balance was calculated by multiplying the arterio-femoral venous concentration difference for individual amino acids by the leg plasma flow.

Table 1. Plasma flow in a group of eight chronic dialysis patients, before (basal), during hemodialysis (60, 120 and 180 min), at the end of dialysis (end), and one hour after the end of hemodialysis (60 min post-dialysis), using a dialysis fluid with (GD) or without glucose (GFD)

	GD	GFD
Basal	1.45 ± 0.3	1.30 ± 0.2
60 min	1.56 ± 0.4	1.27 ± 0.2
120 min	1.63 ± 0.3	1.43 ± 0.2
180 min	1.25 ± 0.3	1.37 ± 0.2
End of dialysis	1.21 ± 0.2	1.30 ± 0.2
60 min post-dialysis	1.23 ± 0.2	1.22 ± 0.2

Results are expressed as ml/min/100 g tissue (mean ± SEM).

Analytical methods

Blood samples for amino acid analysis were kept on ice. After precipitation with 4% SSA, the plasma samples were centrifuged at +8°C and the supernatants were kept frozen (−70°C) until they were analyzed. The plasma concentrations of individual amino acids were measured by the HPLC method, using a pre-column derivatization procedure with orthophthaladehyde [19]. Analyses of amino acids in dialysate were performed in non-deproteinized samples, in accordance with previous descriptions [11] of the same HPLC method. Glucose and urea concentrations in plasma and dialysate were measured using an IL 919 system (Instrumentation Laboratory, Milan, Italy). The concentrations of insulin (normal value <19 μU/ml) and glucagon (normal value <232 pg/ml) in plasma were analyzed, as previously described [20, 21].

Presentation of data

If not stated otherwise, all data are presented as means ± SEM. The Student's *t*-test for paired data was used to evaluate the results. Analysis of variance (ANOVA) for repeated measurements followed by the paired *t*-test, corrected for multiple comparisons, was used. Differences between means of samples with skewed distribution [22] were assessed with the Wilcoxon matched-paired signed-rank test. Correlations were tested by linear regression analysis. A two-tailed P value < 0.05 was considered statistically significant.

Results

Leg plasma flow

The values of plasma flow in the leg determined before, during and 60 minutes after hemodialysis are presented in Table 1. The plasma flow remained constant during and after hemodialysis and no difference was observed between dialysis with or without glucose.

Table 2. Arterial amino acid concentrations (μmol/liter) before (basal), at the end (end) and 60 min after the end (60 min post-dialysis) of hemodialysis, using a dialysis fluid with (GD) or without glucose (GFD)

Amino acid	Dialysis fluid	Basal	End	60 Min post-dialysis
HIS	GD	84.68 ± 5.70	51.59 ± 2.50[a]	61.78 ± 2.90[b]
	GFD	88.80 ± 8.20	60.10 ± 6.40[a]	64.58 ± 6.00[b]
ILE	GD	64.26 ± 5.50	49.06 ± 3.10[a]	57.94 ± 3.50[b]
	GFD	59.86 ± 4.10	64.96 ± 5.90	65.48 ± 3.20
LEU	GD	91.78 ± 7.10	86.74 ± 4.10	100.66 ± 5.27[b]
	GFD	90.78 ± 7.50	112.84 ± 9.20	120.50 ± 6.90
LYS	GD	171.50 ± 19.70	117.13 ± 11.90[a]	145.88 ± 18.30[b]
	GFD	165.63 ± 12.70	131.66 ± 10.80[a]	146.75 ± 12.20[b]
MET	GD	27.70 ± 2.40	23.19 ± 2.80[a]	23.75 ± 2.10
	GFD	22.70 ± 2.90	20.60 ± 4.80	21.76 ± 4.50
PHE	GD	68.16 ± 5.80	52.30 ± 2.90[a]	57.44 ± 3.10
	GFD	62.98 ± 7.10	54.38 ± 4.90	57.99 ± 4.10
THR	GD	141.63 ± 17.20	83.20 ± 7.10[a]	100.03 ± 9.60[b]
	GFD	128.08 ± 15.40	85.90 ± 8.50	93.50 ± 8.10
TYR	GD	39.61 ± 3.30	28.50 ± 1.80[a]	32.99 ± 1.40[b]
	GFD	39.85 ± 3.20	33.03 ± 4.20	35.14 ± 3.80
VAL	GD	181.63 ± 13.30	114.25 ± 4.70[a]	134.25 ± 6.90[b]
	GFD	182.38 ± 13.40	143.35 ± 12.90[a]	155.00 ± 10.10
ALA	GD	244.96 ± 37.50	148.00 ± 15.30[a]	187.75 ± 28.90[b]
	GFD	276.25 ± 35.10	163.88 ± 16.20[a]	190.63 ± 21.10[b]
ARG	GD	105.19 ± 5.30	63.85 ± 2.30[a]	74.20 ± 4.40
	GFD	101.95 ± 6.50	70.04 ± 6.30[a]	79.05 ± 7.10[b]
ASN	GD	53.43 ± 3.70	37.24 ± 1.96[a]	45.01 ± 2.60[b]
	GFD	52.04 ± 4.30	36.88 ± 3.90[a]	41.20 ± 3.60
GLN	GD	740.93 ± 53.10	541.00 ± 33.70[a]	681.38 ± 54.20[b]
	GFD	696.88 ± 40.70	555.50 ± 35.90[a]	625.75 ± 41.90[b]
GLY	GD	294.84 ± 33.00	212.50 ± 25.40[a]	267.50 ± 40.20[b]
	GFD	310.75 ± 33.70	226.63 ± 24.50[a]	266.00 ± 31.30[b]
ORN	GD	71.05 ± 6.90	46.71 ± 5.50[a]	47.56 ± 4.20
	GFD	67.89 ± 3.40	43.01 ± 2.80[a]	48.36 ± 2.80[b]
SER	GD	88.54 ± 4.30	59.19 ± 5.20[a]	74.73 ± 4.40[b]
	GFD	89.70 ± 7.90	67.49 ± 7.90[a]	74.93 ± 8.70
TAU	GD	40.69 ± 2.20	23.66 ± 2.30[a]	30.24 ± 3.60[b]
	GFD	38.74 ± 3.50	24.16 ± 1.90[a]	30.13 ± 1.90[b]
TRP	GD	18.21 ± 1.30	19.24 ± 1.20	20.64 ± 1.20
	GFD	17.76 ± 1.90	18.83 ± 2.10	22.30 ± 2.20[b]
ΣAA basal	GD	2528.76 ± 125.80	1757.34 ± 64.90[a]	2143.70 ± 119.70[b]
	GFD	2493.00 ± 142.50	1913.18 ± 140.60[a]	2139.03 ± 145.50[b]

Data are mean ± SEM.
[a] $P \leq 0.05$ compared to basal values before starting the hemodialysis
[b] $P \leq 0.05$ compared to values at the end of hemodialysis

Plasma amino acid concentrations

The arterial concentrations of the amino acids are given in Table 2. In the basal state, plasma levels of amino acids did not differ between dialysis with or without glucose. Hemodialysis was associated with significant decreases in all measured amino acids, except LEU and TRP in dialysis with glucose and ILE, LEU, MET, PHE, TYR and TRP in dialysis without glucose. The decline in the plasma amino acid concentrations, calculated as the sum of the concentrations of the measured amino acids, was highly significant ($P < 0.001$) and quantitatively similar (approximately 30%) during dialysis with either solution.

After the dialysis the plasma arterial concentrations of most amino acids rose significantly. However, after one hour the plasma concentrations of the amino acids were the same in the GD and GFD groups.

Amino acid exchange in the leg

The duration of hemodialysis (HD) in each patient corresponded to the average duration of the leg exchange when the three or four blood samples were taken at 60 minutes, 120 minutes, 180 minutes, and at the end of the dialysis (180 min, $N = 1$; 210 min, $N = 5$; 240 min, $N = 2$; Table 3).

Compared to basal values, the efflux of most amino acids increased significantly during hemodialysis. In the case of the Σ of the amino acids measured, an increase in the release from 105 ± 104 in the basal state to 295 ± 46 nmol/min/100 g tissue during hemodialysis was observed in GD as compared with an increase from 71 ± 62 to 289 ± 60 nmol/min/100 g tissue in GFD. The magnitude of the increases did not differ significantly between the two types of dialysis. One hour after the end of either form of hemodialysis, the leg efflux of amino acids had decreased slightly, although not significantly, as compared to the end of dialysis.

The presence of glucose in the dialysis fluid did not influence this response since, during as well as after dialysis, the release of amino acids by leg muscle tissues was the same in the GD and GFD groups.

The total release of individual amino acids in each patient during hemodialysis may be calculated as the total area under the

Table 3. Leg exchange of amino acids (nmol/min/100 g tissue) before (basal), during dialysis and 60 min after (60 min post-dialysis) of hemodialysis, using a dialysis fluid with (GD) or without glucose (GFD)

		Basal	During HD[a]	60 Min post-dialysis
HIS	GD	−0.56 ± 3.30	−7.19 ± 1.55[c]	−7.78 ± 4.31
	GFD	1.97 ± 2.20	−3.22 ± 4.57	−5.48 ± 2.40
ILE	GD	−0.79 ± 2.60	−5.92 ± 1.55[c]	−5.31 ± 3.10
	GFD	−1.07 ± 1.40	−3.31 ± 2.05	−8.74 ± 2.90
LEU	GD	0.36 ± 3.95	−9.85 ± 2.66[c]	−10.61 ± 6.30
	GFD	−0.62 ± 2.90	−8.22 ± 3.00	−12.28 ± 5.10
LYS	GD	−2.61 ± 6.90	−18.99 ± 3.57[c]	−13.81 ± 7.70
	GFD	−3.15 ± 4.10	−18.10 ± 3.48[c]	−13.93 ± 6.40
MET	GD	−1.46 ± 1.30	−3.61 ± 0.75	−9.20 ± 4.70
	GFD	−1.64 ± 0.80	−4.80 ± 1.29[c]	−3.19 ± 0.90
PHE	GD	0.54 ± 2.70	−8.32 ± 1.60[c]	−1.68 ± 7.20
	GFD	−0.60 ± 2.60	−6.66 ± 1.65[b]	−4.99 ± 2.40
THR	GD	−4.41 ± 5.00	−15.98 ± 2.11[b]	−13.22 ± 5.10
	GFD	−4.66 ± 2.60	−16.30 ± 2.59[b]	−11.17 ± 3.80
TYR	GD	−2.09 ± 1.50	−5.42 ± 0.38[a]	−5.73 ± 2.50
	GFD	−0.20 ± 1.10	−5.86 ± 1.38[b]	−3.67 ± 1.40
VAL	GD	4.39 ± 7.50	−14.17 ± 4.46[b]	−9.09 ± 8.10
	GFD	4.13 ± 5.50	−10.99 ± 3.84[a]	−10.29 ± 6.90
ALA	GD	−68.45 ± 24.30	−68.02 ± 8.53	−62.73 ± 15.60
	GFD	−52.79 ± 9.70	−76.27 ± 13.30	−65.54 ± 15.10
ARG	GD	−8.47 ± 4.20	−13.48 ± 2.01	−17.63 ± 6.50
	GFD	−1.56 ± 2.90	−14.10 ± 3.03[b]	−11.42 ± 3.50
ASN	GD	−3.77 ± 2.20	−7.05 ± 1.18[a]	−7.18 ± 2.90
	GFD	−1.76 ± 1.30	−7.98 ± 1.75[b]	−6.37 ± 2.10
GLN	GD	−37.33 ± 30.90	−84.69 ± 15.61[a]	−38.56 ± 16.80
	GFD	−22.00 ± 18.70	−80.54 ± 20.44[a]	−62.21 ± 20.00
GLY	GD	−4.63 ± 11.40	−28.41 ± 6.28[a]	−14.48 ± 15.50
	GFD	−7.80 ± 7.60	−31.02 ± 6.81[b]	−19.48 ± 9.50
ORN	GD	6.32 ± 4.10	−2.79 ± 1.61	−3.34 ± 3.20
	GFD	3.23 ± 2.70	−1.62 ± 1.43	−0.97 ± 3.20
SER	GD	7.58 ± 2.70	−1.50 ± 3.21	1.16 ± 4.10
	GFD	8.86 ± 4.10	−0.18 ± 1.79[b]	−2.00 ± 4.20
TAU	GD	9.32 ± 2.90	0.41 ± 1.27[a]	4.38 ± 2.20
	GFD	8.31 ± 3.20	−0.47 ± 0.92[b]	3.56 ± 3.10
TRP	GD	0.24 ± 0.80	−0.46 ± 0.65	−8.68 ± 5.60
	GFD	0.53 ± 1.30	0.71 ± 1.16	0.04 ± 1.10
ΣAA	GD	−105.83 ± 104.30	−295.44 ± 45.66[b]	−223.49 ± 100.40
	GFD	−70.81 ± 62.30	−288.94 ± 59.71[b]	−237.52 ± 76.70

Data are mean ± SEM.
[a] average value of the four time-periods during dialysis, calculated for each subject separately
[b] $P \leq 0.05$ compared to basal value
[c] $P \leq 0.025$ compared to basal value

curve (AUC; nmol/100 g tissue/hemodialysis) of amino acid release in the leg, whereas the incremental area (nmol/100 g tissue/hemodialysis) equals the total AUC − the release at the start of hemodialysis × the duration of hemodialysis (min). Significant positive incremental areas were observed in the GD and GFD groups. In the GD groups significant incremental areas were observed for all amino acids, except MET, ALA, ASN, ORN, SER and TRP, whereas in the GFD group the incremental areas of HIS, ILE, LEU, ORN and TRP did not reach statistical significance. The release of individual amino acids or the sum of all measured amino acids was the same with both types of hemodialysis.

Amino acids in dialysis fluid

Data on free amino acid losses to the dialysate during hemodialysis are presented in Table 4. Similar amounts of amino acids were lost to the dialysate in the GD (8.3 ± 0.9 g) and GFD (7.9 ± 0.4 g; NS) groups. In both groups the quantity of individual amino acids contained in the dialysate showed a high correlation to the average plasma arterial concentration of the individual amino acids during the hemodialysis ($r = 0.975$). Similarly, the total amount of each amino acid in the dialysate was highly correlated to the degree of change in the release of these amino acids by the leg tissues (Δ end-basal) in both the GD ($r = 0.93$, $P < 0.001$) and in the GFD ($r = 0.90$, $P < 0.001$) groups (Fig. 1).

Hormones

The basal plasma concentrations of insulin and glucagon were the same in the GD and GFD groups (Fig. 2). During GD, the plasma concentration of insulin rose significantly, whereas during GFD, the concentration fell significantly. During the post-dialysis period, the levels returned to the initial values in both groups. In contrast the plasma glucagon concentrations showed no significant changes during or after hemodialysis.

Table 4. Total loss of amino acids (μmol) to the dialysate during hemodialysis using dialysis fluid with (GD) or without glucose (GFD)

	GD	GFD
HIS	1867 ± 193	1913 ± 121
ILE	2046 ± 304	2066 ± 289
LEU	2788 ± 286	2913 ± 220
LYS	3279 ± 724	2700 ± 185
MET	859 ± 75	643 ± 55
PHE	1680 ± 221	1571 ± 182
THR	2964 ± 472	2574 ± 268
TYR	861 ± 53	911 ± 59
VAL	4570 ± 471	4887 ± 423
ALA	5701 ± 663	5525 ± 458
ARG	1965 ± 179	1888 ± 96
ASN	1184 ± 152	1160 ± 88
GLN	17529 ± 1438	16228 ± 634
GLY	8093 ± 1195	7656 ± 824
ORN	3396 ± 1403	2924 ± 489
SER	2510 ± 148	2502 ± 239
TAU	665 ± 74	640 ± 40
CIT	2685 ± 401	2653 ± 246
ΣAA	65113 ± 7319	62116 ± 3392

Data are mean ± SEM.

Glucose

As seen in Figure 3, when glucose was present in the dialysis fluid, the plasma glucose gradually rose until the end of dialysis and then fell to the pre-dialysis levels. In contrast, when no glucose was present in the dialysis fluid, the plasma glucose concentration steadily fell during hemodialysis, but it then increased significantly during the post-dialysis period. The changes in the plasma glucose levels during dialysis showed a correlation to the changes in the plasma insulin levels ($r = 0.69$, $P = 0.006$). The former changes were highly correlated to the amount of glucose absorbed from the dialysis fluid or lost to the dialysate ($r = 0.83$, $P < 0.001$). The presence of glucose in the dialysis fluid resulted in an uptake of 29.4 ± 7 g during hemodialysis whereas the absence of glucose in the fluid resulted in a total loss of glucose amounting to 25.6 ± 3.4 g (Fig. 4).

Urea

With both dialysis regimens the concentrations of the pre-dialysis plasma urea and the magnitude of the decline in the concentrations of plasma urea during dialysis were virtually identical (Fig. 5). Likewise, the total amounts of urea in the dialysate were similar (GD 608 ± 59 mmol and GFD 529 ± 62 mmol) (Fig. 6).

During the 60 minute post-dialysis period, the plasma urea level rose rapidly, averaging 1.63 ± 0.23 mmol/liter in the GD group and 1.77 ± 0.17 mmol/liter in the GFD group (not significant).

Discussion

The results of this study confirm that hemodialysis using a glucose-free dialysis fluid is associated with losses of about 8 g of free amino acids into the dialysate and marked decreases in the arterial concentrations of amino acids. In addition, release of amino acids in the leg, increased about threefold during dialysis of glucose-free fluids. The dialytic loss of amino acids, the release of amino acids from the leg, and the loss of urea, were not significantly different with or without glucose in the dialysis fluid.

Regardless of the type of dialysis fluid, the amount of individual amino acids lost to the dialysate showed a high correlation with the changes in the respective plasma amino acid concentrations, indicating that the loss of amino acids into the dialysate contributed to the reduction in the plasma arterial concentrations. In agreement with previous observations [12], the amount of free amino acids lost to the dialysate exceeds the amount normally contained in the entire extracellular compartment. Thus, a substantial amount of amino acids must have been released from the tissues to compensate for the gradual decline in the extracellular amino acid levels. In both the GFD and GD groups the quantities of individual amino acids in the dialysate were highly correlated to the changes in balance of amino acids in the leg tissue, suggesting that muscle amino acids were released down an intracellular-extracellular concentration gradient. However, it cannot be deduced from the present data whether net muscle protein breakdown is involved in this reaction, as suggested [12].

It may be inferred that the amino acid exchange in the leg tissues was not assessed during steady-state conditions, as evidenced by continuous loss of arterial amino acids to the dialysate during hemodialysis with and without glucose. In the present study, calculations of leg exchanges were based on plasma amino acid determinations. However, circulating erythrocytes have been reported to actively participate in amino acid transport [23]. Accordingly, it has been proposed that in the assessment of the true rate of amino acid efflux from peripheral tissues, whole blood amino acid determinations, rather than plasma, should be used [24]. This is especially applicable in conditions where a steady state cannot be obtained [25]. Quantitative estimates should therefore be interpreted with some caution. Assuming that the changes in plasma arteriovenous differences are at least as large as those observed in whole blood, as reported [23], it seems unlikely that the efflux of amino acids from leg tissues has been overestimated. If we further assume that the release of amino acids in the leg reflects that of the total body muscle mass (40% of body wt), the increases observed in the release of amino acids in muscle cannot fully account for the total amount of amino acids lost into dialysate. This suggests that amino acids derived from non-muscle tissues may have partly made up for the falling concentrations of circulating amino acid concentrations.

The release of most amino acids in the leg tissue increased significantly during dialysis of fluids that did not contain glucose. Similarly, during glucose dialysis, comparable increases in the release of amino acids in the leg were observed, which was unexpected since the glucose and insulin concentrations in the plasma rose significantly. Glucose and insulin are known to favor the uptake of amino acids by muscle cells in normal [26] and uremic subjects [27], and insulin reduces proteolysis in skeletal muscle [17].

It has been shown that modest increases of about 10 μU/ml in plasma insulin are needed to promote the cellular uptake of amino acids [28]. The increases observed in the levels of plasma insulin during GD may therefore have been too small (5 to 7 μU/ml) to counterbalance the dialysis-induced tissue release of amino acids. In addition, hypoaminoacidemia may have blunted the antiproteolytic action of insulin [29, 30]. The glucose in the dialysis fluid resulted in similar changes in the plasma urea levels and in the amount of urea removed during hemodialysis, compared to the dialysis of fluids not containing glucose. Moreover, during the post-dialytic period the rate of increase in the plasma urea level was also comparable in both dialysis modalities.

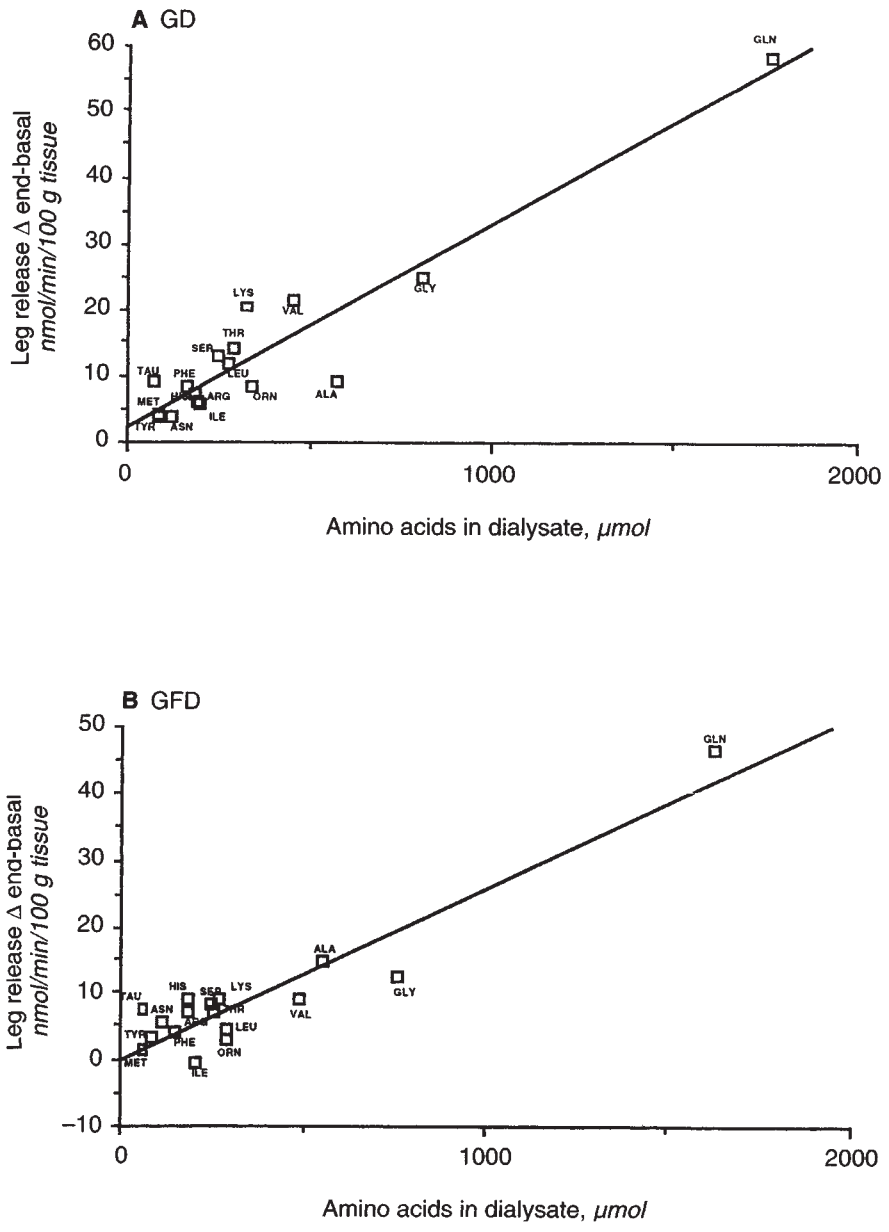

Fig. 1. *Total amount of individual amino acids in dialysate versus changes in leg release of amino acids during hemodialysis using a dialysis fluid with (A, GD; r = 0.93) or without glucose (B, GFD; r = 0.90).*

The results of the present study indicate that the presence of glucose in dialysis fluid in a concentration of 10 mmol/liter does not have a nitrogen-sparing effect and does not reduce dialytic amino acid losses in fasting hemodialysis patients in spite of preventing glucose loss and stimulating insulin secretion. This is in contrast to the few observations earlier reported by Kopple et al [11] suggesting that glucose in the dialysis fluid has an amino acid-sparing effect in fasting patients. In that study the glucose concentration was much higher (25 mmol/liter) and might have induced a more marked stimulation of insulin secretion than in the present study.

Our results are in keeping with previous studies showing that addition of glucose to the dialysis fluid does not reduce amino acid losses or decrease protein catabolism. However, the results are not directly comparable since in the study of Ono, Sasaki and Waki [13] the patients were not fasting. Farrell and co-workers [5, 6] provide no information regarding the food intake, but one has to assume that the patients were non-fasting, since they were studied repeatedly over several weeks. In these studies protein catabolism during dialysis was evaluated by urea kinetic modeling based on blood urea determinations. This method involves various assumptions regarding urea distribution and equilibrium in the body fluids and is therefore inaccurate.

It should be kept in mind that in the present study the period of food deprivation preceding the experiments was no longer than 12 hours, and it cannot be excluded that glucose-free dialysis might have had a more marked catabolic effect after starvation for a longer period of time.

Since hemodialysis implies the rapid clearance of small molecules (that is, urea, amino acids) primarily contained in the

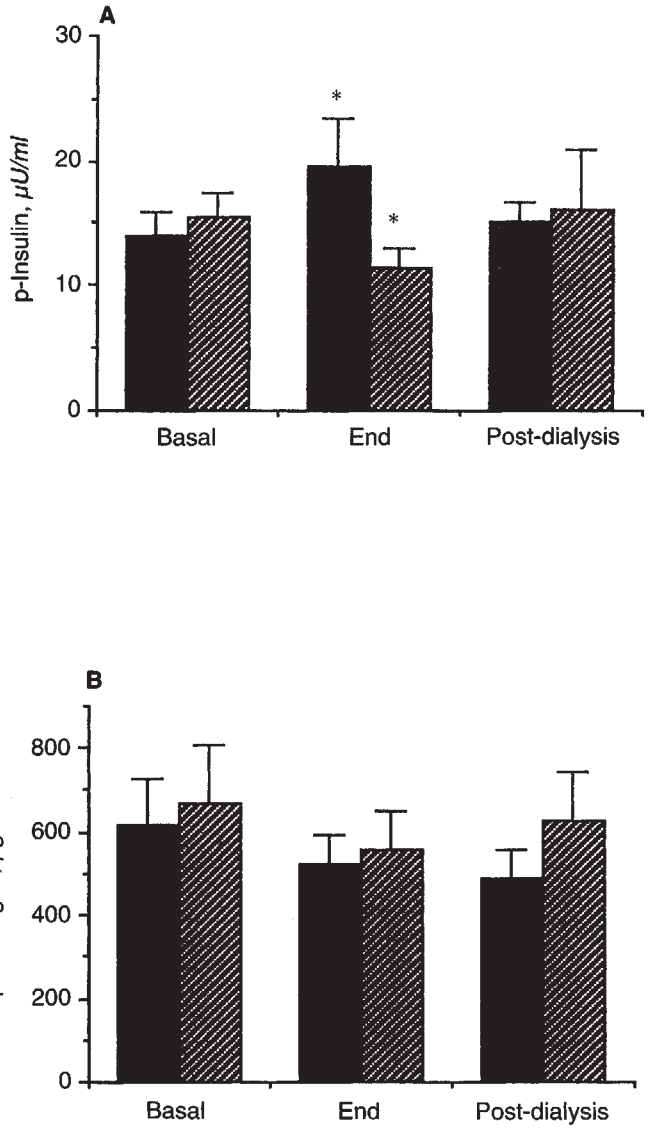

Fig. 2. *Plasma insulin levels (A) and plasma glucagon levels (B) in eight hemodialysis patients, using a dialysis fluid with (■, GD) or without glucose (▨, GFD); * P < 0.05 compared to basal values.*

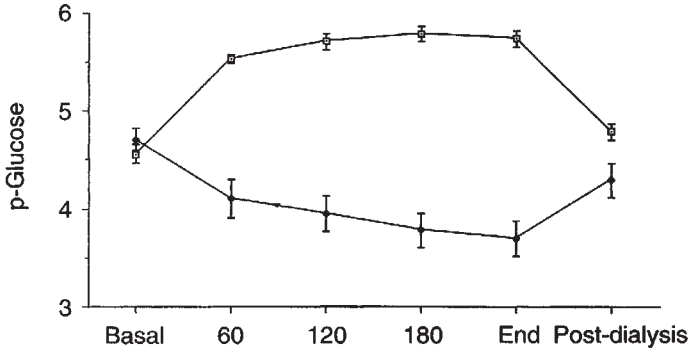

Fig. 3. *Plasma glucose (mmol/liter) during and 60 minutes after hemodialysis, using a dialysis fluid with (⊡, GD) or without glucose (◆, GFD).*

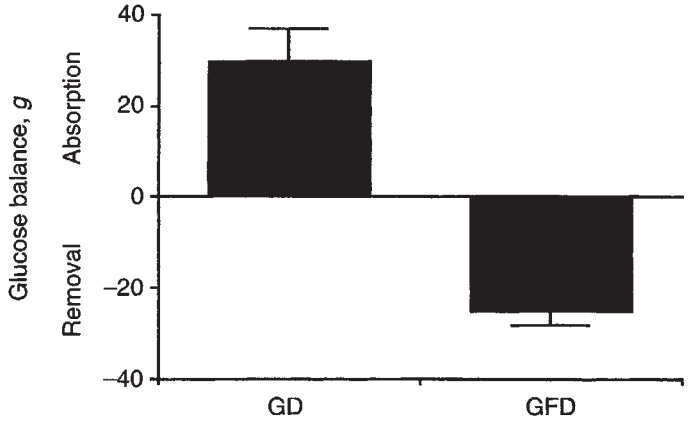

Fig. 4. *Glucose absorption/removal (g) in eight patients, using a dialysis fluid with (GD) or without glucose (GFD).*

extracellular compartment, adjacent compartments will continue to release these molecules during the post-dialytic period. This leads to a rapid rise in plasma concentrations (rebound) until a new steady state is reached. Following hemodialysis similar increases in the arterial concentrations of urea in the plasma occurred in both groups, a finding that accords with a previously reported rebound [31]. The present data do not permit any conclusions to be drawn about whether this rebound also reflects an accelerated urea generation rate due to dialysis-induced catabolism.

The results of the present study concerning changes in plasma glucose, plasma insulin and loss of glucose into the dialysate or the absorption of glucose from the dialysis fluid during hemodialysis are in close agreement with other reports [13, 14, 32]. In accordance with the results of a previous study [14], we found a highly significant correlation between the amount of glucose uptake or loss during the dialysis and the respective changes in the glucose and the insulin concentrations in the plasma.

GFD resulted in only a slight decrease in the plasma glucose levels, which suggests that increased hepatic glucose production must have made up for the loss of approximately 30 g glucose into the dialysate. In normal fasting individuals, 60 to 75% of the hepatic glucose release is derived from glycogenolysis and the rest from gluconeogenesis [33]. It is likely that the improvement in energy balance associated with the addition of glucose to the dialysis fluid may reduce the metabolism of already impoverished glucogenic substrates (mainly hepatic glycogen) in energy-compromized patients.

In a previous study in healthy subjects we showed that the contact between blood and regenerated cellulose membranes with a high capacity for complement-activation, a sign of bioincompatibility, led to a marked increase in muscle protein breakdown. On the other hand, the use of more biocompatible, cellulose acetate membranes was associated with only a slight, but insignificant increase in leg efflux of amino acids [10]. In the present study, using dialysis membranes of identical (cellulose acetate) or comparable (Hemophan) biocompatibility properties, substantial increases in amino acid efflux from the leg tissues were observed

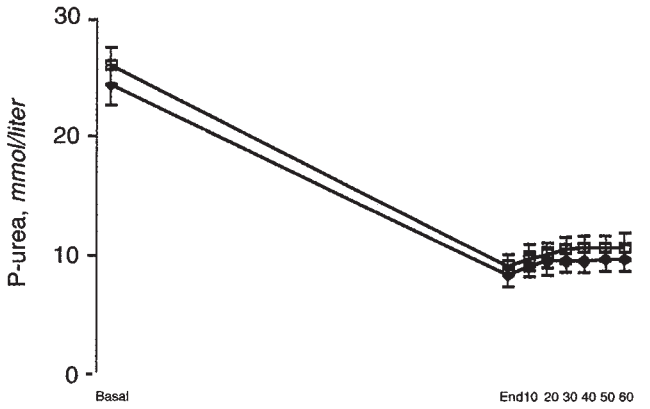

Fig. 5. *Plasma urea levels before, at the end of and during 60 minutes after the end of hemodialysis treatment, using a dialysis fluid with (□, GD) or without glucose (▼, GFD).*

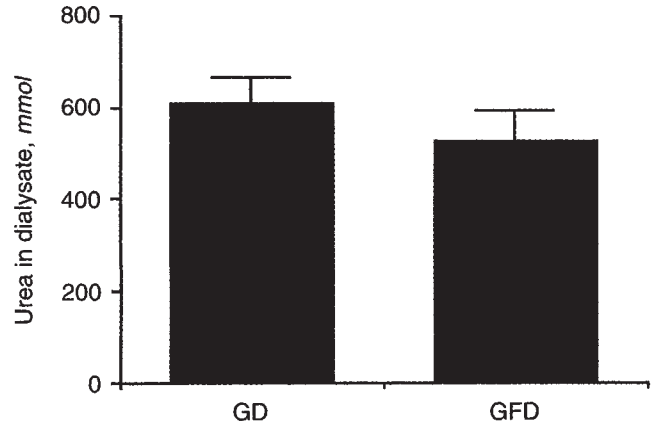

Fig. 6. *Amount of urea in dialysate after hemodialysis treatment, using a dialysis fluid with (GD) or without glucose (GFD).*

during the hemodialysis. Although blood-membrane interaction and/or endotoxins in dialysis fluid may have contributed to the enhanced muscle protein catabolism, our results suggest that the loss of amino acids to the dialysate was the main factor causing the marked increase in the release of amino acids in the leg.

In summary, as compared to dialysis with a fluid not containing glucose, the addition of glucose to the dialysis fluid did not improve nitrogen balance, although a positive energy balance was obtained. In debilitated hemodialysis patients with deficient energy intake and exhausted glycogen stores, the use of a dialysis fluid containing glucose may be of benefit. However, in view of the practical disadvantages associated with the use of glucose-containing dialysis fluid (higher cost, increased bacterial and fungal growth [34]) its routine use in clinical dialysis seems not to be warranted.

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