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Increased albumin and fibrinogen synthesis rate in patients with chronic renal failure

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Increased albumin and fibrinogen synthesis rate in patients with chronic renal failure.

Background. Hypoalbuminemia and hyperfibrinogenemia are frequently observed in patients with chronic renal failure (CRF) and are both associated with cardiovascular diseases. The mechanisms responsible for hypoalbuminemia and hyperfibrinogenemia in CRF are unknown.

Methods. In the present study, both albumin and fibrinogen kinetics were measured in vivo in predialysis patients ($N = 6$), patients on peritoneal dialysis ($N = 7$) and control subjects ($N = 8$) using L-[1-^{13}C]-valine.

Results. Plasma albumin concentration was significantly lower in patients on peritoneal dialysis compared to control subjects ($P < 0.05$). Plasma fibrinogen was significantly increased in both predialysis patients ($P < 0.01$) as well as patients on peritoneal dialysis ($P < 0.001$) in comparison to control subjects. In contrast to albumin, fibrinogen is only lost in peritoneal dialysate and not in urine. The absolute synthesis rates (ASR) of albumin and fibrinogen were increased in patients on peritoneal dialysis (ASR albumin, 125 ± 9 mg/kg/day versus 93 ± 9 mg/kg/day, $P < 0.05$; ASR fibrinogen, 45 ± 4 mg/kg/day versus 29 ± 3 mg/kg/day, $P < 0.01$) compared to control subjects. Albumin synthesis is strongly correlated with fibrinogen synthesis ($r^2 = 0.665$, $P < 0.0001$, $N = 21$). In this study, the observed hypoalbuminemia in patients on peritoneal dialysis is likely not explained by malnutrition, inadequate dialysis, inflammation, metabolic acidosis, or insulin resistance. We speculate that peritoneal albumin loss is of relevance.

Conclusion. Synthesis rate of albumin and fibrinogen are coordinately up-regulated. Both albumin and fibrinogen are lost in peritoneal dialysis fluid. To compensate protein loss, albumin synthesis is up-regulated, but the response, in contrast to predialysis patients, does not fully correct plasma albumin concentrations in peritoneal dialysis patients. The increase in fibrinogen synthesis introduces an independent risk factor for atherosclerosis, since plasma fibrinogen pool is enlarged.

Key words: albumin, fibrinogen, renal failure, peritoneal dialysis, hypoalbuminemia, hyperfibrinogenemia, amino acids.

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Hypoalbuminemia is a strong predictor of mortality and morbidity in patients with chronic renal failure (CRF). Long-term survival in dialysis is low and half of the patients die of cardiovascular complications [1–3]. In particular, low levels of nutritional indices such as albumin, prealbumin, and creatinine have been associated with poor survival in dialysis patients [1, 4, 5]. In peritoneal dialysis patients, several factors are known to affect serum albumin levels [6]. Multivariate analysis demonstrated that low plasma albumin concentration correlates most strongly with markers of inflammation and peritoneal albumin loss [5, 7].

Elevated levels of plasma fibrinogen have also been reported in predialysis [8] as well as in dialysis patients [9] and have been associated with an increased prevalence of coronary heart disease (CHD) both in the normal situation [10] as well as in dialysis patients [11].

The mechanisms responsible for the hyperfibrinogenemia are not fully understood in dialysis patients. Fibrinogen is an acute-phase protein and end-stage renal disease (ESRD) is associated with significant increases in the acute phase [12]. One possibility is that fibrinogen synthesis is increased as a component of the acute-phase response [13]. Albumin synthesis and that of fibrinogen are coordinately up-regulated in the presence of urinary protein losses in nephrotic patients [14], suggesting a second mechanism that may also be recruited in dialysis patients in whom loss of albumin across the peritoneal membrane may significantly reduce albumin concentration by a noninflammatory route.

Table 1. Clinical data of the patients and control subjects

	Renal disease	Age *year*	Gender	Weight *kg*	Body surface area m^2	Daily protein intake *g/kg/day*	Medication
Predialysis patients ($N = 6$)							
1	Chronic pyelonephritis	59	M	82.6	2.0	0.9	1
2	Reflux nephropathy	49	M	72.6	1.9	1.1	1
3	Interstitial nephritis	51	M	85.7	2.1	0.8	—
4	IgA nephropathy	19	F	83.0	1.9	0.8	1
5	Unknown	56	F	74.4	1.9	0.9	—
6	Reflux nephropathy	52	F	70.4	1.8	0.7	2
Mean ± SEM		48 ± 6[a]	3M/3F	78.1 ± 2.6[a]	1.9 ± 0.0[a]	0.8 ± 0.0[a]	
Peritoneal dialysis patients ($N = 7$)							
7	Glomerulonephritis	49	M	87.1	2.1	0.7	2
8	Unknown	54	M	78.3	1.9	1.1	1
9	Interstitial nephritis	54	F	69.3	1.8	1.0	1
10	Unknown	45	F	58.8	1.7	0.8	1
11	Polycystic disease	70	F	71.6	1.8	0.8	2
12	SLE nephritis	39	F	70.3	1.8	n.d.	1, 2, 3
13	Reflux nephropathy	53	F	68.1	1.7	0.8	2
Mean ± SEM		52 ± 4[a]	2M/5F	71.9 ± 3.3[a]	1.8 ± 0.0[a]	0.9 ± 0.0[a]	
Control subjects ($N = 8$)							
Mean ± SEM		45 ± 3	4M/4F	73.3 ± 5.1	1.9 ± 0.1	1.0 ± 0.1	—

SLE is systemic lupus erythematosus; 1 = angiotensin-converting enzyme (ACE) inhibitor, 2 = β-blocker, 3 = anti-inflammatory medication (10 mg prednisone).
[a]Not significant

In this study, we tested the hypothesis whether albumin and fibrinogen synthesis are part of a coordinated up-regulated hepatic response in patients with CRF (predialysis as well as patients on peritoneal dialysis) using endogenous labeling with ^{13}C-valine. Besides, several factors known to affect albumin and fibrinogen synthesis in patients with CRF, including nutritional status, adequacy of dialysis, state of inflammation, metabolic acidosis, insulin resistance, and exogenous protein loss were studied.

METHODS

Subjects

Six predialysis patients (three males, three females), seven peritoneal dialysis patients (two males, five females), and eight control subjects (four males and four females) participated in the study. Two patients in the predialysis group had reflux nephropathy, one patient had interstitial nephritis, one patient had immunoglobulin A (IgA) nephropathy, one patient had chronic pyelonephritis, and the cause of renal disease in one patient was unknown. The patients in the peritoneal dialysis group had glomerulonephritis, interstitial nephritis, polycystic kidney disease, reflux nephropathy, systemic lupus erythematosus (SLE) nephritis, and the cause of renal failure was unknown in two patients. Patients on peritoneal dialysis treatment included those on continuous ambulatory peritoneal dialysis (CAPD) or CAPD in combination with automated peritoneal dialysis (APD). The minimum time on dialysis was 9 months. Only one patient was studied on two occasions (predialysis and on peritoneal dialysis treatment (patients 6 and 13 in Table 1), whereas two other patients from the predialysis group (patients 1 and 2) dropped out (transplantation or died). The other patients had not yet started on peritoneal dialysis. Patients with type 2 diabetes and/or other underlying cardiovascular diseases and patients with volume overload were excluded from the study. The clinical data from the patients and control subjects are shown in Table 1. Only patient 12 was receiving anti-inflammatory medication (10 mg prednisone). Specific medication other than vitamins, erythropoietin, iron supplementation, sodium bicarbonate, and phosphate binder supplementation are presented in Table 1. Patient 8 smoked cigarettes. Before isotope infusion, the patients recorded their food intake for 3 days and collected 24-hour urine for 3 consecutive days. Additionally, the peritoneal dialysis group also collected 24-hour dialysis fluid for 3 days.

Control studies were done in eight healthy subjects (four males, four females). Control subjects also recorded their food intake for 3 days and collected one 24-hour urine sample. Urine was analyzed for urea, creatinine, protein, albumin, and fibrinogen. Dialysis fluid was analyzed for urea, protein, albumin, and fibrinogen. All patients and volunteers agreed to participate after signing an informed consent form, in accordance with the Helsinki Declaration of Human Rights. The Institutional Ethical Committee for Studies in Humans approved this study.

Infusion protocol

The subjects came to the research unit in the morning after a 10-hour fast. Before the start of the infusion, subjects were weighed and two intravenously cannulas were placed for blood sampling and infusion of the labeled valine. No food was given during the tracer infusion and the subjects were only allowed to drink water.

The last bag of peritoneal dialysate was drained for a minimum of 6 hours prior to the start of tracer infusion. During the infusion period, no dialysis was performed.

At baseline (t = 0), predialysis patients received a priming dose of 11.5 μmol/kg L-[1-^{13}C]-valine (isotope mole fraction >0.99) (Mass Trace, Woburn, MA, USA) intravenously in 2 minutes, followed by a continuous infusion of 11.5 μmol/kg/hour L-[1-^{13}C]-valine for 10 hours. The priming dose for patients on peritoneal dialysis was 8 μmol/kg and 15 μmol/kg for control subjects, followed by a continuous infusion 8 μmol/kg/hour and 15 μmol/kg/hour for 10 hours. Blood samples were taken in heparin-containing and citrate-containing tubes from the contralateral arm at t = 0, 30, 60, 180, 300, 360, 420, 480, 540, and 600 minutes. Samples were kept on ice until plasma was separated by centrifugation (15, 3000 rpm at 4°C).

Preinfusion measurements

Plasma albumin, plasma fibrinogen, total protein, bicarbonate, creatinine, glucose, urea in urine, creatinine in urine, and protein in urine were measured with standard laboratory methods on a Vitros 950 (Johnson & Johnson, Clinical Diagnostics, NY, USA). The measurement of albumin was based on the bromcresol green method.

C-reactive protein (CRP) was measured using a high-sensitivity CRP immunonephelometric assay (detection limit = 0.175 mg/L) (Dade Behring, Marburg, Germany). Insulin was measured with a competitive radioimmunoassay using a polyclonal antiinsulin antibody (Caris 46), ^{125}I-Insulin (IM166) (Amersham Biosciences, Roosendaal, The Netherlands), as a tracer and Humuline (YV2632) (AMV Lilly, Indianapolis, IN, USA) as a standard. Albumin in urine and dialysis fluid was measured on a Linx 30 analyzer (Konelab; Thermo Clinical Labsystems, Espoo, Finland). Determination of fibrinogen in urine and dialysis fluid was done using rocket electrophoresis. The amino acid profile was determined using a Biochrom 20 amino acid analyzer (Pharmacia Biotech, Ltd., Cambridge, England).

Isolation of albumin and ketoisovaleric acid (KIV)

The isolation of albumin from heparinized plasma is based on differential solubility in absolute ethanol from trichloroacetic acid (TCA)-precipitated proteins and was performed as described in detail previously [14]. The preparation of KIV prior to measurement was performed according to the method of Rocchiccioli, Leroux, and Cartier [15] with some slight modifications. The modified method has been described in detail elsewhere [16].

Isolation of fibrinogen

Fibrinogen was purified from plasma anticoagulated with citrate based on the method of Vila et al [17]. Briefly, 0.5 mL plasma was added very slowly to 0.5 mL 80 g/L polyethylene glycol 6000 (Merck, Darmstadt, Germany). The mixture was placed on ice and gently shaken for 10 minutes. After centrifugation (10 minutes, 2500 rpm at 4°C) the precipitate was redissolved in 1 mL phosphate buffer (0.01 mol/L Na_2HPO_4 and 0.15 mol/L NaCl, pH 7.4) and subsequently precipitated with 0.5 mL 2 mol/L acetic acid acetate buffer (pH 4.6). This mixture was again placed on ice for 30 minutes and gently shaken. After centrifugation the precipitate was redissolved in 1 mL phosphate buffer (0.036 mol/L Na_2HPO_4, pH 7.8) and precipitated with 330 μL 4 mol/L $(NH_4)_2SO_4$. After centrifugation, the pellet was redissolved in 1 mL phosphate buffer (0.018 mol/L Na_2HPO_4, pH 7.8). A part of the isolated fibrinogen was used to monitor purity and identification. Subsequently, an aliquot of the solution containing ±500 μg fibrinogen was precipitated by adding 1 mL ice-cold 2 mol/L $HClO_4$ in order to remove the free amino acids. Hydrolysis of fibrinogen was done in 0.5 mL 6 mol/L HCl [18], followed by cation exchange chromatography (AG 50W-X8) (Bio-Rad Laboratories, Hercules, CA, USA) and dried under N_2. Derivatization was done according to the method of Husek [19].

Fibrinogen rocket electrophoresis

Fibrinogen rocket electrophoresis was used to assess loss of fibrinogen in urine and dialysis fluid in the studied patients. A fibrinogen calibration curve (standard = 2.5 mg/mL) (Dade Behring) was made in physiologic salt ranging from 16 to 250 mg/L to detect loss of fibrinogen in urine. One milliliter of urine of the patients was freeze-dried and dissolved in 100 μL physiologic salt. A 2 μL sample was loaded on a gel containing 1% agarose M (Amersham Pharmacia Biotech, Uppsala, Sweden) containing 15 μL polyclonal antihuman fibrinogen antibody (Dade Behring). Electrophoresis was done for 16 hours at 200 V and 10 mA per gel using Tris-Tricine, pH 8.6, as electrophoresis buffer, after which the gel was washed for a minimal 6 hours in physiologic salt. After washing, the gel was dried and detection of the samples was done with 0.25% Coomassie staining. Quantitative results are obtained by measuring the distance from the origin to the tip of the peak. The peak height was proportional to the concentration. A spiked urine sample was used as positive control and urine of different control subjects were used as negative controls.

To detect fibrinogen loss in dialysis fluid, a fibrinogen calibration curve was made in physiologic salt ranging from 16 to 250 mg/L. One milliliter dialysis fluid was freeze-dried and dissolved in 100 μL and 200 μL physiologic salt. Samples were run following the same procedure as described above. Unused dialysis fluid and physiologic salt were used as negative controls and a spiked dialysis sample was used as a positive control. The intravariation of the rocket electrophoresis was 2.6% and the intervariation was 5.3%.

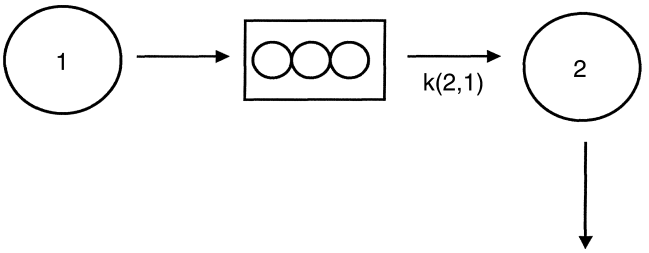

Fig. 1. Model for estimation of albumin and fibrinogen fractional synthesis rates (FSR) for patients and control subjects. Compartment 1 represents the precursor pool and compartment 2 represents the plasma albumin or fibrinogen pool. The k-value represents the rate constant. The equation used for calculation of FSR_day = k (2,1) × 24 × 100; 24 is the conversion of pools/hour to pools/day; 100 is the conversion of pools to %.

Derivatives and mass spectrometric measurements

The preparation of N(O,S) methoxycarbonylmethylester derivatives of albumin and fibrinogen and the derivatization of KIV with MTBSTFA:pyridine (3:1) and mass spectrometric measurements were described in detail elsewhere [16, 20].

Kinetic analysis

Fractional synthesis rates (FSR) for albumin and fibrinogen were calculated from the tracer/tracee data and the data were fitted using the SAAM II software (Simulation Analysis and Modeling, Seattle, WA, USA). The absolute synthesis rate (ASR), which is the amount of protein synthesized per day, was calculated as the product of the FSR and the plasma pool (plasma volume × plasma concentration). To adjust for different body weights, the quantities are expressed per kilogram body weight. Plasma volumes in all subjects were calculated from body surface area (BSA) using the formulas described by Hurley [21].

Calculation of the FSR for albumin and fibrinogen was done using a two-compartment model (Fig. 1), where compartment 1 is the precursor pool and compartment 2 represents the protein pool. The delay was approximately 30 minutes for albumin as well as fibrinogen. The equation used for calculation of the FSR/day (%/day) = k(2,1) × 24 × 100. Since steady state is achieved, subtraction of external loss from the ASR results in the absolute catabolic rate (ACR) and presumes that all direct losses of albumin are measured. The fractional catabolic rate (FCR) was calculated by dividing the ACR by the plasma pool size.

Statistical analysis

Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) assessed the significance of differences and multiple comparisons was done using Bonferroni *t* test. If normality test or equal variance test failed, a Kruskal-Wallis one-way ANOVA on ranks was performed and multiple comparisons was done using Dunn's test. Correlations were performed by linear regression analysis. A P value <0.05 was considered to be statistically significant.

RESULTS

Baseline data

The plasma values of total protein, albumin, fibrinogen, CRP, insulin, and bicarbonate are shown in Table 2. Plasma albumin is significantly lower in peritoneal dialysis patients than in control subjects (33.0 ± 0.9 g/L versus 36.2 ± 0.5 g/L, $P < 0.05$). In contrast to albumin, plasma fibrinogen concentration was significantly greater both in predialysis patients (4.8 ± 0.3 g/L, $P < 0.01$) and peritoneal dialysis patients (5.2 ± 0.4 g/L compared to control 3.0 ± 0.2 g/L, $P < 0.001$). While fibrinogen levels tended to be greater in patients who were treated with peritoneal dialysis when compared to those who had not yet begun treatment (4.8 ± 0.3 g/L versus 5.2 ± 0.4 g/L), these differences did not achieve significance. Plasma bicarbonate was not significantly lower in predialysis patients compared to peritoneal dialysis patients and control subjects (21.6 ± 2.3 mmol/L versus 26.1 ± 1.5 mmol/L versus 24.0 ± 1.0 mmol/L). No significant difference is found for total protein, CRP, and plasma volume in the three groups (predialysis versus peritoneal dialysis versus control subjects).

Protein losses in urine and dialysis fluid are shown in Table 3. The reported value is the mean of 3 days urine collection and dialysis fluid collection in the patient groups. Proteinuria and albuminuria was constant over the 3 days as also found for protein loss, albumin loss, and fibrinogen loss in dialysis fluid. Mean urinary protein losses in predialysis patients was 3.9 ± 1.0 g/day (range, 1.4 to 7.2 g/day) and 0.8 ± 0.2 g/day (range, 0.3 to 1.5 g/day) for dialysis patients and was significantly greater in both groups compared to control subjects (3.9 ± 1.0 g/day, $P < 0.01$; and 0.8 ± 0.2 g/day versus control subjects $<0.1 \pm 0.0$ g/day, $P < 0.01$). Urinary protein loss was also significant different between predialysis and peritoneal dialysis patients (3.9 ± 1.0 g/day versus 0.8 ± 0.2 g/day, $P < 0.05$). The urinary loss of albumin was significantly greater in both patient groups compared to control subjects (predialysis patients, albumin 24 ± 6 mg/kg/day versus $<0.1 \pm 0.0$ mg/kg/day, $P < 0.001$; and patients on peritoneal dialysis, albumin 4 ± 1 mg/kg/day versus $<0.1 \pm 0.0$ mg/kg/day, $P < 0.001$).

Albuminuria in predialysis patients was significantly greater compared to patients on peritoneal dialysis (24 ± 6 mg/kg/day versus 4 ± 1 mg/kg/day, $P < 0.01$). Mean protein loss in dialysis fluid was 6.2 ± 0.9 g/day (range, 3.3 to 9.9 g/day). The mean loss of albumin in dialysis fluid was 52 ± 5 mg/kg/day (range, 30 to 67 mg/kg/day) and 0.7 ± 0.2 mg/kg/day for fibrinogen (range, 0.2 to

Table 2. Biochemical parameters in plasma from chronic renal failure (CRF) patients and control subjects

	Total protein *g/L*	Albumin *g/L*	Fibrinogen *g/L*	C-reactive protein *μg/mL*	Insulin *mE/L*	HOMA-R	$[HCO_3^-]$ bicarbonate *mmol/L*	Plasma volume *mL/kg*
Predialysis patients ($N = 6$)								
1	80.2	40.1	6.6	10.8	16	4.0	22.0	38
2	80.1	40.0	4.9	3.6	30	9.1	25.9	40
3	64.4	35.6	4.0	3.4	18	4.2	19.3	38
4	60.4	30.1	4.7	3.7	26	5.0	12.4	33
5	80.0	42.5	4.4	1.7	7	1.6	28.3	36
6	76.0	38.4	4.2	1.4	20	4.9	21.4	35
Mean ± SEM	74.2 ± 3.3[d]	37.8 ± 1.8[d]	4.8 ± 0.3[b]	4.1 ± 1.4[d]	19.5 ± 3.3[c]	5.1 ± 0.9[b]	21.6 ± 2.3[d]	37 ± 1[d]
Peritoneal dialysis patients ($N = 7$)								
7	59.3	28.7	4.5	1.0	5	1.2	25.0	38
8	71.3	36.1	6.6	8.6	12	3.1	22.0	39
9	65.8	33.2	4.6	0.6	15	3.4	33.8	37
10	73.7	35.0	4.1	6.4	9	2.1	23.8	40
11	65.2	35.0	7.1	9.4	13	3.4	24.2	35
12	65.0	31.8	5.1	2.9	31	7.4	24.7	37
13	65.1	31.7	5.0	8.9	13	3.7	29.4	36
Mean ± SEM	66.4 ± 1.7[d]	33.0 ± 0.9[a]	5.2 ± 0.4[c]	5.4 ± 1.4[d]	14.0 ± 3.1[b]	3.4 ± 1.7[a]	26.1 ± 1.5[d]	37 ± 1[d]
Control subjects ($N = 8$)								
Mean ± SEM	67.4 ± 1.5	36.2 ± 0.5	3.0 ± 0.2	3.2 ± 1.3	5.0 ± 0.0	1.5 ± 0.9	24.0 ± 1.0	39 ± 1

[a] $P < 0.05$, compared to control subjects; [b] $P < 0.01$, compared to control subjects; [c] $P < 0.001$, compared to control subjects; [d] Not significant

Table 3. Biochemical parameters in urine and dialysis fluid from patients and control subjects

	Urinary loss		Dialysis fluid				
	Protein *g/day*	Albumin *mg/kg/day*	Protein *g/day*	Albumin *mg/kg/day*	Fibrinogen *mg/kg/day*	Creatinine clearance *mL/min/1.73 m²*	Kt/V
Predialysis patients ($N = 6$)							
1	7.0	33	—	—	—	8	—
2	2.6	20	—	—	—	7	—
3	1.4	6	—	—	—	11	—
4	7.2	50	—	—	—	9	—
5	3.5	23	—	—	—	9	—
6	1.8	11	—	—	—	5	—
Mean ± SEM	3.9 ± 1.0[a]	24 ± 6[b]	—	—	—	8 ± 1[a]	—
Peritoneal dialysis patients ($N = 7$)							
7	1.5	8	9.9	67	1.8	—	1.70
8	1.5	6	7.9	63	0.3	—	2.55
9	0.3	1	4.5	50	0.3	—	1.90
10	0.3	1	4.6	49	0.5	—	1.70
11	0.7	1	7.5	64	1.1	—	1.99
12	0.8	1	3.3	30	0.2	—	1.70
13	0.8	9	5.6	40	0.8	—	2.19
Mean ± SEM	0.8 ± 0.2[a,c]	4 ± 1[b,d]	6.2 ± 0.9	52 ± 5	0.7 ± 0.2	—	1.96 ± 0.12
Control subjects ($N = 8$)							
Mean ± SEM	<0.1 ± 0.0	<0.1 ± 0.0	—	—	—	97 ± 12	—

[a] $P < 0.01$, compared to control subjects; [b] $P < 0.001$ compared to control subjects; [c] $P < 0.05$, compared to predialysis subjects; [d] $P < 0.01$, compared to predialysis patients

1.8 mg/kg/day). Mean total protein loss in patients on peritoneal dialysis was 7.0 ± 1.0 g/day (range, 4.1 to 11.4 g/day) or 95 ± 11 mg/kg/day (range, 58 to 131 mg/kg/day). Mean total albumin loss was 4.1 ± 0.6 g/day (range, 2.1 to 6.6 g/day) or 56 ± 5 mg/kg/day (range, 31 to 75 mg/kg/day). In predialysis patients, albumin loss was 45% ± 4% (range, 27% to 57%) of total protein loss and 61% ± 4% (range, 52% to 73%) for patients on peritoneal dialysis.

Creatinine clearance calculated, using the Cockcroft-Gault formula, was significantly decreased in predialysis patients compared to control subjects (8 ± 1 mL/min/1.73 m² versus 97 ± 12 mL/min/1.73 m², $P < 0.01$). The mean Kt/V value in the peritoneal dialysis group, where V was estimated by the formula of Watson and Watson, was 1.96 ± 0.12 (range, 1.70 to 2.55).

Synthesis rate of albumin in patients with CRF and in control subjects

Albumin kinetics in predialysis patients and patients on peritoneal dialysis are shown in Table 4. The ASR of albumin tended to be greater in both patient groups compared to control subjects (110 ± 11 mg/kg/day and

Table 4. Kinetics of albumin and fibrinogen

	FSR albumin *%/day*	FSR fibrinogen *%/day*	ASR albumin *mg/kg/day*	ASR fibrinogen *mg/kg/day*	FCR albumin *%/day*	FCR fibrinogen *%/day*
Predialysis patients ($N = 6$)						
1	8.6	24.6	129	61	6.4	24.6
2	9.7	21.6	157	43	8.5	21.6
3	7.4	18.2	100	28	6.9	18.2
4	8.9	18.1	88	28	3.8	18.1
5	6.3	16.9	95	26	4.7	16.9
6	6.6	15.8	88	23	5.7	15.8
Mean ± SEM	7.9 ± 0.6[d]	19.2 ± 1.3[d]	110 ± 11[d]	35 ± 6[d]	6.0 ± 0.7[d]	19.2 ± 1.3[d]
Peritoneal dialysis patients ($N = 7$)						
7	10.0	23.7	108	40	3.0	22.6
8	11.9	22.0	166	56	7.0	21.9
9	7.7	18.1	94	31	3.5	18.0
10	8.3	27.9	116	45	4.8	27.6
11	11.6	21.6	144	54	6.4	21.1
12	9.5	17.6	123	36	4.5	17.5
13	11.1	29.9	126	53	6.8	29.4
Mean ± SEM	10.0 ± 0.6[b,c]	23.0 ± 1.7[d]	125 ± 9[a]	45 ± 4[b]	5.1 ± 0.6[d]	22.6 ± 1.7[d]
Control subjects ($N = 8$)						
Mean ± SEM	6.7 ± 0.6	24.6 ± 1.6	93 ± 9	29 ± 3	6.7 ± 0.6	24.6 ± 1.6

Abbreviations are: ASR, absolute synthesis rate; FSR, fractional synthesis rate.
[a]$P < 0.05$ compared to control subjects; [b]$P < 0.01$ compared to control subjects; [c]$P < 0.05$ compared to predialysis subjects; [d]not significant

125 ± 9 mg/kg/day versus 93 ± 9 mg/kg/day) and was significantly greater in peritoneal dialysis patients. The FSR of albumin also tended to be greater in both patient groups compared to control subjects (7.9 ± 0.6%/day and 10.0 ± 0.6%/day versus 6.7 ± 0.6%/day, $P < 0.01$), but was only significantly greater in peritoneal dialysis patients. Albumin FSR was also significantly higher in patients on peritoneal dialysis compared to predialysis patients (7.9 ± 0.6%/day and 10.0 ± 0.6%/day, $P < 0.05$). No significant difference was found for albumin FCR in both patient groups compared to control subjects.

Synthesis rate of fibrinogen in patients with CRF and in control subjects

Fibrinogen kinetics in patients with CRF and control subjects are shown in Table 4. Fibrinogen ASR is significantly higher in the peritoneal dialysis group compared to control subjects (45 ± 4 mg/kg/day versus 29 ± 3 mg/kg/day, $P < 0.01$) and tends to be increased in the predialysis group (35 ± 6 mg/kg/day versus 29 ± 4 mg/kg/day).

No significant difference was found for fibrinogen FSR in patients and control subjects (Table 4).

Correlations

Considering the data in control subjects and patients together, several parameters were tested. Albumin synthesis strongly correlated with fibrinogen synthesis ($r^2 = 0.665$, $P < 0.0001$, $N = 21$) (Fig. 2) but not with plasma albumin concentration ($r^2 = 0.003$, $N = 21$). Plasma fibrinogen strongly correlated with the ASR of fibrinogen ($r^2 = 0.643$, $P < 0.0001$, $N = 21$) and ASR of albumin ($r^2 = 0.498$, $P < 0.001$, $N = 21$). No significant correlation

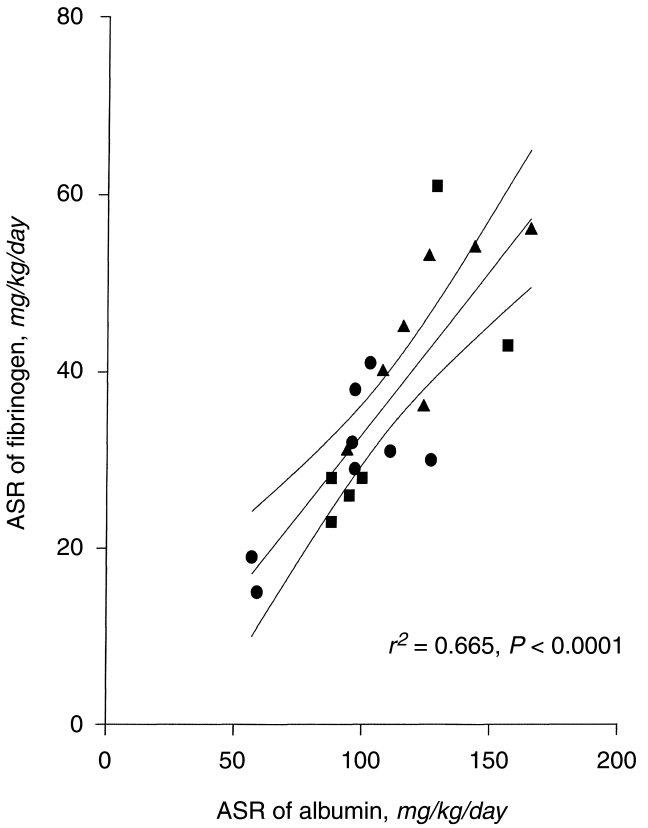

Fig. 2. Relationship between absolute synthesis rate (ASR) of fibrinogen (mg/kg/day) and the ASR of albumin (mg/kg/day) for predialysis patients ($N = 6$) (■), peritoneal dialysis patients ($N = 7$) (▲), and control subjects ($N = 8$) (●). The 95% confidence limits of the entire group are shown on either side of the regression line ($r^2 = 0.665$, $P < 0.0001$, $N = 21$).

was found for plasma insulin and albumin synthesis ($r^2 = 0.126$, $P = 0.11$, $N = 21$).

In the control group, albumin synthesis correlated with fibrinogen synthesis ($r^2 = 0.527$, $P < 0.05$, $N = 8$). No significant correlation was found for albumin synthesis and CRP ($r^2 = 0.201$, $P = 0.31$, $N = 8$) or fibrinogen synthesis and CRP ($r^2 = 0.165$, $P = 0.36$, $N = 8$).

In the patient groups, albumin synthesis correlated with fibrinogen synthesis ($r^2 = 0.636$, $P < 0.01$, $N = 13$). Plasma fibrinogen also strongly correlated with the ASR of fibrinogen ($r^2 = 0.614$, $P < 0.01$, $N = 13$) and the ASR of albumin ($r^2 = 0.636$, $P < 0.01$, $N = 13$). Total albumin loss strongly correlated with total protein loss ($r^2 = 0.930$, $P < 0.0001$, $N = 13$). Total albumin loss ($r^2 = 0.338$, $P = 0.037$, $N = 13$), but not total protein loss ($r^2 = 0.250$, $P = 0.08$, $N = 13$) correlated with plasma albumin concentration. Albumin ASR did not correlate with total albumin loss ($r^2 = 0.09$, $N = 13$) or total protein loss ($r^2 = 0.140$, $N = 13$). Furthermore, no correlation was found for albuminuria ($r^2 = 0.001$, $N = 13$), proteinuria ($r^2 = 0.03$, $N = 13$) and plasma fibrinogen concentration. Albumin synthesis did not correlate with plasma insulin ($r^2 = 0.006$, $P = 0.80$).

DISCUSSION

In the present study, using stable isotopes, we showed that both albumin synthesis and fibrinogen synthesis were increased in patients with CRF. A strong correlation was found between the synthesis rates of both liver derived proteins in patients and control subjects ($r^2 = 0.665$, $P < 0.0001$, $N = 21$) as well as in both groups separately ($r^2 = 0.636$, $P < 0.01$, $N = 13$ and $r^2 = 0.527$, $P < 0.05$, $N = 8$), suggesting a tightly coordinated up-regulated process. This finding is also observed in nephrotic patients and in hemodialysis patients, suggesting a common mechanism may be acting in these divergent patient groups [13, 22].

In our patient group, a correlation was found between total albumin loss and plasma albumin ($r^2 = 0.338$, $P < 0.05$, $N = 13$), suggesting that albumin loss is a major determinant for plasma albumin levels as also reported by others [5, 7, 23]. The increased rate of albumin synthesis in these patients off sets the albumin loss and is likely to be regulated in a manner to defend plasma albumin concentration. In the predialysis group, the small increase in albumin synthesis (20%) is sufficient to compensate urinary albumin loss, resulting in normal plasma albumin concentrations. In peritoneal dialysis patients, however, albumin synthesis is significantly greater than in control subjects (35%), while plasma albumin concentration is not normalized, suggesting inability to increase albumin synthesis rate to higher levels or the existence of other defense mechanisms. In this study, plasma volume was calculated from BSA and was not estimated with the Evans Blue method as recently described in our group for nephrotic patients [14]. Due to the toxic properties of the dye Evans Blue, as stated in the guidelines for controlling safety hazards in hospitals, the Ethical Committee of our hospital did not give their consent for such experiment. Since it has been reported that plasma volume is usually increased in patients with CRF [23, 24], we chose to calculate the data based on normal plasma volume. This results in underestimation than overestimation of the reported data.

Fibrinogen synthesis was increased in the peritoneal dialysis group. We hypothesize that fibrinogen synthesis is increased as part of a coordinated response of liver derived proteins such as occurs in the nephrotic syndrome. Of 13 patients, 11 were prescribed medication, consisting of a β-blocker, an angiotensin-converting enzyme (ACE) inhibitor, or a combination of both drugs. Both drugs are known to reduce plasma fibrinogen levels [25, 26], yet fibrinogen levels remained significantly elevated. In contrast to albumin, fibrinogen is lost in peritoneal dialysis fluid, but not in urine as observed in our patients and also increases in plasma despite the losses. Despite the fibrinogen loss, fibrinogen FCR is normal, suggesting that the increase in plasma fibrinogen concentration results from increased synthesis rate alone as also found in patients with the nephrotic syndrome for fibrinogen [14], lipoprotein(a) [Lp(a)] [27] and α_2-macroglobulin [28].

Several factors may affect albumin and fibrinogen metabolism in patients with CRF, including nutritional status, adequacy of dialysis, state of inflammation, metabolic acidosis, insulin resistance, and exogenous protein loss [5, 7].

Malnutrition has been reported to be a potential cause of hypoalbuminemia in patients with CRF [29], since a low protein intake decreases albumin synthesis [30]. In this report, albumin ASR in three of six predialysis patients and in six of seven peritoneal dialysis patients was higher than mean albumin ASR in control subjects. Furthermore, daily protein intake in all the patients studied was close to normal and dialysis adequacy was sufficient (mean Kt/V = 1.96 ± 0.12, $N = 7$), suggesting that the hypoalbuminemia in peritoneal dialysis group is not explained by malnutrition or inadequate dialysis.

It had recently been recognized that inflammation rather than protein malnutrition plays a role in albumin synthesis in patients on hemodialysis [31]. In contrast to other studies of both predialysis patients [12] and those with ESRD [7, 32], our patients had CRP values that approached the normal range. Indeed, there was no significant difference among these groups. However, plasma CRP in one control subject was 10 mg/L. Exclusion of this subject results in a mean plasma CRP value of 2.1 ± 0.9 mg/L that is in agreement with values reported for healthy controls [33]. No significant differences were observed after recalculation of the data, suggesting that it is unlikely that inflammation has contributed substan-

tively to either albumin levels or fibrinogen levels in this group of patients.

Metabolic acidosis has been recognized as an important stimulus for protein catabolism and could also contribute to hypoalbuminemia. In normal individuals, albumin synthesis was reduced during metabolic acidosis [34]. Since plasma bicarbonate is not different in control subjects and patients, despite an increased albumin synthesis rate in the latter group, these data suggest that corrected metabolic acidosis is of minor importance for hypoalbuminemia in patients with CRF [35]. In one patient (patient 4, predialysis group), plasma bicarbonate was 12.4 mmol/L and this patient had the lowest serum albumin concentration (30.1 g/L) and lowest synthesis rate (88 mg/kg/day), suggesting that metabolic acidosis may have contributed to hypoalbuminemia is this patient. However, no correlation was found between plasma bicarbonate and plasma albumin ($r^2 = 0.01$, $P = 0.70$), as reported by others [29].

Several studies clearly showed that the action of insulin is markedly impaired in patients with CRF [36, 37] and exogenous insulin administration increases albumin synthesis in humans [38]. The reduced action of insulin to increase albumin synthesis could therefore be of relevance. Plasma insulin levels and homeostasis model assessment of insulin resistance (HOMA-R) index, but not albumin synthesis, tended to be higher in predialysis patients compared to patients on dialysis. This might suggest a higher degree of insulin insensitivity in the predialysis group, implying that intensive glucose loading during peritoneal dialysis has no or little effect on insulin resistance. Additionally, no significant correlation was found for plasma insulin and albumin synthesis rate in the whole group ($r^2 = 0.126$, $P = 0.11$, $N = 21$) or in the patient group ($r^2 = 0.006$, $P = 0.80$). It is important to notice that, despite absence of direct evidence for either of these factors, the occurrence of an indirect effect of these factors cannot completely excluded.

The present data demonstrate that albumin synthesis is 20% higher in predialysis patients and 35% higher in peritoneal dialysis patients. Renal failure per se does not reduce albumin synthetic rate [39]. In hemodialysis patients, the increase in albumin synthesis rate is similar as observed in our patients, suggesting no effect of dialysis modality on albumin synthesis [22, 29]. In the nephrotic syndrome, however, the increase in albumin synthesis rate described previously was much higher (100%) [14], compared to patients on peritoneal dialysis, despite similar amount of external protein loss in both patient groups. This phenomenon might be explained by lower net amino acid availability in dialysis patients [40, 41]. Alternatively, serum albumin concentration was significantly less in the nephrotic patients, perhaps providing a greater stimulus for albumin synthesis in that group.

Patients with CRF have reduced plasma levels of branched-chain amino acids and keto analogs [42–44] in contrast to nephrotic patients, where only the keto acid pool is reduced [43]. In order to increase these pools, oral supplementation with amino acids or keto acids, intravenous administration of amino acids or addition of amino acids to dialysis fluid could be useful. Amino acid and keto acid supplementation increases albumin synthesis in rats [44, 45]. In humans, supplementation of a combination of amino acids and keto analogs of essential amino acids to patients with CRF, resulted in an increase in plasma albumin and plasma transferrin concentration [46–48]. Unfortunately, plasma fibrinogen concentration was not measured. In peritoneal dialysis, supplementation of amino acids in dialysis fluid is possible. Recently, we studied albumin kinetics in two patients (patients 9 and 10) using an experimental 1% glycerol amino acid dialysis fluid during 4 weeks, but no effect on plasma albumin concentration was found (31.7 g/L and 35.1 g/L). In one patient (patient 9), no change of albumin synthesis was found (93 mg/kg/day), whereas the other patient (patient 10) showed an increase in albumin synthesis rate (148 mg/kg/day), despite similar concentrations of branched-chain amino acids in both patients before and after dialysis with the experimental 1% glycerol amino acid dialysis fluid (Baxter, Utrecht, The Netherlands) (140 and 109 μmol/L valine, 65 and 56 μmol/L leucine, and 34 and 29 μmol/L isoleucine, respectively). Furthermore, in two other studies a marginal effect of amino acid supplementation via the peritoneum on plasma albumin and transferrin levels was found [49, 50]. This suggests that amino acid supplementation is likely not useful for peritoneal dialysis patients with a protein intake that is close to normal.

CONCLUSION

Albumin and fibrinogen synthesis are coordinately up-regulated in CRF. In peritoneal dialysis patients both albumin and fibrinogen are lost in dialysis fluid. Albumin synthesis is up-regulated, but the response is insufficient to maintain a normal albumin concentration in dialysis patients. In contrast, patients with near-ESRD are capable of increasing albumin synthetic rate sufficiently to maintain a normal albumin concentration despite significant urinary losses. In this study, the observed hypoalbuminemia in peritoneal dialysis patients is not explained by malnutrition, inadequate dialysis, inflammation, metabolic acidosis, or insulin resistance, speculating that peritoneal albumin loss is of relevance. Since fibrinogen synthesis is positively correlated with plasma fibrinogen concentration, this linkage may contribute to the risk of cardiovascular disease in patients with renal failure.

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