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Proportionate increase of fibrinogen and albumin synthesis in nephrotic patients: Measurements with stable isotopes

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Proportionate increase of fibrinogen and albumin synthesis in nephrotic patients: Measurements with stable isotopes. Hyperfibrinogenemia is a common feature of the nephrotic syndrome, and contributes to increased tendency for thrombosis and atherosclerosis. Its genesis is not certain, but the increase in liver fibrinogen mRNA in nephrotic rats indicates increased synthesis. Data in humans are scarce. We presently compared synthesis rates of fibrinogen and albumin in nephrotic adults ($N = 7$; plasma albumin 22.3 ± 0.7 g/liter, proteinuria 12 g/day) and healthy control subjects ($N = 8$) using a primed/continuous infusion of the stable isotope L-[1-^{13}C]valine for six hours. Absolute synthesis rate (ASR) of fibrinogen was 31 ± 3 mg/kg/day in nephrotic subjects and 21 ± 1 mg/kg/day in control subjects ($P < 0.05$), and positively correlated with plasma fibrinogen ($P = 0.0317$). The plasma fibrinogen pool was disproportionately increased in the nephrotic patients (271 ± 30 mg/kg) compared to the controls (126 ± 8 mg/kg), suggesting decreased fractional catabolic rate as well. The ASR of albumin was increased from 71 ± 4 mg/kg/day in the controls to 160 ± 19 mg/kg/day in the patients ($P < 0.0001$), and strongly correlated with the ASR of fibrinogen ($P = 0.0046$). Plasma α_2-macroglobulin was also elevated and correlated with the albumin synthesis rate, whereas plasma serum amyloid A and C-reactive protein were not elevated. These data suggest that in nephrotic patients the increased albumin synthesis is associated with an increase in synthesis of a specific and coordinated group of proteins, among which is fibrinogen.

The nephrotic syndrome is characterized by proteinuria (> 3.5 g/day) and a reduction in the concentration of albumin and other proteins of intermediate molecular weight [1]. In contrast, the concentration of lipoproteins [2–7] and several proteins of high molecular weight such as fibrinogen and macroglobulins are increased [1, 8–9]. Part of the reason for this difference is that albumin, transferrin and IgG pass through the damaged glomerulus [1] while large proteins, including IgM, α_1- and α_2-macroglobulins, fibrinogen, factor XIII, fibronectin and larger lipoproteins, do not pass through it to any significant extent [8–11].

Key words: fibrinogen, albumin, isotope measurement, nephrotic syndrome.

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The concentration of proteins in plasma may be increased either as a consequence of an increased synthesis, a decreased catabolic rate, or both. While the rates of synthesis of many plasma proteins are increased in the nephrotic syndrome [8, 11–18], this is not true for all proteins [13, 18, 19]. Plasma fibrinogen concentration is increased, and evidence from nephrotic rats suggests that an increased rate of synthesis of this protein may contribute to its increased plasma concentration. mRNAs coding for fibrinogen and albumin in the liver are increased in rats with the nephrotic syndrome [8, 13]. Furthermore, the rate of transcription of their genes is also increased in livers of nephrotic rats [8], suggesting coordinate increases in the synthesis of these two proteins in the nephrotic syndrome. It is, however, not certain whether these results can be translated to humans, since no direct assessments have been done.

The aim of the present study was to simultaneously measure the synthesis rates of fibrinogen and albumin in patients with the nephrotic syndrome and to compare them with measurements in a control group. For this purpose, we used a primed/continuous infusion of the stable isotope labeled valine, and measured its enrichment in the purified proteins using gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS). Besides the relationship between fibrinogen and albumin synthesis and their respective plasma protein levels, we also measured the concentration of three other acute phase proteins [α_2-macroglobulin, C-reactive protein (CRP) and serum amyloid A (SAA)], since it was suggested that a group of proteins is up-regulated coordinately in the nephrotic syndrome [8].

METHODS

Subjects

Seven patients (five males) with a nephrotic syndrome (proteinuria > 3.5 g/day and plasma albumin concentration < 30 g/liter) were studied. Their mean age was 50 years (range 31 to 63) and mean body wt 83.8 kg (range 68.6 to 92.0). All patients had a stable nephrotic syndrome of at least three months duration (mean duration 19 months, range 3 to 54 months). Six patients had membranous glomerulopathy and one had minimal change disease (indicated by biopsy). Patients were prescribed a diet containing 0.8 g protein/kg body wt/day and 100 mmol sodium/

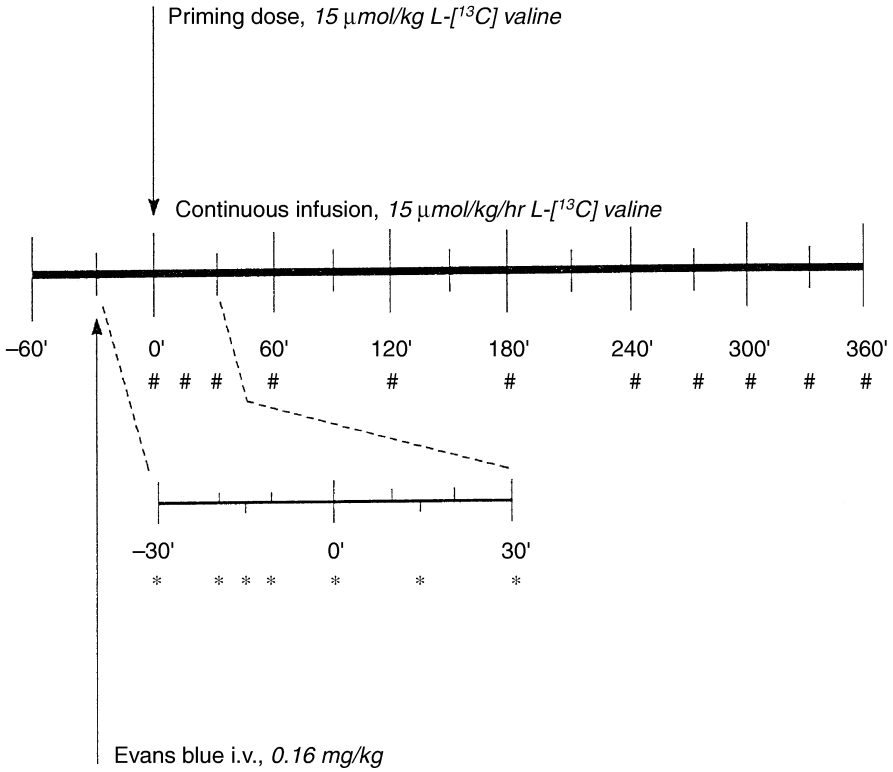

Fig. 1. Study protocol. Studies were performed after the subjects had fasted for 12 hours. After at least 30 minutes of recumbency, an intravenous dose of Evans blue was given (t = −30′). At t = 0, a priming dose, directly followed by a continuous infusion of L-[1-^{13}C] valine during 360′ was given. Symbols (* and #) indicate blood sampling points for determination of plasma volume and protein synthesis, respectively.

day. Besides diuretics, they used no medication, or medication was stopped at least two weeks before the infusion study (see below). Control studies were done in eight healthy subjects (four males), mean age 32 years (range 25 to 41), mean weight 76.7 kg (range 64.1 to 103.4) under similar dietary conditions. One day before the infusion study, the subjects collected 24-hour urine specimens that were analyzed for urea, creatinine, protein and albumin.

Patients and volunteers agreed to participate after informed consent in accordance with the Helsinki Declaration of Human Rights. The study was approved by the Institutional Ethical Committee for studies in Humans.

Materials

L-[1-^{13}C] valine (isotope mole fraction > 0.99; MassTrace, Woburn, MA, USA) was dissolved in sterile 0.9% saline and sterilized through a 0.22 μm filter. All chemicals were obtained from Riedel de Haën (Seelze, Germany) unless otherwise indicated.

Infusion protocol

The subjects came to the research unit in the morning after a 12 hour fast. Bilateral cubital arm veins were cannulated for blood sampling and for injection of Evans blue and the labeled valine. No food was given during the tracer infusions, and subjects were only allowed to drink water. After the subjects were in supine position for at least 30 minutes, baseline blood samples were taken and an i.v. dose of evans blue was given. After 10, 15, 20, 30, 45 and 60 minutes, samples were taken to measure the Evans blue distribution volume, an index for plasma volume [20]. At t = 0, a priming dose of 15 μmol/kg L-[1-^{13}C] valine was administered intravenously over two minutes, followed by a continuous infusion of 15 μmol/kg/hr L-[1-^{13}C] valine during six hours. Blood samples were collected into heparine-containing tubes and into citrate containing tubes. Samples were taken from the contralateral arm at indicated intervals (Fig. 1) and kept on ice (maximum 1 hr) until plasma was separated by centrifugation (20 min, 3000 × g, 4°C). Plasma samples were stored at −20°C until further preparation. During the infusion period, 11 samples were drawn for determination of the labeled/unlabeled ratio of valine (tracer/tracee ratio) in plasma and for the determination of the tracer/tracee ratio in albumin and fibrinogen.

Biochemical analysis

Isolation of plasma amino acids from plasma and proteins. Free amino acids in plasma were isolated from 750 μl heparin plasma by deproteinization with 750 μl 20% trichloric acetic acid (TCA). After centrifugation (10 min, 2000 × g, room temperature) the supernatant was directly applied to cation-exchange columns as described below.

Albumin was isolated from plasma based on differential solubility in absolute ethanol from TCA-precipitated proteins according to the method of Korner and Debro [21]. Briefly, 0.5 ml heparin plasma was deproteinized with 0.5 ml 20% TCA. After centrifugation (10 min, 2000 × g, room temperature), the pellet was redissolved in 1 ml ethanol. After a second centrifugation, an aliquote of the supernatant was used to monitor protein purity and identification, using a 4 to 15% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gradient and visualization by silver staining. Electrophoretically obtained bands

were compared with commercially obtained human albumin (Behringwerke AG, Marburg, Germany). The purified albumin exhibited as a single band corresponding to human albumin (not shown). For quantification of albumin a Lowry method [22] was used. Recovery of the isolation procedure was about 80%. To remove free amino acids, a second aliquot of the ethanolic protein solution containing 300 μg protein was evaporated under N_2 at 45°C until dry. The residue was redissolved in 1 ml 0.3 M NaOH at 37°C for 30 minutes. The albumin was reprecipitated with 1 ml 2 M $HClO_4$ and subsequently with 1 ml 0.2 M $HClO_4$ at 4°C before hydrolysis.

Fibrinogen was purified from citrate plasma based on the method of Vila et al [23]. Briefly, 0.5 ml plasma was added to 0.5 ml 80 g/liter polyethylene glycol 6000 (Merck, Darmstadt, Germany). The mixture was placed on ice and gently shaken for 10 minutes. After centrifugation (10 min, 1800 $\times$ g at 4°C) the precipitate was redissolved in 1 ml phosphate buffer (0.01 M Na_2HPO_4; BDH Chemicals Ltd, Poole, UK), 0.15 M NaCl, pH 7.4) and subsequently precipitated with 0.5 ml 2 M 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 M Na_2HPO_4, pH 7.8, 200,000 U/liter aprotinin; Sigma Chemical, St. Louis, MO, USA), and precipitated with 330 μl $(NH_4)_2SO_4$ (4 mol/liter). After centrifugation the pellet was redissolved in 1 ml phosphate buffer (0.018 M Na_2HPO_4, 200,000 U/liter aprotinin, pH 7.8). A part of the isolated fibrinogen was used to monitor purity and identification using the same gel system as described for albumin. Electrophoretically obtained bands were compared with commercially obtained human fibrinogen (American Diagnostica Inc., Greenwich, CT, USA). Purified fibrinogen showed two electrophoretic bands, corresponding to the high and low molecular weight form of fibrinogen (Fg1 = 340,000 Da and Fg2 = 320,000 Da). To verify the purity of fibrinogen we also reduced the isolated fibrinogen. Samples were prepared by mixing 10 μl purified fibrinogen with 40 μl of a reducing buffer containing 5% SDS, 10% β-mercaptoethanol, 5% glycerol, and 0.2% bromophenol blue and heating the mixture for five minutes at 100°C. The reduced firinogen showed three electrophoretic bands with a molecular weight of 67,000, 58,000 and 47,000 Da that can be assigned to fibrinogen Aα, Bβ and γ chains. For quantification of the fibrinogen a Lowry method [22] was used. Recovery of this isolation procedure was about 80%. Subsequently, an aliquot of the solution containing 300 μg of the fibrinogen was precipitated by adding 1 ml 2 M $HClO_4$. After centrifugation the precipitate was used for hydrolysis.

Hydrolysis of both albumin and fibrinogen was done in 0.5 ml 6 N HCl for 24 hours at 110°C in sealed tubes [24]. The hydrolysates and the supernatant after deproteinization of plasma were applied to columns containing 2 ml of the cation-exchange resin, AG 50W-X8 (200 to 400 mesh), H^+ form (Bio-Rad Laboratories, Richmond, CA, USA), that had been prewashed with aqua-dest. After washing with 10 ml of aqua-dest, the aminoacids were eluted with 6 ml of 6 N NH_4OH. The eluate was dried at 45°C under N_2 and the dried samples were kept at 4°C until derivatization.

Determination of ^{13}C-valine enrichment. The N(O,S)-methoxycarbonyl methyl esters of the isolated amino acids were prepared according to the method of Hušek [25]. For each series of analyses a standard curve was used, prepared by disolving weighted amounts of L-[1-^{13}C] valine (infused tracer) and commercially available L-valine in aqua-dest. Two standard curves were made, one ranging between 0 and 0.25 tracer/tracee molar ratio and one ranging between 0 and 0.025 tracer/tracee molar ratio. The solutions were divided into aliquots and dried under a stream of nitrogen and stored at 4°C until derivatization. The N(O,S)-methoxycarbonyl methyl ester derivative of plasma-free aminoacids was analyzed by GC/MS on a Hewlett-Packard HP 5890 type II gas chromatograph interfaced to a JEOL JMS AX 505 mass spectrometer (JEOL, Tokyo, Japan). Separation of derivatized L-valine from other derivatized amino acids was achieved on a CP Sil 19CB capillary column (Chrompack, Middelburg, the Netherlands). The column effluent was ionized by chemical ionization with NH_3, and resulting positively charged fragments were monitored at m/z 190 and m/z 191. Each sample was analyzed in triplicate (CV $<$ 5%).

Aminoacids isolated from hydrolysates of fibrinogen and albumin were analyzed as their N(O,S)-methoxycarbonyl methyl ester derivative by GC/C/IRMS on a Varian 3400 gas chromatograph, equipped with a CuO combustion oven and interfaced to a DELTA S isotope ratio mass spectrometer (Finnigan-MAT, Bremen, Germany). Baseline separation of derivatized L-valine from other derivatized amino acids, essential in this method, was achieved on a CP Sil 19CB capillary column. The column effluent was combusted and the resulting CO_2 was monitored at m/z 44 and 45. This GC/C/IRMS method resembles a comparable GC/C/IRMS method published by Yarasheki et al [26] that was developed for the N-acetyl n-propyl ester of leucine.

Pre-infusion measurements. Pre-infusion plasma concentrations of urea, creatinine, and albumin were measured with standard laboratory methods on a Ektachem 950 (Johnson & Johnson, Clinical Diagnostics, NY, USA). The measurement of albumin was based on the broomcresol green method. C-reactive protein (CRP), urinary urea, urinary creatinine proteinuria and albuminuria were measured with standard laboratory methods on a Hitachi-911 (Boehringer, Mannheim, Germany). The colloid osmotic pressure (COP) was measured using a 4400 colloid osmometer (Wescor Inc., Logan, UT, USA). Fibrinogen was measured using the Von Clauss method [27]. Serum amyloid A (SAA) was measured using an ELISA (Hemagen Diagnostics, Waltham, MA, USA) and α_2-macroglobulin was measured using a routine nephelometric method (Behringwerke AG, Marburg, Germany).

Calculations and statistics

The mass spectrometry intensity ratios, either at m/z 191 and m/z 190 for the GC/MS measurements or at m/z 45 and m/z 44 for the GC/C/IRMS measurements, were converted to tracer/tracee molar ratios by means of the appropiate calibration curves depicting the relevant intensity ratio as a function of the molar ratio of weighed amounts of L-[1-^{13}C] valine/L-valine. For the GC/MS measurements the calibration curve ranged from 0 to 0.25 tracer/tracee molar ratio and for the GC/C/IRMS measurements the calibration curve ranged from 0 to 0.025 tracer/tracee molar ratio was used. The fractional synthesis rate (FSR) of albumin and fibrinogen were calculated from the tracer/tracee data [28] according the formula and guidelines discussed by Foster et al [29] and Parhofer et al [30]: the data were fitted to $A(t) = A_p\,[1 - e^{-k(t-d)}]$, where A(t) is the tracer/tracee ratio of valine at time t (in hours), A_p is the steady-state plasma tracer/tracee ratio of

Table 1. Biochemical parameters in plasma and urine of nephrotic patients and controls

	Patients ($N = 7$)	Controls ($N = 8$)	P value
Plasma urea *mmol/liter*	7.7 ± 1.3 (4.1–14.3)	3.7 ± 0.3 (2.4–4.8)	<0.01
Plasma creatinine *μmol/liter*	121 ± 12 (89–164)	75 ± 4 (60–89)	<0.01
Plasma albumin *g/liter*	22.3 ± 0.7 (19.9–24.4)	37.3 ± 0.8 (33.7–40.2)	<0.0001
Plasma COP *mm Hg*	11.2 ± 0.4 (9.5–12.3)	24.6 ± 0.5 (22.4–26.2)	<0.0001
Plasma fibrinogen *g/liter*	6.5 ± 0.5 (4–8.04)	3.1 ± 0.2 (2.2–4.1)	<0.0001
Plasma α_2-macroglobulin *g/liter*	3.69 ± 0.51 (2.0–6.1)	1.61 ± 0.09 (1.3–2.0)	<0.0001
Plasma SAA *mg/liter*	9.7 ± 2.8 (3.0–27.0)	7.1 ± 1.4 (1–11.5)	NS
Plasma volume *ml/kg*	40 ± 2 (34–46)	40 ± 2 (32–46)	NS
Proteinuria *mg/kg/day*	139 ± 22 (80–240)	<1.5	<0.0001
Albuminuria *mg/kg/day*	111 ± 16 (53–182)	ND	<0.0001
Creatinine clearance *ml/min*	86 ± 7 (62–109)	131 ± 11 (84–177)	<0.01
Urinary urea *mmol/kg/day*	3.4 ± 0.3 (1.7–4.6)	4.3 ± 0.3 (2.8–5.7)	NS

Values are expressed as mean ± SEM (range). Abbreviations are: ND, not done, NS, not significant; COP, colloid osmotic pressure; SAA, serum amyloid A. P values were determined by t-test.

Table 2. Fibrinogen pool and fractional synthesis rate (FSR) and absolute synthesis rate (ASR) of fibrinogen and albumin in nephrotic patients and controls

	Patients ($N = 7$)	Controls ($N = 8$)	P value
Fibrinogen pool *mg/kg*	271 ± 30 (136–370)	126 ± 8 (90–157)	<0.0001
Fibrinogen FSR *%/day*	11.6 ± 0.9 (8.9–14.7)	16.9 ± 0.6 (13.9–18.5)	<0.0001
Fibrinogen ASR *mg/kg/day*	31 ± 3 (23–45)	21 ± 1 (14–27)	<0.05
Albumin FSR *%/day*	17.1 ± 1.3 (12.7–22.0)	4.8 ± 0.3 (3.3–6.4)	<0.0001
Albumin ASR *mg/kg/day*	160 ± 19 (107–241)	71 ± 4 (53–88)	<0.0001

Values are expressed as mean ± SEM (range).
P values were determined by t-test.

valine, d is a delay time and k represents the FSR of the protein. Calculations were done using SPSS software. 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 (wt), these quantities are expressed per kg body wt.

The significance of differences was assessed by Student's t-test. Data are expressed as means ± SEM and means of the difference with 95% confidence intervals.

RESULTS

Baseline data

The plasma values of urea, creatinine, albumin, COP, fibrinogen, α_2-macroglobulin and SAA of patients and controls are shown in Table 1. Judged from plasma urea and creatinine, the patients had mildly disturbed renal function. Plasma albumin and COP were decreased, plasma fibrinogen and α_2-macroglobulin were about twice normal, whereas plasma SAA was normal. C-reactive protein (CRP), an index of inflammation, was also in the normal range (< 5 mg/liter) in both patients and controls. Twenty-four-hour urinary collections showed that the nephrotic patients displayed considerable proteinuria. Urine urea excretion, as an index of protein intake, was not significantly different from that in the control subjects. Plasma volume was also comparable.

Synthesis rate of fibrinogen and albumin in patients and control subjects

The absolute synthesis rate (ASR) of fibrinogen was significantly increased by about 50% in the patients compared to the control subjects (Table 2). By inference, assuming steady state and no external loss, fibrinogen catabolism was increased to the same extent. However, the fibrinogen pool was increased disproportionately. Therefore, the FSR of fibrinogen (and, by inference, its fractional catabolic rate, FCR) was significantly decreased to 11.6 ± 0.9%/day, compared to measurements in the control subjects, 16.9 ± 0.6%/day ($P < 0.0001$). The ASR of albumin was twice that measured in control subjects. Different from the FSR of fibrinogen, the FSR of albumin was greatly increased.

Correlations

Considering the data in controls and patients together, several correlations were found. Importantly, the ASR of albumin correlated with the ASR of fibrinogen ($r^2 = 0.79$, $P < 0.0001$; Fig. 2). Further, the ASR of fibrinogen correlated directly with plasma concentration of fibrinogen ($r^2 = 0.70$, $P = 0.0001$; Fig. 3) and negatively with plasma albumin ($r^2 = 0.39$, $P = 0.0127$) and COP ($r^2 = 0.40$, $P = 0.0115$). The ASR of albumin correlated negatively with plasma albumin concentration ($r^2 = 0.56$, $P = 0.0014$; Fig. 4), and COP ($r^2 = 0.59$, $P = 0.0008$) and positively with plasma fibrinogen concentration ($r^2 = 0.75$, $P < 0.0001$) and plasma α_2-macroglobulin concentration ($r^2 = 0.82$, $P < 0.0001$), but not with other acute phase proteins, SAA and CRP.

Given the large variation in ASR of albumin and fibrinogen in the patient group (Fig. 2), we also evaluated correlations within this group separately. Again, there was a strong correlation between the ASR of albumin and the ASR of fibrinogen ($r^2 = 0.83$, $P = 0.0046$), and positive correlations were found between the ASR of fibrinogen and plasma fibrinogen concentration ($r^2 = 0.64$, $P = 0.0317$). Within this group, no correlations were found between the ASR of albumin and plasma albumin concentration, COP, plasma fibrinogen concentration, plasma α_2-macroglobulin concentration, and proteinuria. More importantly, there was a wide range in ASR of albumin in the patient group despite a

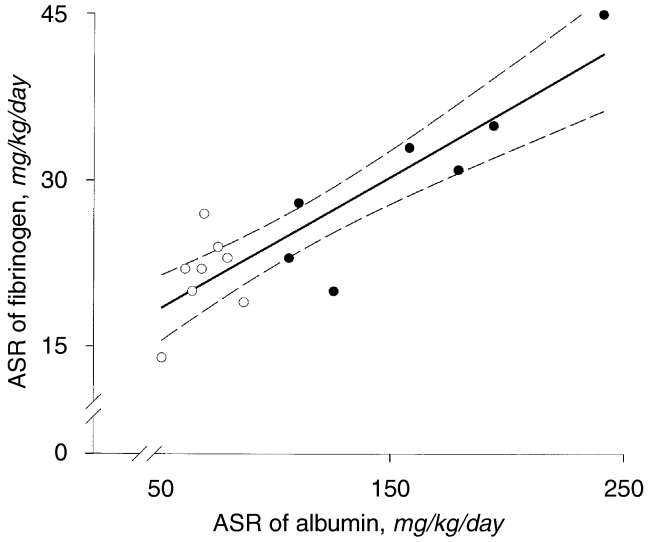

Fig. 2. Relationship between the absolute synthesis rate (ASR) of fibrinogen (mg/kg/day) and the ASR of albumin (mg/kg/day) for nephrotic patients (●, $N = 7$) and controls (○, $N = 8$). The 95% confidence limits of the entire group are shown on either side of the regression line. $r^2 = 0.79$, $P < 0.0001$ ($N = 15$).

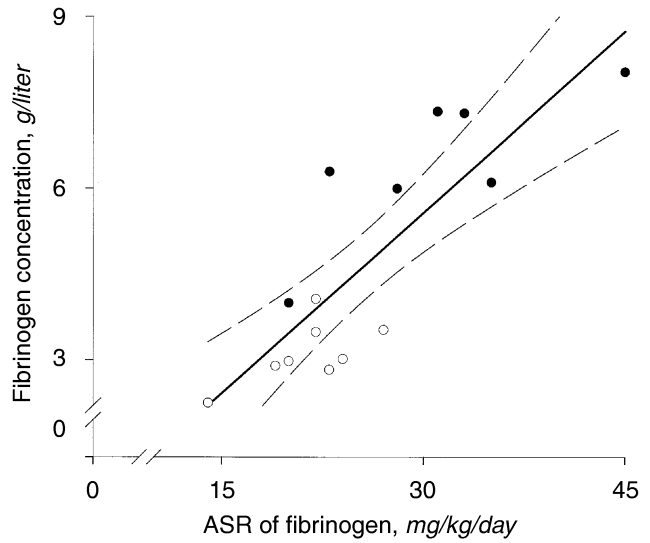

Fig. 3. Relationship between plasma fibrinogen concentration (g/liter) and the absolute synthesis rate (ASR) of fibrinogen (mg/kg/day). The 95% confidence limits of the whole group are shown on either side of the regression line. $r^2 = 0.70$, $P = 0.0001$ ($N = 15$). Symbols are: (○) controls, $N = 8$; (●) patients, $N = 7$.

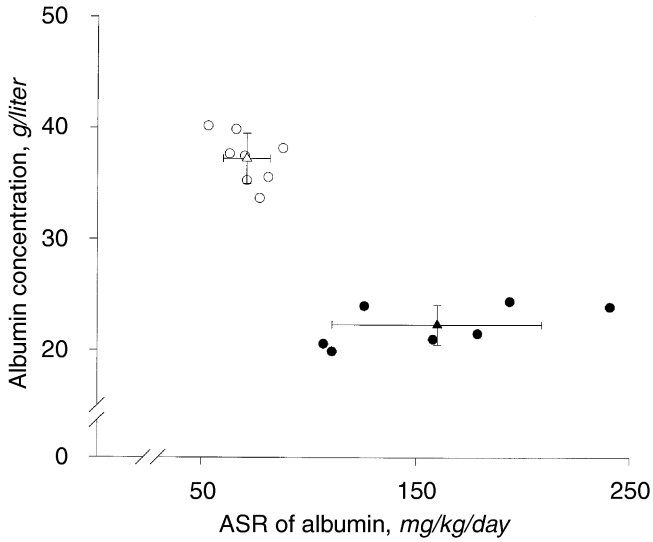

Fig. 4. Absolute synthesis rate (ASR) of albumin (mg/kg/day) and its plasma concentration in both controls (○ $N = 8$) and patients (●, $N = 7$). The error bars indicate ± 1 SD.

relatively narrow range in plasma albumin concentrations (Fig. 4). Plasma CRP and SAA concentrations were normal in the patient group and did not correlate with the ASR of albumin.

DISCUSSION

The present study clearly demonstrates that whereas plasma albumin is reduced and plasma fibrinogen is increased in the nephrotic syndrome, the synthesis rate of both proteins is increased and interrelated. This implies that in the nephrotic syndrome synthesis of albumin and fibrinogen is probably regulated by a common signal. We also found that in the nephrotic subjects the plasma fibrinogen concentration is directly correlated to the absolute synthesis rate of fibrinogen. The FSR of fibrinogen and thus the fractional catabolic rate (FCR) (assuming steady state and no external loss) is decreased compared to controls, suggesting that a reduced FCR of fibrinogen also contributes to the increased plasma levels of this protein.

In this study, albumin and fibrinogen synthesis were determined by using a primed/continuous infusion model and GC/C/IRMS as the detection technique. There are only few reports on measurements of albumin and fibrinogen synthesis rates in nephrotic patients. Some investigators used decay curves of I-labeled proteins [31, 32]. However, using decay curves is not ideal, since I-labeled protein may be denaturated and the labeling itself may contribute to modification of the protein. Measurement of synthesis with use of a flooding dose [17] also has limitations, since administration of large dosages of aminoacid may directly stimulate protein synthesis [33]. We therefore used a primed/continuous infusion model. Instead of the commonly used leucine, we used L-[1-^{13}C] valine as the stable isotope. Valine does not stimulate insulin, a well known factor stimulator of protein synthesis *in vivo* [34, 35]. Other advantages of valine are that it is the most soluble essential aminoacid, and that protein valine content is generally high. Separation of valine from the other aminoacids by gas chromatography is technically feasable, whereas leucine and isoleucine cannot be easily separated. The presently calculated FSR of fibrinogen (16.9 ± 0.6%/day) and FSR of albumin (4.8 ± 0.3%/day) in normal subjects are lower than generally published [34–43]. However, this is an artifact since we used the tracer/tracee ratio of valine in plasma *per se*, whereas others made a correction for the presumed lower ratios in the cell compartment [33, 44].

To our knowledge, this is the first study reporting direct measurement of fibrinogen synthesis in nephrotic patients. Takeda and Chen [31] evaluated fibrinogen decay with ^{131}I-labeled fibrinogen in patients with the nephrotic syndrome. Since

the catabolic rate was increased, they supposed that the increase in plasma fibrinogen must be due to an increased synthesis. The present data firmly establish that plasma fibrinogen is increased due to increased synthesis of fibrinogen. Since the relatively large fibrinogen molecule is not filtered in the diseased glomerulus in the nephrotic syndrome, there is no urinary loss of this protein [11]. This makes it likely that fibrinogen catabolism is increased to the same extent. Increased fibrinogen catabolism is compatible with increased fibrinogen use in the nephrotic syndrome, for which our data thus give indirect support. Interestingly, the FSR of fibrinogen in this study was lower than in normal subjects. This implies that the increase in the fibrinogen pool in nephrosis cannot be fully explained by increased synthesis of fibrinogen. Assuming that fractional synthesis and catabolism of fibrinogen are in equilibrium, our data suggest that a decrease in fractional fibrinogen catabolism also contributes to the hyperfibrinogenemia of the nephrotic syndrome.

The observed increase in albumin synthesis in this patient group is in agreement with findings in both patients [14, 17, 32] and animals [15, 16, 45–47] with the nephrotic syndrome. Our observation that there is no relationship between albumin synthesis rate and plasma albumin concentration in the patient group is similar to what has been found by others [14, 17, 32]. However, a wide range in (elevated) synthesis rates of albumin was found, despite about similarly (decreased) plasma albumin concentrations. This suggests that: (*1*) the net albumin loss contributed variably to the fall in plasma albumin, even though the resulting plasma albumin concentrations were similar; (*2*) albumin synthesis was increased to its maximal level in each patient, and this homeostatic mechanism failed to normalize albumin plasma concentrations, even though the extravascular albumin pool and thus the effective albumin distribution are strongly decreased [32]; and (*3*) other factors besides plasma albumin or COP influence the albumin synthesis rate in nephrotic syndrome [17, 48]. We did not find a relationship between proteinuria and albumin synthesis rate in the nephrotic patients. On the other hand, a positive correlation between proteinuria and the albumin synthesis rate has been found in both nephrotic humans [49] and rats [15] by estimation of albumin synthesis from ^{125}I albumin disappearance. Since the latter measurements were made at steady state over many days we have to consider that the presently used method, which measures synthesis for only six hours, does not take into account possible diurnal variations.

An important conclusion that we can draw independently of any possible disturbing affect of diurnal variations in synthesis rates is that in the nephrotic syndrome the synthesis of fibrinogen and albumin are increased proportionally. This is in contrast with the increased fibrinogen synthesis in type I diabetes mellitus, which has been found to vary inversely with that of albumin [35]. In the case of fibrinogen, the increased synthesis rate is not a response to a changed plasma level, as might be hypothesized for albumin, but instead increased plasma fibrinogen concentrations are a consequence of the increased synthesis. That plasma α_2-macroglobulin concentrations are also increased and correlate with the ASR of albumin suggests that synthesis of this protein may also be increased, and reflects a more global change in the pattern of protein synthesis and secretion by the liver in the neprotic syndrome. With regard to increased synthesis rates of both albumin and fibrinogen, this change represents a reversal of the relationship between the synthesis rate of these two proteins (which is usually reciprocal and not proportional [35]). Apparently, the nephrotic syndrome is an instance in which syntheses of both a positive and a negative acute phase protein are increased in concert.

Neither CRP (115 to 140 kDa) nor SAA (220 to 235 kDa) levels were increased in the nephrotic patients. While it might be possible that, like albumin, their synthesis rates are increased but fail to replace urinary losses, we feel this explanation to be unlikely. Serum amyloid A (SAA) does not circulate in its free form, but is tightly bound to high density lipoprotein (HDL) [50]. While small amounts of HDL are lost in the urine in the nephrotic syndrome [51], this most likeley represents reduction in renal processing of filtered apo A-I and not net renal filtration and loss of HDL [52]. Thus, the opportunity for increased renal losses of SAA is not great. Second, unlike either albumin or fibrinogen, the concentrations of both of these proteins are capable of increasing by several orders of magnitude when synthesis is stimulated [53]. Therefore, normal levels of SAA and CRP in nephrotic patients most likely reflect unchanged hepatic secretion rates. Based on these considerations, we speculate that in the nephrotic syndrome hepatic secretion of a specific cohort of proteins is stimulated, probably by a similar signal.

Some caution is required concerning the younger age in the control subjects of our study, and the possible effects of age on protein synthesis. As regards albumin synthesis, it has been shown in a study using stable isotopes that, whereas the serum albumin diminished slightly with age, FSR and ASR did not differ between young adults and elderly subjects [54]. Plasma levels of fibrinogen are related to age in adults, and show an increase of 3 to 10%/10 years [55–56]. Whether this is due to an increased synthesis or decreased catabolism is not known. Nonetheless, it is unlikely that the observed 50% increase in fibrinogen synthesis and doubling of plasma fibrinogen in the nephrotic subjects reflect a physiological age-related synthesis increase.

Plasma fibrinogen concentration in the nephrotic syndrome draws much attention since epidemiological studies have shown that a high plasma fibrinogen concentration is an independent risk factor for atherosclerotic disease [57–61] and a predictor of mortality in patients with coronary heart disease [62]. Elevation of the plasma fibrinogen level is a consistent and significant abnormality observed in patients with the nephrotic syndrome [63–65] and these increased concentrations are associated with hypercoagulability, hyperviscosity and increased platelet aggregation [66]. In addition, increased plasma fibrinogen concentrations may contribute to the progression of renal disease [9]. There are only a few drugs successful in decreasing plasma fibrinogen [67–68], and no data are yet available on whether such drugs are beneficial in the nephrotic syndrome. Insight in the signal that causes hyperfibrinogenemia may add to further leads for development of rational therapy. Two clinically important consequences arise from our observations. First, finding a relationship between the ASR of fibrinogen and albumin in patients with the nephrotic syndrome may be relevant for interventions that will augment albumin synthesis. For example, increased dietary protein intake, which may augment the albumin synthesis rate in the nephrotic patient [16], may also increase fibrinogen synthesis and therefore the plasma fibrinogen concentration. Thus, strategies aimed at increasing the albumin synthesis rate may have an unfavorable side effect compared to strategies aimed at reducing the defect in glomerular permselectivity. Second, our data suggest that plasma

fibrinogen may be a better marker for albumin synthesis (and thus total albumin loss) than proteinuria. Whether that also holds true for mild proteinuria remains to be seen, but if it does, then elevated plasma fibrinogen concentrations found in patients with only mild proteinuria can be interpreted as an indication that net renal albumin loss is greater than indicated by the proteinuria.

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APPENDIX

Abbreviations used in this article are: ASR, absolute synthesis rate; GC/C/IRMS, gas chromatography combustion isotope ratio mass spectrometry; SAA, serum amyloid A; CRP, C-reactive protein; TCA, trichloric acetic acid; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; COP, colloid osmotic pressure; FSR, fractional synthesis rate; FCR, fractional catabolic rate; HDL, high density lipoprotein.

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