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Effects of dietary protein restriction on fibrinogen and albumin metabolism in nephrotic patients

Mauro Giordano, Pierpaolo De Feo, Paola Lucidi, Emanuela dePascale, Gelsomina Giordano, Domenico Cirillo, Giuseppe Dardo, Salvatore Santo Signorelli, and Pietro Castellino

Istituto di Clinica Medica "L. Condorelli," University of Catania, Catania; Dipartimento di Medicina Interna, Scienze Endocrine e Metaboliche, University of Perugia, Perugia; and Istituto di Medicina Interna e Nefrologia, Second University of Naples, Naples, Italy

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Background. Nephrotic syndrome (NS) is characterized by profound changes in albumin and fibrinogen levels. Dietary protein restriction has been advocated in the treatment of patients with NS, but its effects on albumin and fibrinogen metabolism have not been fully elucidated.

Methods. We evaluated the effects of dietary protein restriction on endogenous leucine flux (ELF), fibrinogen and albumin metabolism in seven patients with NS who consumed either a normal protein diet (NPD; 1.20 ± 0.06 g/kg/day), or a low protein diet (LPS; 0.66 ± 0.04 g/kg/day) for four weeks. Seven normal subjects served as controls. The postabsorptive ELF value, fractional synthesis rate (FSR) and absolute synthesis rate (ASR) of both albumin and fibrinogen were evaluated during the last 120 minutes of a five-hour 5,5,5-D3-L-leucine infusion.

Results. During the NPD regimen. ELF was increased, serum albumin was reduced, plasma fibrinogen was increased, albumin FSR and ASR were both increased, fibrinogen FSR was normal, and fibrinogen ASR was greater in patients with NS compared to controls. In patients with NS the LPD regimen reduced proteinuria, ELF, albumin FSR and ASR, plasma fibrinogen levels, fibrinogen ASR, and increased serum ulbumin levels. Dietary-induced changes in albumin and fibrinogen synthesis were significantly correlated ($r = 0.719$, $P < 0.05$).

Conclusions. Patients with NS treated with LPD show: (*1*) a reduction of proteinuria, albumin ASR and FSR, with an increase in serum albumin levels and its intravascular pool; (*2*) a decrease of fibrinogen ASR, with a reduction in both plasma fibrinogen levels and intravascular pool; and (*3*) a reduced rate of whole body proteolysis.

Key words: proteinuria, nephrotic syndrome, chronic renal disease, progressive renal disease, hyperfibrinogenemia.

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The nephrotic syndrome is a complex dysmetabolic renal disease. Principal features of the syndrome include proteinuria (>3.5 g/day) and albuminuria and hypoalbuminemia. In contrast to albumin, fibrinogen is a protein with a high molecular weight that is not filtered by the damaged glomerulus of nephrotic patients. Hyperfibrinogenemia is often observed in these patients, and it can actively contribute to hypercoagulability, hyperviscosity and increased platelet aggregation [1]. High fibrinogen levels may also accelerate the progression of renal disease [2]. The rate of albumin synthesis is increased in nephrotic subjects [3], and it has been recently demonstrated that the synthesis of fibrinogen is increased proportionally to that of albumin [4]. In addition, in rats with experimental nephrotic syndrome, hepatic levels of mRNAs encoding for fibrinogen and albumin are both stimulated [5].

Few data are available on the mechanisms of action and metabolic effects of therapeutic regimens that may concomitantly increase serum albumin levels and decrease plasma fibrinogen concentration. In this regard, the optimal dietary protein intake for nephrotic patients is still a matter of discussion. Since nephrotic syndrome is associated with chronic protein depletion, high dietary protein intake has been advocated to avoid protein malnutrition [6, 7]. However, when nephrotic patients were fed a high protein diet, urinary protein excretion increased without a concomitant rise in serum albumin concentration [6, 7]. On the contrary, in patients with established nephrosis and preserved glomerular filtration rate (GFR), a low protein diet induced a decline in urinary albumin excretion and an improvement in serum albumin concentration with a reduction in albumin synthesis [8]. These changes were associated with a decline in hepatic albumin synthesis.

The effects of changes in dietary protein intake on

fibrinogen synthesis and plasma fibrinogen levels have not been investigated.

The present study therefore was undertaken to determine whether dietary protein restriction may induce coordinate and potentially advantageous changes in albumin and fibrinogen metabolism in patients with nephrotic syndrome.

METHODS

Patient population

Seven healthy normal volunteers (controls; 4 males and 3 females; age 37 ± 3 years; height 172 ± 4 cm; weight 68 ± 4 kg, ideal body weight 107 ± 4%) and seven patients with nephrotic syndrome (4 males and 3 females; age 39 ± 3 years; height 166 ± 3 cm; weight 68 ± 6 kg, ideal body weight 108 ± 8%) who were referred to our unit for transcutaneous renal biopsy participated in the study protocol. Eligibility criteria of nephrotic patients included: 20 to 50 years of age, urinary protein excretion >3.5 g/24 h, and no evidence of endocrine or other major organ system disease, as determined by medical history, physical examination, and routine laboratory tests. Renal biopsy specimens were evaluated prior to their inclusion in the study. The etiology of renal diseases were as follows: membranoproliferative glomerulonephritis ($N = 3$), membranous nephropathy ($N = 2$), amyloidosis ($N = 1$), and focal segmental glomerulosclerosis ($N = 1$). Other than vitamins, patients did not take any medication for the entire duration of the study. The purpose and potential risks of the study were explained to all subjects, and their voluntary written consent was obtained before their participation.

Experimental protocol

Control subjects participated in one study and were instructed to consume a weight-maintaining diet providing about 35 to 38 Cal/kg/day and containing about 250 to 300 g of carbohydrates and 1.1 g/kg/day of protein for at least seven days prior to their participation in the study.

Patients with established nephrosis participated in two separate experimental protocols performed at a four- to five-week interval after they had been maintained on each of the two different dietary regimens for about four weeks (27 ± 1 days). On the first dietary regimen (normal protein diet, NPD) patients were instructed to consume a weight-maintaining diet providing about 35 to 38 Cal/kg/day and containing 1.1 g of protein/kg/day. For the second dietary regimen (low protein diet, LPD) patients were instructed to consume a similar caloric intake, but the dietary protein was reduced to 0.55 g/kg/day and more than 65% of the ingested protein was of a high biologic value. In addition, during both dietary regimens, patients received 1 gram of dietary protein intake for each gram of daily protein excretion. The amount of dietary protein provided to replace urinary protein excretion was maintained constant during both dietary regimens. The NPD dietary carbohydrates and lipids represented 55 and 25% of the total caloric intake, respectively. The LPD contribution to total calories was increased to 60 and 30%, respectively. During the NPD regimen, dietary phosphate and calcium intake were 1490 ± 65 and 1021 ± 92 mg, respectively, and decreased to 844 ± 61 and 730 ± 85 mg after the LPD period. To verify compliance to the diet, during each four-week study period all patients were invited to visit our clinical unit weekly with their dietary diary, and a 24-hour urinary specimen was obtained to determine urinary protein and nitrogen excretion levels.

Metabolic studies were performed with the subject in a post-absorptive state after a 12-hour overnight fast. In nephrotic patients the metabolic analysis was performed after each four-week dietary period, which were started in random order and completed by all patients. At the end of the dietary periods two consecutive, 24-hour urinary collections also were obtained to determine urinary protein excretion. On the day of the study, an 18-gauge polyethylene catheter was inserted into an antecubital vein for the infusion of all test substances, and a second catheter was placed retrogradely into a wrist vein for blood sampling. The hand was kept in a heated box at 60°C to densure arterialization of the venous blood. At 08:00, a prime (0.6 mg/kg bolus) continuous (1.2 mg/kg/h) infusion of 5,5,5-D3-L-leucine (Mass Trace, Woburn, MA, USA) was started and continued for five hours by a Harvard syringe pump (Harvard Apparatus, Ealing, South Natick, MA, USA). Ten milliliters of blood were collected at −15, 0, 180, 210, 240, 270 and 300 minutes to measure the plasma concentration and enrichment of leucine, α-ketoisocaproic acid (KIC) and the enrichment of D3-leucine bound to plasma albumin and fibrinogen. At the end of the continuous infusion of leucine period, plasma volume was determined by the Evans blue dye dilution method. Briefly, a bolus of approximately 4 mL of 0.9% NaCl solution containing 5 mg/mL of sterile, pyrogen-free Evans blue dye (BDH Laboratory Supplies, UK) was injected into an antecubital vein. Blood was drawn every ten minutes from 10 to 60 minutes to measure the Evans blue dye in the serum. In nephrotic patients, during the second hour of the leucine infusion period, respiratory exchange measurements also were performed by continuous indirect calorimetry for 45 minutes. Briefly, a plastic ventilated hood was placed over the head of the subject and made air tight around the neck. A slightly negative pressure was maintained in the canopy to avoid loss of the expired air, and the carbon dioxide and oxygen content of the expired air were continuously measured.

Analytical determinations

Leucine and KIC were extracted from plasma samples as previously described [9]. Enrichments and concentrations of plasma leucine and KIC were determined on their t-butyldimethylsilyl derivatives using gas chromatography-mass spectrometry (GCMS) in the electron impact (EI) ionization mode (GC8000, MS Voyager Finnigan; ThermoQuest Italia, Milan, Italy), monitoring the ions 302 and 305 for leucine and 301 and 304 for KIC [10]. Plasma albumin and fibrinogen were purified as previously described in detail [11, 12]. To evaluate plasma volume, serum samples were added with an equal volume of ~4000 D, polyethyleneglycol (24 g/dL; J.T. Baker, Deventer, Netherlands) solution for precipitation of non-albumin proteins. Samples and standards were centrifuged for 10 minutes at 3000 rpm. Supernatants from samples and standards were then read at 620 nm of wavelength [13] using a spectrophotometer (Ciba-Corning Diagnostics Limited, Halstead, Essex, UK). Serum albumin concentration was determined by a standard bromcresol green method [14] (ALB plus, Roche Diagnostics, Mannheim, Germany) on a Hitachi 747 (Hitachi, Milano, Italy). Plasma chronometric determination of fibrinogen was obtained in citrate plasma using the clotting method of Clauss [15] on Hemolab Fibrinomat (bioMèrieux SA, Lyon, France). Urinary protein excretion was measured on 24-hour urine samples using a modification of Coomassie Brilliant Blue method (Total Protein Test Kit; Bio-Rad Laboratories SRL, Milan Italy) [16]. Urinary albumin excretion was measured in 24-hour urine samples using an immunoturbidimetric assay (Tina-quant Albumin; Roche Diagnostics, Mannheim, Germany) on a Hitachi 911 analyzer.

Calculations and statistics

The enrichments of leucine and KIC were expressed as the tracer-to-tracee ratio (TTR), accounting for isotopomer skewed distribution and spectra overlapping when appropriate. Whole body leucine flux was calculated as the rate of appearance (Ra; μm/kg/min) of leucine as follows: $Ra = I/Ep$, where I is the isotope infusion rate of leucine, and Ep is the plasma enrichment (TTR) of KIC. Estimates of the whole-body leucine kinetic were determined from the data obtained during the last two hours of the study (from 180 to 300 min) at the isotopic and metabolic steady state [17]. Albumin and fibrinogen fractional synthesis rate (FSR) were calculated by dividing the slope of the increase in the enrichment of leucine bound to albumin or fibrinogen by the enrichment of plasma KIC over the last two hours of the study. The absolute intravascular albumin and fibrinogen synthesis rates (ASRs) were estimated multiplying albumin or fibrinogen FSR by the total intravascular albumin or fibrinogen content. To evaluate plasma volume, after the Evans Blue dye injection the concentration at time zero was extrapolated. The estimated concentration at time zero was used to calculate plasma volume by the standard dilution formula: PV (mL) = dose of EBD (μg) injected/ serum concentration of EBD (μg/mL) extrapolated at time zero [13]. Dietary protein intake in nephrotic patients and compliance with the diet were evaluated from weekly determinations of 24-hour urinary nitrogen excretion according to the formula: urinary nitrogen = urine urea nitrogen + nonurea nitrogen, where 1 g is urinary nitrogen = 6.25 g of protein and nonurea nitrogen excretion = 30 mg/kg/day [18]. Urinary protein loss was added to the above formula. Oxygen consumption and carbon dioxide production were determined with a Deltatrac M 100 (Datex, Helsinki, Finland). Energy expenditure was calculated from calorimetric data using standard formulas [19]. Protein oxidation was evaluated from the urinary nitrogen excretion rate. Its value was employed to calculate non-protein oxygen consumption and carbon dioxide production. Glucose and lipid oxidation were derived from non-protein oxygen consumption and carbon dioxide production using standard formulas [19].

All values are expressed as the mean ± standard error. Comparison between the groups (inter-group) was performed using analysis of variance. Comparison of normal protein diet treatment and low protein diet treatment results within the nephrotic study group (intra-group) were performed using the Student *t* test for paired data.

Table 1. Clinical characteristics of healthy control subjects and calorimetric data of nephrotic patients after a four week period on a normal protein diet (NPD) or low protein diet (LPD)

	Controls NPD	Nephrotic NPD	Nephrotic LPD
Protein intake *g/kg/day*	1.15 ± 0.06	1.20 ± 0.06	0.66 ± 0.04[a]
BMI *kg/m²*	25.4 ± 2	24.7 ± 2	24.2 ± 2
BUN *mg/dL*	13.8 ± 3	21.8 ± 3	15.3 ± 3[a]
Serum creatinine *mg/dL*	0.87 ± 0.3	1.39 ± 0.3	1.42 ± 0.3
Creatinine clearance *mL/min*	124 ± 16	87 ± 14	86 ± 12
Proteinuria *g/day*	—	10.2 ± 1.8	6.5 ± 1.2[a]
Albuminuria *g/day*	—	7.2 ± 1.4	5.0 ± 1.1[a]
RQ	—	0.80 ± 0.01	0.83 ± 0.02
Carbohydrate Ox *mg/kg/min*	—	1.06 ± 0.1	1.23 ± 0.1
Lipid Ox *mg/kg/min*	—	0.78 ± 0.05	0.81 ± 0.06
Protein Ox *mg/kg/min*	—	0.71 ± 0.04	0.39 ± 0.03[a]
BEE *Kcal/kg per day*	—	21.2 ± 0.9	19.4 ± 0.8[a]

Abbreviations are: BMI, body mass index; RQ, respiratory quotient; Ox, basal substrate oxidation; BEE, basal energy expenditure. Values are mean ± SE.
[a] $P < 0.03$ LPD vs. NPD

RESULTS

Clinical and nutritional characteristics of nephrotic patients

In nephrotic patients during the normal protein diet period, the protein intake (as evaluated from urinary nitrogen excretion) was not different from control subjects, while it was significantly reduced during the low protein diet regimen (Table 1). Blood urea nitrogen was signifi-

cantly lower after the low protein diet regimen. Serum creatinine concentration and body mass index did not change while the subjects consumed either the normal or low protein diets. After the low protein diet, 24-hour proteinuria and albuminuria levels in all nephrotic patients decreased by an average of −38 ± 5 and −34 ± 6%, respectively (both $P < 0.03$). In response to the low protein diet regimen, basal protein oxidation and energy expenditure decreased in all nephrotic subjects by an average of 45 ± 6 and 5 ± 0.1%, respectively (both $P < 0.03$); postabsorptive carbohydrate and lipid oxidation rose by 16 ± 3 and 5 ± 0.9%, respectively, but these changes did not achieve statistical significance. During the normal protein diet regimen, plasma volume in nephrotic patients was significantly increased (3296 ± 219 mL/1.72 m^2, $P < 0.01$ vs. controls) in comparison with that of control subjects (2731 ± 155 mL/1.72 m^2). After the low protein diet regimen, plasma volume in nephrotic patients did not change (3375 ± 210 mL/1.72 m^2).

Endogenous leucine flux

In control subjects the endogenous leucine flux, an index of whole body protein breakdown, was 2.15 ± 0.06 μmol/kg/min and was moderately increased in nephrotic patients during the normal diet regimen (2.66 ± 0.1 μmol/kg/min, $P < 0.05$ vs. controls). After the low protein diet regimen a slightly significant reduction in endogenous leucine flux was observed (2.45 ± 0.1 μmol/kg/min; $P < 0.05$ vs. controls).

Albumin synthesis

Serum albumin levels in controls were 4.20 ± 0.1 g/dL. In nephrotic patients the serum albumin concentration was 2.88 ± 0.3 g/dL while they consumed a normal protein diet ($P < 0.01$ vs. controls) and increased to 3.06 ± 0.2 during the low protein period ($P < 0.03$ vs. NPD; Fig. 1). The total plasma albumin pool in control subjects was 115 ± 3 g/1.73 m^2. In nephrotic patients during the normal protein diet period, the plasma albumin pool was significantly reduced (94 ± 7 g/1.73 m^2, $P < 0.01$ vs. controls). After the low protein diet period, the plasma albumin pool increased to 103 ± 7 g/1.73 m^2 in nephrotic patients ($P < 0.03$ vs. NPD). The fractional synthesis rate of albumin was 9.05 ± 1% in control subjects. In nephrotic patients consuming a normal dietary protein intake, the fractional synthesis rate of albumin was markedly increased to 19.1 ± 2%/day ($P < 0.03$ vs. controls), and after the low protein diet period it decreased to 14.4 ± 1%/day ($P < 0.03$ vs. NPD). While the absolute synthesis rate of albumin averaged 10.5 ± 1 g/1.73 m^2/day in control subjects, it was markedly increased in nephrotic patients during the normal protein diet regimen (18.2 ± 2 g/1.73 m^2/day, $P < 0.03$ vs. controls). In response to the low protein regimen, in nephrotic patients the albumin synthesis rate decreased to 14.9 ± 2 g/1.73 m^2/day ($P < 0.03$ vs. NPD).

Fibrinogen synthesis

Plasma fibrinogen levels in controls were 240 ± 16 mg/dL. In nephrotic patients the plasma fibrinogen concentration was 430 ± 29 mg/dL while they consumed a normal protein diet ($P < 0.01$ vs. controls) and this decreased to 373 ± 24 mg/dL after consuming the low protein diet ($P < 0.03$ vs. NPD; Fig. 2). The total plasma fibrinogen pool in control subjects was 6.71 ± 0.5 g/1.73 m^2. In nephrotic patients during the normal protein diet period it was significantly increased (14.3 ± 0.8 g/1.73 m^2, $P < 0.03$ vs. controls) and after the low protein diet period plasma fibrinogen pool decreased to 12.7 ± 0.7 g/1.73 m^2 ($P < 0.03$ vs. NPD). The fractional synthesis rate of fibrinogen was 28.5 ± 2% in control subjects and was similar in nephrotic patients during normal protein regimen (31.0 ± 5%/day). After the low protein period it did not change significantly (23.8 ± 3%/day, P = NS vs. NPD and vs. controls). The absolute synthesis rate of fibrinogen averaged 1.93 ± 0.3 g/1.73 m^2/day in control subjects. In nephrotic patients, the fibrinogen synthesis rate was markedly increased during a normal protein diet regimen (4.61 ± 0.6 g/1.73 m^2/day, $P < 0.03$ vs. controls), while in response to the low protein regimen, it decreased to 3.00 ± 0.3 g/1.73 m^2/day ($P < 0.03$ vs. NPD).

Correlations

In nephrotic subjects after the low protein diet, the decreased values of the daily protein intake positively correlated with those of 24-hour proteinuria ($y = 5.052x + 1.239$, $r = 0.740$, $P < 0.05$), the decrease in the daily albumin absolute synthesis rate positively correlated with those of 24-hour albuminuria ($y = 0.370x + 0.207$, $r = 0.712$, $P < 0.05$), and the decrease in daily albumin absolute synthesis rate positively correlated with those values of the daily fibrinogen absolute synthesis rate ($y = 0.343x + 0.506$, $r = 0.719$, $P < 0.05$; Fig. 3). The total decreased daily albumin and fibrinogen absolute synthesis rates induced by a low protein diet positively correlated with the decline in the whole-body leucine flux ($r = 0.86$, $P < 0.01$).

DISCUSSION

Our study shows that nephrotic subjects consuming a normal protein intake are characterized by a decreased albumin intravascular pool, with increased albumin absolute synthesis and fractional synthesis rates. The absolute synthesis rate of fibrinogen is increased and is associated with higher plasma fibrinogen levels and total intravascular pool. Following a low protein diet regimen, providing ~0.6 g/kg/day (plus 1 gram protein per gram of protein-

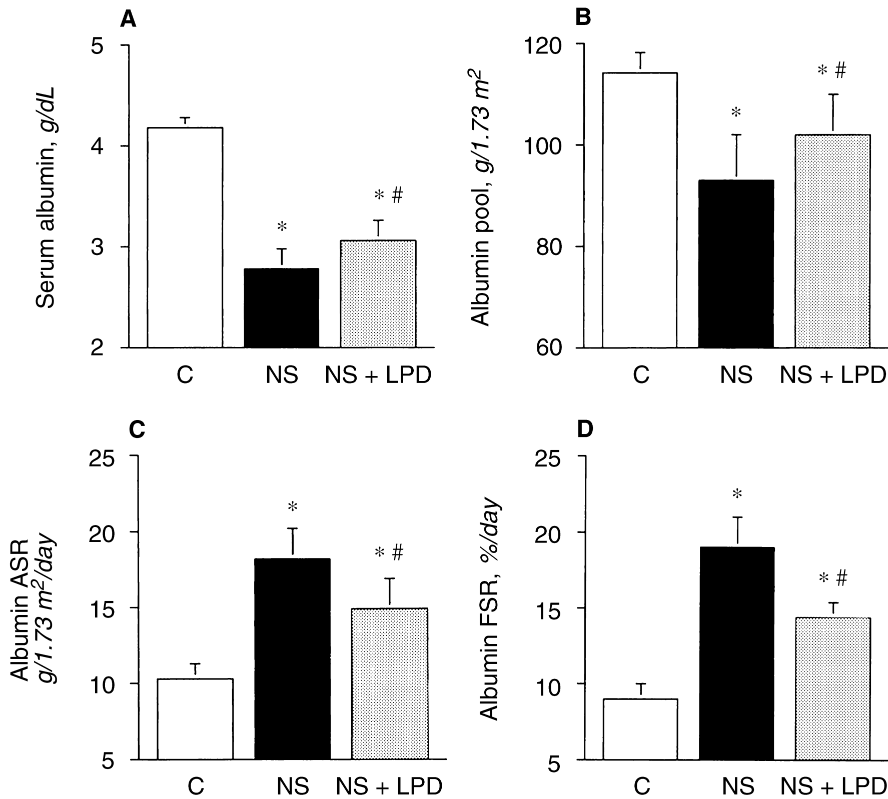


Fig. 1. Serum albumin concentration (*A*), intravascular albumin pool (*B*), albumin absolute synthesis rate (*C*), and albumin fractional synthesis rate (*D*) in control subjects (C) and nephrotic syndrome patients (NS) while consuming a normal protein diet, and in nephrotic syndrome patients after placement on a low protein diet (NS + LPD). Values are mean ± SEM. $*P < 0.03$ vs. controls and $\#P < 0.05$ vs. NS.

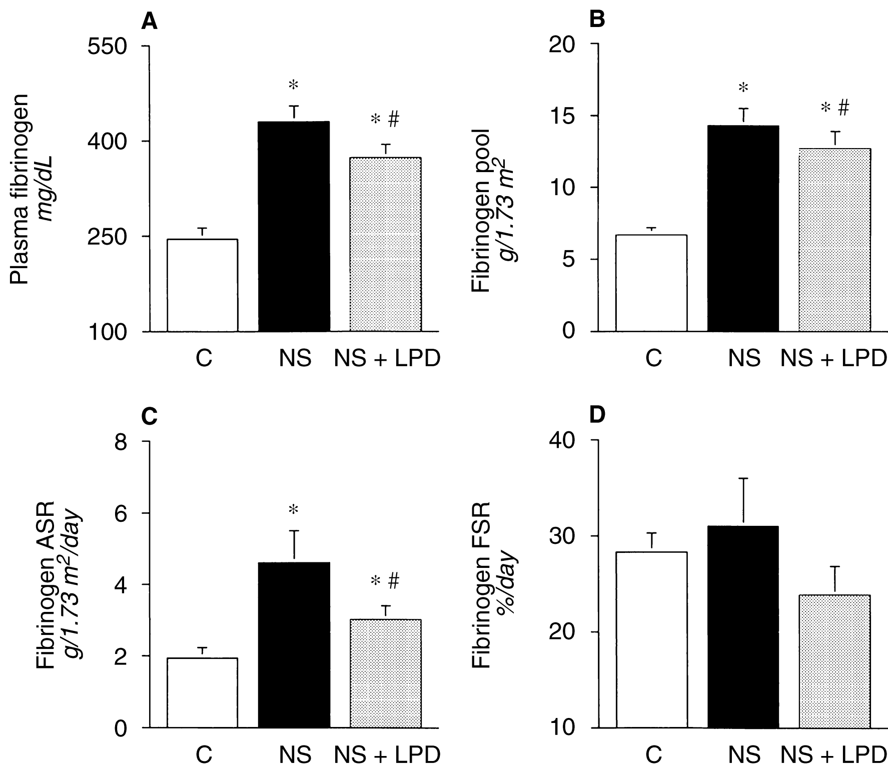
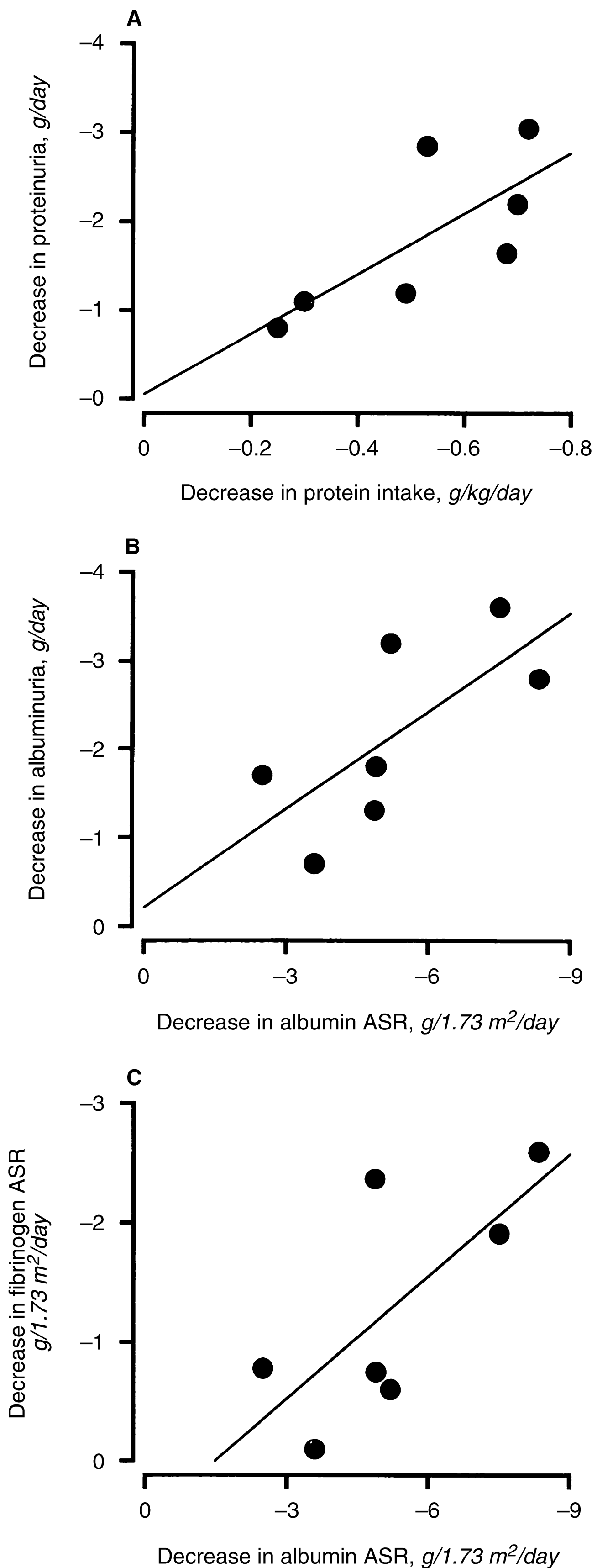


Fig. 2. Plasma fibrinogen concentration (*A*), intravascular fibrinogen pool (*B*), fibrinogen absolute synthesis rate (*C*), and fibrinogen fractional synthesis rate (*D*) in control subjects (C) and nephrotic syndrome patients (NS) while consuming a normal protein diet, and in nephrotic syndrome patients after placement on a low protein diet (NS + LPD). Values are mean ± SEM. $*P < 0.03$ vs. controls and $\#P < 0.05$ vs. NS.



uria), 24-hour proteinuria and albuminuria rates are decreased, and the observed derangement in both albumin and fibrinogen metabolism are in part reversed. These changes significantly correlated and are associated with a reduction in whole-body protein breakdown.

The optimal dietary protein intake for adults with nephrotic syndrome has yet to be established. Previous studies in nephrotic children demonstrated that, following an high dietary protein intake, their urinary protein excretion increased, while serum albumin and total protein concentrations did not ameliorate significantly [6]. If the need of a high protein intake can be excluded, few studies have evaluated whether a restriction from a normal to low dietary protein intake may ameliorate protein homeostasis. No data are available on the effects of changes in dietary protein intake on fibrinogen metabolism. Kaysen et al demonstrated that in nephrotic patients maintained on a reduced dietary protein intake, serum albumin concentration and intravascular albumin mass increased [8]. Our data are in agreement with their report [8], and show that a restriction in dietary protein intake from ~1.1 to ~0.6 g/kg (plus 1 gram protein per gram of proteinuria) is followed by a significant reduction in proteinuria, as well as in the absolute synthesis and fractional synthesis rates of albumin.

Concerns have been raised that a dietary protein restriction in patients with nephrotic syndrome may favor the onset of protein malnutrition [6, 7]. However, Maroni et al demonstrated that nephrotic patients consuming a protein restricted diet provided 0.8 grams of protein per kilogram (plus 1 gram protein per gram of proteinuria) can adapt to dietary protein restriction by activating a response that includes postprandial stimulation of protein synthesis, inhibition of protein breakdown, and a reduction in amino acid oxidation [20]. In agreement with Maroni et al's study, our present study showed a complex metabolic adaptation in the nephrotic patients during the low protein diet regimen with a reduction in postabsorptive whole body proteolysis, protein oxidation, and adaptive changes in carbohydrate and lipid utilization. We also observed a slight reduction in the basal energy expenditure rate after the low protein diet. In this regard, we have previously demonstrated a correlation between amino acid administration, protein synthesis and energy expenditure in normal subjects [21].

Lim et al observed a significant correlation between

Fig. 3. In nephrotic patients, relationship between the decreases (obtained after 4 weeks of a low protein dietary regimen) in the daily protein intake versus 24 hours of proteinuria (*A*; $r = 0.740$, $P < 0.05$), in the daily albumin absolute synthesis rate versus 24 hours of albuminuria (*B*; $r = 0.712$, $P < 0.05$), and in the daily albumin absolute synthesis rate versus daily fibrinogen absolute synthesis rate (*C*; $r = 0.719$, $P < 0.05$).

dietary protein intake and protein degradation in nephrotic patients who maintained a nitrogen equilibrium with an intake of 0.84 g of protein and 33 kcal/kg per day [22]. Recently, de Sain-van der Velden et al showed that whole body valine flux and oxidation were similar in nephrotic patients and in control subjects who consumed a diet providing 0.8 g/kg of protein per day [23]. In the present study endogenous leucine flux was higher in nephrotic patients than in control subjects. Differences in the isotope employed (D_3-L-leucine vs. 1-^{13}C-valine) does not allow a complete comparison between the kinetics data of the two studies. However, the data of de Sain-van der Velden et al on whole body protein turnover in the presence of an elevated hepatic albumin and fibrinogen synthesis, implies a reduced rate of extra-hepatic protein synthesis [23]. In contrast, our present study is compatible with the maintenance of a normal extra-hepatic protein synthesis. Previous data of Maroni et al [20] and Lim et al [22] also provide evidence for a complex adaptive response that total body protein is conserved in nephrotic patients. We similarly observed a positive correlation ($r = 0.86$, $P < 0.01$) between the total decrease in albumin and fibrinogen synthesis induced by the low protein diet and a decline in the whole body protein breakdown. Clearly, more studies are needed to evaluate the interplay between protein intake, hepatic and extra-hepatic protein synthesis in nephrotic syndrome.

The mechanism(s) by which the low protein diet regimen may reduce the urinary albumin excretion rate in nephrotic patients cannot be inferred from the present data. However, kinetic data of albumin metabolism point to an improvement in the glomerular ultrafiltration function rather than to an increased tubular reabsorption of protein. In fact, in the present study the rise in the intravascular albumin pool is associated with a decline in the absolute and fractional albumin synthesis rates. Thus, the low protein diet regimen ameliorates the vicious cycle of the nephrotic syndrome by reducing the glomerular filtration of protein. This antiproteinuric effect increases the concentration of several plasma proteins, including albumin, which in turn mitigates the compensatory rise of the albumin synthesis rate. Of interest, we observed a positive relationship between decreases in the 24-hour albuminuria (induced by dietary protein restriction) and albumin absolute synthesis rates (Fig. 3). The data suggest that under the present experimental conditions, in spite of the persistence of an hypoalbuminemic state, the individual rate of hepatic albumin synthesis is regulated by its catabolic rate. Since the reduction in proteinuria was obtained via a restricted dietary protein intake, it cannot be excluded that the reduced albumin synthesis may represent, at least in part, an adaptive response to a reduced availability of substrate. The effect of a non-dietary correction of proteinuria may contribute to a better understanding of this issue.

Our study also reports elevated fibrinogen levels secondary to an increased synthesis rate of fibrinogen in nephrotic patients on normal protein diet, a finding that is in agreement with previous reports [4, 24]. Fibrinogen is a high molecular weight protein and, in contrast to albumin, it is not filtered by the damaged glomerulus of the nephrotic patients; thus, its elevated synthesis leads to an increase in plasma fibrinogen levels and the intravascular pool. It is known that hyperfibrinogenemia may contribute to hypercoagulability, hyperviscosity and increased platelet aggregation [1], and it can accelerate the progression of renal disease in nephrotic patients [2]. The most relevant finding of the present study is the reduction of the fibrinogen synthesis rate following dietary protein restriction. The mechanism(s) of the decline in fibrinogen synthesis following dietary protein restriction are at present unknown. However, it is possible that the hepatic mechanism(s) that regulates the synthesis of both albumin and fibrinogen is induced by a common signal. In their comprehensive study, de Sain-van der Velden et al report that the absolute synthesis rates of albumin and fibrinogen are positively correlated [3]. The present findings are in agreement with that study [3]. In addition, our data show a positive correlation between the individual changes in albumin and fibrinogen synthesis rates induced by dietary protein restriction (Fig. 3). These data support the hypothesis that albumin and fibrinogen synthesis are regulated in parallel fashion in nephrotic patients. This is at variance to what is found in other metabolic diseases [25], and may represent a peculiar feature of nephrotic syndrome in which the liver response to a hypoalbuminemic state leads to an elevated synthesis of both the negative acute phase protein (albumin) and positive acute phase protein (fibrinogen). This hypothesis has recently been proposed by Kang et al, who reported that in rats the hepatic response to albuminuria is characterized by increased mRNA level encoding for both negative acute phase proteins, including albumin, and the positive acute phase protein fibrinogen, which correlated with one another, suggesting that they are coordinately controlled [5]. To our knowledge the present finding represents the first experimental evidence that this type of regulation of albumin and fibrinogen metabolism is present and can be advantageously modulated in nephrotic patients.

Taken together, the present data suggest that a protein-restricted diet providing ~0.6 g/kg of protein (plus 1 g protein per g of proteinuria) can induce a significant reduction in proteinuria with an increase in both serum albumin levels and the intravascular albumin pool, and that these changes are associated with a decreased plasma fibrinogen concentration and intravascular fibrinogen pool. Changes in proteinuria are significantly

correlated with the rate of albumin synthesis. The data also provide evidence of a coordinate regulation of hepatic albumin and fibrinogen synthesis in nephrotic patients that can be advantageously modulated by dietary protein intake.

Reprint requests to Mauro Giordano, M.D., Ph.D., Istituto di Clinica Medica "L. Condorelli," Via Plebiscito, 628, 95124 - Catania, Italy.
E-mail: giordmau@tin.it

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