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Dietary protein intake does not affect IgG synthesis in patients with nephrotic syndrome

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Abstract

Background. Low plasma IgG levels have long been reported as an important complication of the nephrotic syndrome. Few studies *in vivo* have evaluated IgG synthesis in nephrotic patients and no data are available on the effect of dietary protein restriction on the rate of IgG synthesis.

Methods. We compared the IgG synthesis rates of seven nephrotic patients who assumed, for 4 weeks, either a normal protein diet (NPD) (1.20 ± 0.06 g/kg/day) or a low-protein diet (LPD) (0.66 ± 0.04 g/kg/day) with those of seven normal subjects (matched for age and body mass index). The post-absorptive fractional synthesis rate (FSR) and absolute synthesis rate (ASR) of IgG were evaluated during the last 120 min of a 5 h 5,5,5-D3-L-leucine infusion.

Results. Compared with controls, in nephrotic patients the plasma IgG levels and pool were significantly reduced ($P < 0.05$), while IgG FSR and ASR were increased by 4- and 2.5-fold, respectively ($P < 0.05$). The LPD regimen did not affect plasma IgG FSR, ASR, circulating concentrations and intravascular pool ($P = NS$). There was a significant negative correlation between plasma IgG FSR and the IgG intravascular pool in nephrotic patients evaluated during both the NPD ($r = -0.828$; $P < 0.05$) and LPD ($r = -0.861$; $P < 0.05$) regimens.

Conclusions. Nephrotic syndrome patients with low plasma IgG levels have increased IgG FSR and ASR which are not affected by reduced dietary protein intake.

Keywords: IgG metabolism; low protein diet; proteinuria

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Introduction

A low plasma immunoglobulin G (IgG) concentration has long been reported as an important complication in nephrotic syndrome and is supposed to be responsible for a higher incidence of infection in nephrotic patients. It has been suggested that reduced IgG concentrations in nephrotic patients might be partially due to an impairment of IgG synthesis rate [1–3]. In addition, no data are available on the potential effects of dietary protein restriction on the IgG synthesis rate. This information is relevant from a clinical point of view given the prescription of low-protein diets (LPDs) in nephrotic patients. In fact, it has been demonstrated that IgG synthesis is increased by ∼40% after a mixed meal containing amino acids, whereas it remains unchanged during the absorption of an amino acid-lacking meal [4,5]. The strict relationship between immune defence and protein nutrition is probably responsible for the reduced serum IgG concentrations [6,7] that have been reported in protein malnutrition, thus outlining the need to establish whether LPDs could also impair IgG synthesis in nephrotic patients.

The present study was therefore performed in order to evaluate the absolute synthesis rate (ASR) and fractional synthesis rate (FSR) of IgG in hypogammaglobulinaemic nephrotic patients and to observe the impact of dietary protein restriction on IgG synthesis.

Materials and methods

Patient population

Seven healthy normal volunteers (controls) and seven patients with nephrotic syndrome who were referred to our unit for transcutaneous renal biopsy participated in the study protocol (Table 1). Eligibility criteria of nephrotic patients included: age 20–50 years, urinary protein excretion

Table 1. Summary of clinical characteristics of healthy control subjects and nephrotic patients after a 4 week period on a normal protein diet (NPD) or low protein diet (LPD) regimen

	Controls NPD	Nephrotic NPD	Nephrotic LPD
n (males)	7 (4)	7 (4)	7 (4)
Age (years)	37 ± 3	39 ± 3	39 ± 3
Height (cm)	172 ± 4	166 ± 3	166 ± 3
Weight (kg)	68 ± 4	68 ± 6	66 ± 6
Protein intake (g/kg per day)	1.15 ± 0.06	1.20 ± 0.06	$0.66 \pm 0.04**$
BMI (kg/m^2)	25.4 ± 2	24.7 ± 2	24.2 ± 2
Serum creatinine (mg/dl)	0.87 ± 0.3	1.39 ± 0.3	1.42 ± 0.3
Serum urea (mg/dl)	30.4 ± 6	47.9 ± 6	$33.7 \pm 6**$
Plasma albumin (g/dl)	4.2 ± 0.1	$2.9 \pm 0.3*$	$3.1 \pm 0.2*$
Plasma IgG (g/dl)	1.20 ± 0.08	$0.76 \pm 0.13*$	$0.75 \pm 0.07*$
Proteinuria (g/1.73 m^2/day)	–	$9.9 \pm 1.9*$	$6.6 \pm 1.7**$
Albuminuria (g/1.73 m^2/day)	–	$6.9 \pm 1.3*$	$5.0 \pm 1.2**$

BMI = body mass index.
Values are mean $\pm$ SE; $*P < 0.01$ vs controls; $**P < 0.03$ vs nephrotic NPD.

>3.5 g/24 h, plasma IgG levels <900 mg/dl, and no evidence of endocrine or other major organ system disease, as determined by medical history, physical examination and routine laboratory tests. Patients with diseases known to increase IgG synthesis (amyloidosis SAA, myeloma, hepatitis C-positive patients with mixed cryos, etc.) were excluded from the study. Renal biopsy specimens were evaluated prior to their participation in the study. Other than vitamins, patients were not taking 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. The study protocol was reviewed and approved by the Ethical Committee of the Second University of Naples.

Experimental protocol

Control subjects participated in one study and were instructed to consume a weight-maintaining diet providing ∼35–38 kcal/kg per day and containing ∼250–300 g of carbohydrate and 1.1 g/kg per day of protein for at least 7 days prior to their participation in the study.

Patients with established nephrosis participated in two separate experimental protocols performed at a 4–5 week interval after they had been maintained on each of the two different dietary regimens for ∼4 weeks (27 ± 1 days). On the first dietary regimen (normal protein diet; NPD), patients were instructed to consume a weight-maintaining diet providing ∼35–38 kcal/kg per day and containing 1.1 g of protein/kg per day. On the second dietary regimen (LPD), patients were instructed to consume a similar caloric intake but the dietary protein was reduced to 0.6 g/kg per day and >65% of the ingested proteins were of a high biological value. In addition, during both dietary regimens, patients received 1 g 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. On the NPD, dietary carbohydrates and lipids represented 55 and 25% of the total caloric intake, respectively. On the LPD, their contribution to total calories was increased to 60 and 30%, respectively. In order to verify compliance with the diet, during each 4 week dietary regimen, all patients were invited to return to our clinical unit weekly with their dietary diary and 24 h urinary collection specimens were obtained to determine urinary protein and nitrogen excretion.

Metabolic studies were performed in the post-absorptive state after a 12 h overnight fast. In nephrotic patients, the study was performed after each 4 week period of dietary regimen, which were started in random order and completed in all patients. At the end of the dietary periods, two consecutive 24 h urinary collections were also obtained to determine urinary protein excretion. On the day of the study, a 19 gauge 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, and the hand was kept in a heated box at 60°C to ensure arterialization of the venous blood [8]. At 08:00 h, a prime (0.6 mg/kg bolus) followed by a continuous (1.2 mg/kg/h) infusion of 5,5,5-D3-L-leucine (Mass Trace, Woburn, MA) was begun and continued for 5 h by a Harvard syringe pump (Harvard Apparatus, Ealing, South Natick, MA). A 6 ml aliquot of blood was collected at −15, 0, 180, 210, 240, 270 and 300 min to measure the plasma concentration and enrichment of leucine, α-ketoisocaproic acid (KIC) and the enrichment of D3-leucine bound to plasma IgG.

At the end of leucine continuous infusion period, plasma volume was determined by the Evans blue dye dilution method. Briefly, a bolus of ∼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 10 min from 10 to 60 min for measurement of Evans blue dye in the serum. The present paper represents an extension of a study previously reported [9].

Analytical determinations

Leucine and KIC were extracted from plasma samples as previously described [10] after the addition of 50 μl/ml plasma of norleucine [160 μmol/ml] and 20 μl/ml of α-ketocaproate (20 μmol/ml) as internal standards. Enrichments and concentrations of plasma leucine and KIC were determined on their t-butyldimethylsilyl derivatives using gas chromatography–mass spectrometry (GC–MS) in electron impact (EI) ionization mode (GC8000,

MS Voyager Finningan, ThermoQuest Italia, Milan, Italy), monitoring the ions 302 and 305 for leucine and 301 and 304 for KIC [11]. Plasma IgGs were purified as previously described in detail [4,5]. Briefly, plasma IgGs were purified from 2 ml of serum using a protein A–Sepharose high performance affinity gel, packed in a column C 10/10 (Pharmacia AB, Uppsala, Sweden) that specifically binds the subclasses 1, 2 and 4 of human IgG [12]. Before loading the sample, lipids were removed by exane extraction, and five volumes of phosphate buffer 0.02 M were used for gel equilibration. After loading the sample, the column was washed with three column volumes of starting buffer, followed by two column volumes of starting buffer plus 0.5 M NaCl to remove non-specifically bound proteins. The IgG fraction was eluted with 1.5 column volumes of 0.1 M citric acid, pH 3.3 [13]. The purity of the IgG fraction was tested by SDS–PAGE using standard human IgG (Sigma Immunochemicals). Serum IgGs were determined by a turbidimetric method with precipitation of polyclonal anti-bodies (Dade-Behring Inc. Deerfield, IL).

To evaluate plasma volume, serum samples were added with an equal volume of ~4000 Da, polyethyleneglycol (J. T. Baker, Deventer, The Netherlands) solution (24 g/dl) for precipitation of non-albumin proteins. Samples and standards were centrifuged for 10 min at 3000 r.p.m. Supernatants from samples and standards were then read at 620 nm wavelength [14] using a spectrophotometer (Ciba-Corning Diagnostics Ltd, Halstead, Essex, UK).

Calculations and statistics

The enrichments of leucine and KIC was expressed as the tracer to tracee ratio (TTR), accounting for isotopomer skewed distribution and spectra overlapping when appropriate. The IgG FSR was calculated dividing the slope of the increase in the enrichment of leucine bound to IgG by the enrichment of plasma KIC over the last 2 h of the study, at the isotopic and metabolic steady state [4,5]. The intravascular

IgG ASR was estimated by multiplying the IgG FSR by the total intravascular IgG content [4,15].

To evaluate plasma volume, after Evans blue dye injection, the concentration at time zero was extrapolated. The estimated concentration at time zero was used to calculate the plasma volume by the dilution formula: PV (ml) = dose of EBD (μg) injected/serum concentration of EBD (μg/ml) extrapolated at time zero [14]. Dietary protein intake in nephrotic patients and compliance with the diet were evaluated from weekly determination of 24 h urinary nitrogen excretion according to the formula: urinary nitrogen = urinary urea nitrogen + non-urea nitrogen, where 1 g of urinary nitrogen = 6.25 g of protein and non-urea nitrogen excretion = 30 mg/kg per day [16]. Urinary protein loss has been added to the above formula.

All values are expressed as the mean ± SE. Comparison between the groups (inter-group) was performed using analysis of variance. Comparison of NPD and LPD treatment results within the nephrotic study group (intra-group) was performed using the Student's *t*-test for paired data.

Results

The clinical characteristics of nephrotic patients are reported in Table 1. The aetiologies of renal diseases were as follows: membranoproliferative glomerulonephritis ($n = 3$), membranous nephropathy ($n = 2$), amyloidosis ($n = 1$) and focal segmental glomerulosclerosis ($n = 1$). Plasma IgG levels in controls (Figure 1) were 1.20 ± 0.08 g/dl. Individual raw data of nephrotic patients are reported in Table 2. The plasma IgG concentration in nephrotic patients (Figure 1) was 0.76 ± 0.13 g/dl while consuming the NPD ($P < 0.01$ *vs* controls) and did not change significantly during the LPD period (0.75 ± 0.07 g/dl). The plasma IgG circulating pool in control subjects (Figure 1) was 32.7 ± 2 g/1.73 m^2. Plasma IgG pool in nephrotic
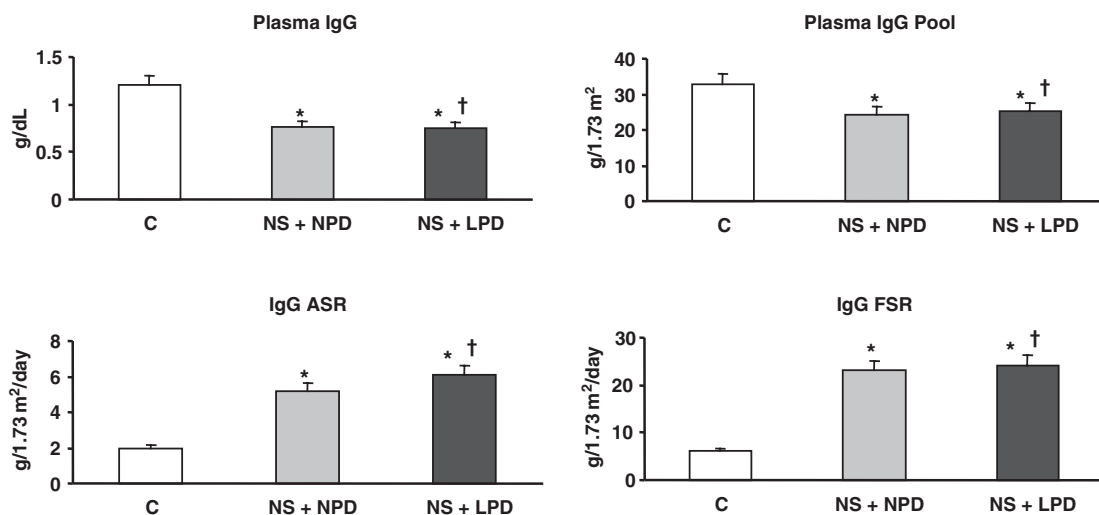

Fig. 1. Plasma IgG concentration, intravascular IgG pool, IgG absolute synthesis rate and IgG fractional synthesis rate in control subjects (C) and nephrotic patients while consuming a normal protein diet (NS + NPD), and in nephrotic patients after having been placed on a low protein diet (NS + LPD). Values are mean ± SEM. *$P < 0.05$ *vs* controls; †P not significant *vs* NS ± NPD.

Table 2. Individual raw data of nephrotic patients after a 4 week period of a normal protein diet (NPD) or low protein diet (LPD) regimen

	Plasma IgG (g/dl)		IgG pool (g)		IgG ASR (g/day)		IgG FSR (%/day)	
	NPD	LPD	NPD	LPD	NPD	LPD	NPD	LPD
Patient 1	0.71	0.83	31	36	4.4	4.6	14	13
Patient 2	1.36	0.57	33	30	3.6	5.6	11	19
Patient 3	0.52	0.63	20	24	6.4	4.2	32	18
Patient 4	1.17	1.05	36	27	6.7	6.7	18	25
Patient 5	0.46	0.77	16	23	2.8	5.7	18	25
Patient 6	0.60	0.91	18	18	6.9	6.9	38	38
Patient 7	0.52	0.49	17	16	5.2	5.1	30	32
Mean	0.76	0.75	25	25	5.1	5.6	23	24
SE	0.1	0.1	3	3	0.6	0.4	4	3

patients (Figure 1) during the NPD period was significantly reduced (24.4 ± 4 g/1.73 m^2, $P < 0.01$ vs controls). After the LPD period, the plasma IgG pool did not change significantly (25.2 ± 3 g/1.73 m^2) (Figure 1). The FSR of IgG was $6.2 \pm 0.2\%$/day in control subjects (Figure 1). In nephrotic patients consuming an NPD, the FSR of IgG was markedly increased to $23.1 \pm 4\%$/day ($P < 0.03$ vs controls) and after the LPD period it did not change significantly ($24.2 \pm 4\%$/day) (Figure 1). The ASR of IgG averaged 2.0 ± 0.1 g/1.73 m^2/day in control subjects and it was markedly increased in nephrotic patients during the NPD regimen (5.2 ± 0.7 g/1.73 m^2/day, $P < 0.03$ vs controls) (Figure 1). In response to the LPD regimen, no difference was observed in IgG synthesis rate (6.1 ± 1.3 g/1.73 m^2/day) (Figure 1).

In nephrotic patients, there was a significant inverse correlation between the IgG FSR and the intravascular IgG pool during both the NPD ($r = -0.828$; $P < 0.05$) and LPD ($r = -0.861$; $P < 0.05$) regimens (Figure 2); no relationship was found between the degree of proteinuria or albuminuria and plasma IgG concentration, IgG pool, IgG ASR or IgG FSR.

Discussion

The results of this study demonstrate that during a normal protein intake, nephrotic subjects with a marked decrease of the IgG intravascular pool have an increase in IgG FSR and ASR, which are not reduced by an LPD regimen ($\sim$0.6 g/kg of protein plus 1 g of protein per g of proteinuria). The present data demonstrate that, compared with normal subjects, nephrotic patients have an $\sim$25% reduced intravascular IgG pool and an $\sim$3-fold increased IgG ASR. The demonstration, in these patients, of a marked increase in IgG FSR and ASR rules out the possibility of an impairment in IgG synthesis worsening the hypogammaglobulinaemia. In this regard, we observed a close relationship (Figure 2) between the reduction in intravascular IgG pool and the increment in IgG FSR, suggesting that low IgG concentrations stimulate IgG synthesis. Thus,
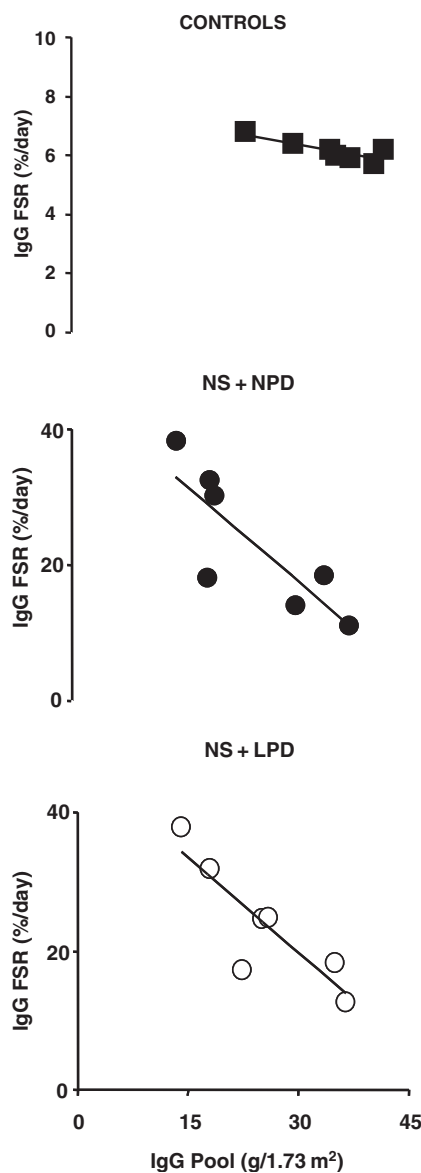

Fig. 2. Correlations between IgG FSR and the plasma IgG circulating pool in control subjects (filled squares) ($r = -0.852$; $P < 0.05$) and in nephrotic patients evaluated during both a normal (NS + NPD) (filled circles) ($r = -0.828$; $P < 0.05$) and a low protein diet (NS + LPD) (open circles) ($r = -0.861$; $P < 0.05$) regimen.

the 4-fold increase in fractional IgG synthesis rates, observed in nephrotic patients, may represent a central defensive mechanism that prevents severe hypogammaglobulinaemia.

Our findings confirm the results of a previous *in vitro* study performed using circulating mononuclear cells from nephrotic patients that reported an increase in synthetic IgG activities [15]. *In vivo*, few data are available on the γ-globulin synthesis rate in nephrotic states. Studies on nephrotic rats have demonstrated that in contrast to a liver-derived protein, such as albumin, hypogammaglobulinaemia did not induce an increase in IgG synthesis [1]. In humans, the results of earlier studies, performed using radiolabelled γ-globulins, showed increased IgG synthesis rates in nephrotic patients [17,18]. The reason for this difference is not clear. However, our data are in agreement with those of the earlier study of Waldmann *et al.* who reported a 3-fold increase in fractional metabolic rate of IgG in nephrotic patients [18].

The beneficial effect of a reduced protein intake on proteinuria has been suggested in nephrotic patients [19,20]. Potentially, an LPD might impair the observed increase in IgG synthesis of nephrotic patients because it has been shown that IgG synthesis is dependent on adequate dietary amino acid supply [5]. Thus, we examined the effect of 4 weeks of protein-restricted diet providing ∼0.6 g/kg of protein (plus 1 g protein per gm of proteinuria). This dietary regimen resulted in a marked reduction of the protein excretion rate and partially corrected the abnormal rates of whole body proteolysis, albumin and fibrinogen synthesis, as previously reported [9]. In the present study, during dietary protein restriction, even if proteinuria was reduced, no increases in plasma IgG or IgG pool were observed. However, it cannot be excluded that a period longer than 4 weeks of an LPD regimen may be required to observe a reduction in IgG catabolism with a consequent change in plasma IgG levels. Interestingly, the results of the present study demonstrate that this dietary protein supply was sufficient to maintain the increase in IgG FSR and ASR, and that these were similar to those estimated with the NPD. Thus, our data may suggest that in nephrotic patients, similarly to the effect observed on albumin metabolism [9,19], an LPD has no adverse effect on IgG metabolism. This represents a novel finding since in nephrotic patients, no data have been reported previously on the effect of dietary protein restriction on IgG synthesis rate.

In conclusion, the marked stimulation of IgG synthesis may represent an important mechanism that prevents further dramatic falls in circulating IgG levels. Finally, this vital mechanism is not impaired by an LPD regimen.

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