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ARTICLE

Effects of dietary protein restriction on albumin and fibrinogen synthesis in macroalbuminuric type 2 diabetic patients

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Abstract
Aims/hypothesis Diabetic nephropathy is associated with hypoalbuminaemia and hyperfibrinogenaemia. A low-protein diet has been recommended in patients with diabetic nephropathy, but its effects on albumin and fibrinogen synthesis are unknown.
Methods We compared the effects of a normal (NPD; 1.38±0.08 g kg^{-1} day^{-1}) or low (LPD; 0.81±0.04 g kg^{-1} day^{-1}) -protein diet on endogenous leucine flux (ELF), albumin and fibrinogen synthesis (L-[5,5,5,-$^2H_3$]leucine infusion), and markers of inflammation in nine type 2 diabetic patients with macroalbuminuria. Six healthy participants on NPD served as control participants.
Results In comparison with healthy participants, type 2 diabetic patients on an NPD had similar ELF, reduced serum albumin (38±1.1 vs 42±0.8 g/l; $p<0.05$), similar fractional synthesis rates (FSR) and absolute synthesis rates (ASR) of albumin, and both increased plasma fibrinogen concentration [10.7±0.6 vs 7.2±0.5 μmol/l (3.64±0.22 vs 2.45±0.18 g/l); $p<0.05$] and fibrinogen ASR [11.03±1.17 vs 6.0±1.8 μmol 1.73 m^{-2} day^{-1} (3.7±0.4 vs 1.9±0.3 g 1.73 m^{-2} day^{-1}); $p<0.01$]. After LPD, type 2 diabetic patients had the following changes in comparison with NPD: reduced proteinuria (2.74±0.4 vs 4.51±0.8 g/day; $p<0.05$), ELF (1.93±0.08 vs 2.11±0.08 μmol kg^{-1} min^{-1}; $p<0.05$) and total fibrinogen pool; increased serum albumin (42±1 vs 38±1 g/l; $p<0.01$) and albumin ASR (14.1±1 vs 9.9±1 g 1.73 m^{-2} day^{-1}; $p<0.05$); and reduced plasma IL-6 levels, which were correlated with albumin ASR ($r=-0.749$; $p<0.05$).
Conclusions/interpretation LPD in type 2 diabetic patients with diabetic nephropathy reduces low-grade inflammatory state, proteinuria, albuminuria, whole-body proteolysis and ASR of fibrinogen, while increasing albumin FSR, ASR and serum concentration.

ISRCTN ID no: CCT-NAPN-16911

Keywords Absolute synthesis · Albumin · Diabetic nephropathy · Fibrinogen · Fractional synthesis · Leucine · Low-grade inflammation · Low-protein diet · Macroalbuminuria · Type 2 diabetes

Abbreviations

ASR	absolute synthesis rate
CRP	C-reactive protein
ELF	endogenous leucine flux
FSR	fractional synthesis rate
KIC	α-ketoisocaproic acid
LPD	low-protein diet

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NPD normal-protein diet
TTR tracer/tracee ratio

Introduction

Diabetes has become the most common single cause of end-stage renal disease in the USA and Europe [1]. Diabetic nephropathy develops in approximately 40% of all type 2 diabetic patients and is mainly characterised by persistent albuminuria and elevated blood pressure [2]. Once patients with microalbuminuria progress to macroalbuminuria (overt nephropathy), they are likely to progress to end-stage renal disease [3]. Diabetic nephropathy is often associated with low-grade inflammation [4], hyperfibrinogenaemia [5], dyslipidaemia and high incidence of cardiovascular morbidity and mortality [6, 7].

The course of diabetic nephropathy can be ameliorated by optimal glucose control, intensive blood pressure treatment with renin–angiotensin system blockade and reduction of plasma lipids [8]. When chronic kidney disease occurs, additional therapeutic strategies, such as low-protein diet (LPD) regimen, are indicated [1]. Recently, it has been reported that type 2 diabetic nephropathy is associated with abnormal albumin and fibrinogen synthesis [5]. In non-diabetic patients with nephrotic syndrome, albumin and fibrinogen metabolism are increased [9] and are ameliorated by LPD regimen [10]. At present, the effects of a low-protein regimen on albumin and fibrinogen metabolism in type 2 diabetic patients with diabetic nephropathy have not been evaluated.

The present study was therefore performed to establish the effects of LPD regimen on albumin and fibrinogen synthesis, on whole-body protein breakdown and on markers of low-grade inflammation in type 2 diabetic patients with macroalbuminuria.

Methods

Patient population Six healthy normal volunteers (controls; three men, three women; age 37±3 years; BMI 23±0.5 kg/m^2) and nine type 2 diabetic patients with nephropathy (six men, three women; age 60±2 years; BMI 33±2 kg/m^2) participated in the study protocol. The clinical characteristics of the diabetic participants at baseline are reported in Table 1. Eligibility criteria of type 2 diabetic patients included: BMI <35 kg/m^2; proteinuria >3 g/day; absence of urinary tract infection or other renal diseases. All diabetic patients were using ACE inhibitors, insulin and hypolipidaemic agents during the study to ensure best blood pressure and metabolic control. Diagnosis of diabetic nephropathy was made in all patients in accordance with guidelines on duration of diabetes, presence of proteinuria and diabetic retinopathy [11]. There was no evidence of endocrine or other major organ system disease, as determined by medical history, physical examination and routine laboratory tests. Eligible patients entered a run-in period of about 2 months, during which they were treated to achieve best metabolic and blood pressure control according to American Diabetes Association guidelines [1]. In particular, all diabetic participants were treated with ACE inhibitors (ramipril). The experimental protocol was reviewed and approved by the ethical committee of the Second University of Naples. Potential risks of the study were explained to all participants and their voluntary written consent was obtained before their participation.

Table 1 Clinical characteristics of type-2 diabetic patients at baseline (NPD)

	Type 2 diabetic patients
Number (men)	9 (6)
Age (years)	60±2
BMI (kg/m^2)	33±2
Known duration of diabetes (years)	15±3
HbA_{1c} (%)	7.3±0.9
Total cholesterol (mmol/l)	5.3±0.11
HDL-cholesterol (mmol/l)	1.2±0.03
Triacylglycerol (mmol/l)	1.9±0.28
Creatinine clearance rate (ml min^{-1} 1.73 m^{-2})	30±3
Insulin treatment	9
Statin treatment	9
ACE inhibitor treatment	9

Values are mean±SD or *n*.

Experimental protocol There was a control group for one study. For at least 7 days prior to their participation, they were instructed to consume a weight-maintaining diet providing about 146–159 kJ kg^{-1} day^{-1} (35–38 kcal kg^{-1} day^{-1}) and containing about 250 to 300 g carbohydrate kg^{-1} day^{-1} and 1.2 g protein kg^{-1} day^{-1}. Diabetic patients participated in two separate experimental protocols performed at an interval of 4 to 5 weeks, after they had been maintained on each of the two different dietary regimens for about 4 weeks (30±2 days). For the first dietary regimen, normal-protein diet (NPD), patients were instructed to consume a weight-maintaining diet providing about 146–159 kJ kg^{-1} day^{-1} (35–38 kcal kg^{-1} day^{-1}) and containing 1.2 g protein kg^{-1} day^{-1}. For the second dietary regimen (LPD), patients were instructed to consume a similar energy intake, but dietary protein was reduced to 0.8 g kg^{-1} day^{-1}, with more than 65% of ingested protein being of high biological value. The amount of dietary protein provided to replace urinary protein excretion was

maintained constant during both dietary regimens. On normal protein intake, dietary carbohydrate and lipid represented 50 and 25% of the total energy intake, respectively. On low protein intake, the contribution of lipid to total energy was increased to 35% (by increasing the amount of unsaturated fat). During normal protein intake, dietary phosphate and calcium intake were 1512±68 and 1016±97 mg/day, respectively, and decreased to 872±63 and 743±79 mg/day during the low protein intake period. In order to verify compliance with the diet, all patients were invited to return to our clinical unit once weekly during each 4 week dietary regimen and bring a 2 day record of their weighed diets with them [12]. On the same days as those of the weighed diet records, patients collected 24 h urinary collection specimens to determine urinary protein and nitrogen excretion. The 24 h urinary nitrogen output was considered the criterion standard for protein intake evaluation [12].

Metabolic studies were performed in post-absorptive state after a 12 h overnight fast. In diabetic 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, 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 ensure arterialisation of the venous blood. At 08:00 hours, a prime (0.6 mg/kg bolus) continuous (1.2 mg kg^{-1} h^{-1}) infusion of L-[5,5,5,-$^{2}H_3$]leucine (Cambridge Isotope Laboratories, Andover, MA, USA) was begun and continued for 5 h by a syringe pump (Harvard Apparatus, Ealing, South Natick, MA, USA). At −15, 0, 180, 210, 240, 270 and 300 min, 10 ml blood were collected to measure the plasma concentration and enrichment of both leucine and α-ketoisocaproic acid (KIC), and the enrichment of [$^{2}H_3$] leucine bound to plasma albumin and fibrinogen. At the end of continuous leucine infusion period, plasma volume was determined by the Evans blue dye dilution method. Briefly, a bolus of approximately 4 ml of normal saline (9 g/l NaCl) solution containing 5 mg/ml sterile, pyrogen-free Evans Blue dye (ICN Biomedicals, Aurora, OH, USA) 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. In diabetic patients, during the second hour of leucine infusion, respiratory exchange measurements by continuous indirect calorimetry were also performed for 45 min. Briefly, a plastic ventilated hood was placed over the head of the participant and made airtight around the neck. A slight negative pressure was maintained inside the hood to avoid loss of the expired air. The carbon dioxide and oxygen content of the expired air were measured continuously.

Analytical determinations Leucine and KIC were extracted from plasma samples as previously described [13]. Enrichments and concentrations of plasma leucine and KIC were determined on their *t*-butyldimethylsilyl derivatives using gas chromatography–mass spectrometry in electron impact ionisation mode (GC8000, MS Voyager Finningan; ThermoQuest Italia, Milan, Italy), monitoring the ions 302 and 305 for leucine and 301 and 304 for KIC [14]. Plasma albumin and fibrinogen were purified as previously described in detail [15, 16]. To evaluate plasma volume, serum samples were added with an equal volume of polyethylene glycol (~4,000 Da; J. T. Baker, Deventer, Holland) solution (240 g/l) for precipitation of non-albumin proteins. Samples and standards were centrifuged for 10 min at 750×*g* (3,000 rpm). Supernatant fractions from samples and standards were then read at 620 nm [17] using a spectrophotometer (Ciba-Corning Diagnostics Limited, Halstead, UK). Serum albumin concentration was determined by standard Bromcresol Green method [18] (ALB plus; Roche Diagnostics, Mannheim, Germany) on a Hitachi 747 (Milan, Italy). Plasma chronometric determination of fibrinogen was obtained in citrate plasma using the clotting method of Clauss [19] on a Hemolab Fibrinomat (bioMérieux, Lyon, France) [20]. Serum samples for IL-6 and C-reactive protein (CRP) were determined in duplicate using commercially available immunosorbent kits (Human IL-6 ELISA; Diaclone Tepnel Lifecodes, Stamford, CT, USA; CRP Ultra; Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL, USA). Urinary protein excretion was measured in 24 h urine samples using a modification of the Coomassie Brilliant Blue method [21] (Total Protein Test Kit; Bio-Rad Laboratories, Milan, Italy). Urinary albumin excretion was measured in 24 h urine samples using immunoturbidimetric assay (Tina-quant Albumin; Roche Diagnostics, Mannheim, Germany) on an autoanalyser (Hitachi 717; Boehringer Mannheim Diagnostic, Indianapolis, IN, USA).

Calculations and statistics The enrichment of leucine and KIC was expressed as the tracer/tracee ratio (TTR), accounting for isotopomer skewed distribution and spectra overlapping when appropriate. Whole-body endogenous leucine flux (ELF) was calculated as the rate of appearance of leucine (μmol kg^{-1} min^{-1}) as follows: $R_a = I/E_p$, where R_a is the rate of appearance, I is the isotope infusion rate of leucine and E_p is the plasma enrichment (TTR) of KIC. The estimates of whole-body leucine kinetic were determined on the data obtained during the last 2 h of the study (180–

300 min) at the isotopic and metabolic steady state [22]. Albumin and fibrinogen fractional synthesis rates (FSR) were calculated by dividing the slope of the increase in enrichment of leucine bound to albumin or fibrinogen by the enrichment of plasma KIC over the last 2 h of the study. Absolute synthesis rate (ASR) for intravascular albumin and fibrinogen were estimated by multiplying albumin or fibrinogen FSR by total intravascular albumin or fibrinogen content. 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 plasma volume by standard dilution formula: plasma volume (ml) = dose of Evans Blue dye (μg) injected/serum concentration of Evans Blue dye (μg/ml) extrapolated at time zero [17]. Dietary protein intake in diabetic patients and compliance with the diet were evaluated from weekly determination of 24 h urinary nitrogen excretion according to the formula: urinary nitrogen = urine urea nitrogen + non-urea nitrogen, where 1 g urinary nitrogen=6.25 g protein, and non-urea nitrogen excretion=30 mg kg^{-1} day^{-1} [23]. Urinary protein loss was added to the above formula. Oxygen consumption and carbon dioxide production were determined with a Deltatrac M 100 calorimeter (Datex, Helsinki, Finland). Energy expenditure was calculated from calorimetric data using standard formulas [24]. Protein oxidation was evaluated from 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 [24].

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

Results

Clinical characteristics and renal function Blood pressure in type 2 diabetic patients was 132±4 and 78±2 mmHg and did not change while consuming LPD (133±4 and 80±2 mmHg). Their blood urea nitrogen was elevated in comparison with healthy control participants (16.4±5 and 5.0±3 mmol/l, respectively; $p<0.01$ vs control) and significantly decreased after LPD (13.2±4 mmol/l; $p<0.01$ vs NPD). In type 2 diabetic patients, serum creatinine was elevated in comparison to healthy controls (247.5±4 and 76.9±3 μmol/l, respectively; $p<0.01$ vs control) and did not change significantly after LPD (212.2±3 μmol/l). Creatinine clearance in type 2 diabetic patients did not change significantly between NPD (30±3 ml min^{-1} 1.73 m^{-2}) and LPD (32±4 ml min^{-1} 1.73 m^{-2}) treatments. Similarly, HbA_{1c} (7.3±0.9%) did not change significantly after the LPD (7.3±0.8%). In addition, total cholesterol, HDL-cholesterol and triacylglycerol (5.3±0.11, 1.2±0.03 and 2.0±0.28 mmol/l, respectively) did not change significantly in type 2 diabetic patients between NPD and LPD (5.1±0.11, 1.2±0.02 and 1.7±0.27 mmol/l, respectively).

After LPD, 24 h proteinuria and albuminuria decreased from 4.5±0.8 (NPD) to 2.7±0.4 and from 2.5±0.4 (NPD) to 1.6±0.2 g/day, respectively ($p<0.01$ for both). After both NPD and LPD, plasma volume of type 2 diabetic patients was significantly increased (NPD 3067±158, LPD 3011±152 ml/1.72 m^2) in comparison with that of control participants (2728±148 ml/1.72 m^2, $p<0.05$).

Protein intake, endogenous leucine flux and protein oxidation As shown in Fig. 1, in type 2 diabetic patients during NPD, protein intake (evaluated by urinary nitrogen excretion) did not differ from control participants (1.38±0.08 and 1.27±0.07 g kg^{-1} day^{-1} respectively), but was significantly reduced during LPD (0.81±0.04 g kg^{-1} day^{-1}; $p<0.01$ vs NPD).

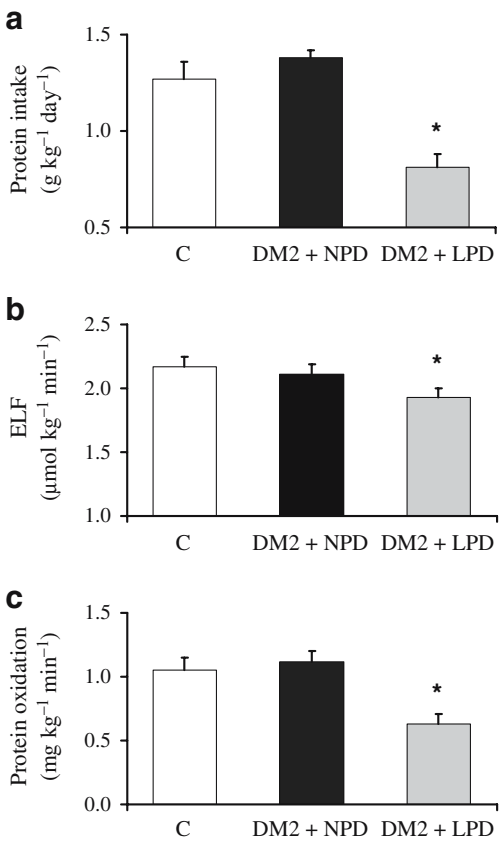

Fig. 1 Protein intake (**a**), ELF (**b**) and protein oxidation (**c**) in type 2 diabetic patients while consuming a NPD (DM2 + NPD) and after LPD (DM2 + LPD). Values are mean±SEM. *$p<0.05$ vs NPD. C, control

In control participants, ELF (an index of whole-body protein breakdown) was 2.17±0.07 μmol kg^{-1} min^{-1} and did not differ in comparison with type 2 diabetic patients assuming NPD (2.11±0.08 μmol kg^{-1} min^{-1}). In type 2 diabetic patients, LPD significantly reduced ELF (1.93± 0.08 μmol kg^{-1} min^{-1}; $p<0.05$ vs NPD).

In type 2 diabetic patients basal protein oxidation was 1.12±0.1 mg kg^{-1} min^{-1} and markedly decreased to 0.63± 0.08 mg kg^{-1} min^{-1} after LPD ($p<0.01$ vs NPD).

Albumin synthesis Figure 2 shows that serum albumin levels in control participants were 42±0.8 g/l. In type 2 diabetic patients, serum albumin concentration was reduced in comparison to healthy participants (38±1.1 g/l during NPD; $p<0.05$ vs control) and returned to normal values (42±1.1 g/l) after LPD ($p<0.05$ vs NPD).

Total plasma albumin pool in control participants was 114±3 g/1.73 m^2. In type 2 diabetic patients during NPD plasma albumin pool was similar to controls (118±7 g/ 1.73 m^2). After LPD, plasma albumin pool in type 2 diabetic patients increased to 129±7 g/1.73 m^2, ($p<0.05$ vs NPD and vs control).

FSR of albumin was 9.0±0.5% per day in control participants. In type 2 diabetic patients during NPD, FSR of albumin was similar to that of controls (8.4±0.9% per day), increasing after LPD to 11.0±1% per day ($p<0.05$ vs NPD).

ASR of albumin in control participants averaged 10.3± 0.7 g 1.73 m^{-2} day^{-1}. In type 2 diabetic patients during NPD, ASR of albumin was similar to that of controls (9.9± 1 g 1.73 m^{-2} day^{-1}), increasing after LPD to 14.1±1 g 1.73 m^{-2} day^{-1} ($p<0.05$ vs NPD).

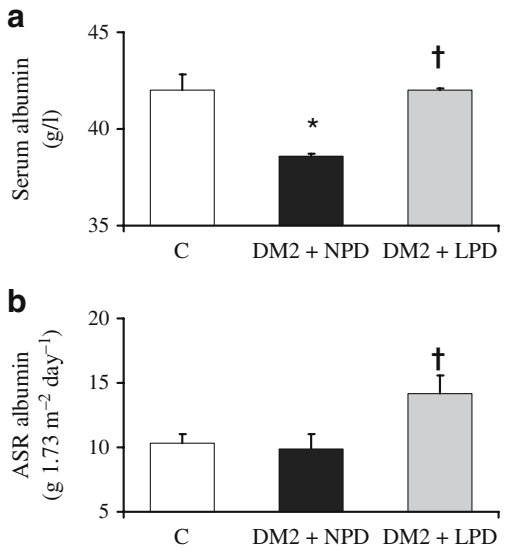

Fig. 2 Serum albumin concentration (**a**) and ASR albumin (**b**) in control patients (C) and in type 2 diabetic patients while consuming a NPD (DM2 + NPD) and after LPD (DM2 + LPD). Values are mean±SEM. *$p<0.05$ vs control; †$p<0.05$ vs NPD

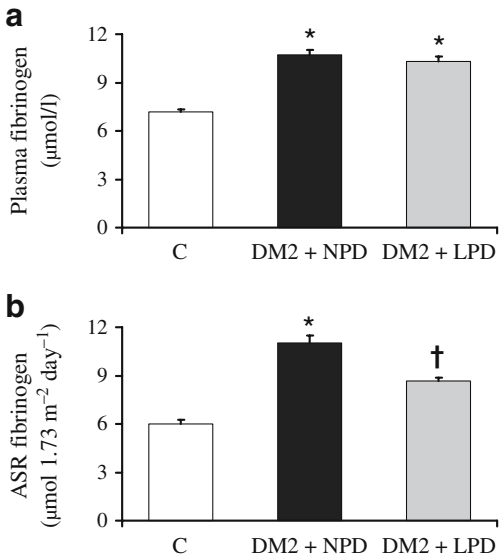

Fig. 3 Plasma fibrinogen concentration (**a**) and fibrinogen ASR (**b**) in control patients (C) and in type 2 diabetic patients while consuming a NPD (DM2 + NPD) and after LPD (DM2 + LPD). Values are mean±SEM (to convert μmol to g divide by 2.94). *$p<$ 0.05 vs control; †$p<0.05$ vs NPD

Fibrinogen metabolism As seen in Fig. 3, plasma fibrinogen concentration in control participants averaged 7.2± 0.5 μmol/l (2.45±0.18 g/l). In type 2 diabetic patients during NPD, plasma fibrinogen concentration increased to 10.7±0.6 μmol/l (3.64±0.22 g/l) ($p<0.01$ vs control) and dropped after LPD to 10.3±0.6 μmol/l (3.52±0.19 g/l) ($p=$ 0.10 vs NPD).

Total plasma fibrinogen pool in control participants was 19.7±1.5 μmol/1.73 m^2 (6.7±0.5 g/1.73 m^2). In type 2 diabetic patients during NPD, plasma fibrinogen pool increased to 32.6±2.4 μmol/1.73 m^2 (11.1±0.8 g/1.73 m^2; $p<0.01$ vs control), decreasing after LPD to 29.4± 2.1 μmol/1.73 m^2 (10.0±0.7 g/1.73 m^2; $p=0.08$ vs NPD).

FSR of fibrinogen was 28.3±2% per day in control participants. In type 2 diabetic patients during NPD, it was increased at 34.2±3% per day ($p<0.05$ vs NPD). After LPD, it returned to values similar to those of healthy control participants (28.2±2% per day).

ASR of fibrinogen averaged 6.0±1.8 μmol 1.73 m^{-2} day^{-1} (1.9±0.3 g 1.73 m^{-2} day^{-1}) in control subjects. In type 2 diabetic subjects, ASR of fibrinogen was markedly increased during NPD [11.03±1.17 μmol 1.73 m^{-2} day^{-1} (3.7±0.4 g m^{-2} day^{-1})] ($p<0.01$ vs controls). After the LPD, ASR of fibrinogen slightly decrease to 8.67± 0.58 μmol 1.73 m^{-2} day^{-1} (2.9±0.2 g 1.73 m^{-2} day^{-1}; $p<$ 0.05 vs NPD).

Low-grade inflammation In type 2 diabetic patients, serum CRP concentration was 4.5±1.0 mg/l during NPD ($p<0.05$ vs control) and decreased to 2.9±0.6 mg/l during LPD ($p<$ 0.05 vs NPD). Similarly, serum IL-6 concentration was

5.3±2 pg/ml during NPD ($p<0.05$ vs control) and decreased to 4.5±1 pg/ml during LPD ($p<0.05$ vs NPD). The increment of albumin ASR induced by LPD positively correlated with the decline in serum CRP ($r=0.653$, $p<0.05$) and IL-6 levels ($r=0.708$, $p<0.05$).

Discussion

The present study shows that in type 2 diabetic patients with macroalbuminuria, a protein-restricted diet providing about 0.8 g kg^{-1} day^{-1} significantly reduced proteinuria (39%), albuminuria (37%), the breakdown and oxidation of whole-body protein, markers of low-grade inflammation and fibrinogen ASR, while augmenting the synthesis and concentration of serum albumin. The antiproteinuric effect of LPD occurred independently of the concomitant ACE inhibition treatment. Several studies in type 1 diabetes patients with varying stages of nephropathy have shown that protein restriction reduces albuminuria and the progression of GFR decline [25–28]. On the other hand, few studies have evaluated the effect of LPD in type 2 diabetic patients with macroalbuminuria. In agreement with the present data, these studies reported a beneficial effect of LPD on proteinuria [8, 29]. Thus, the beneficial effect of moderate protein restriction on proteinuria, which appears to be independent of concomitant ACE-inhibition treatment, may play an important role in the management of diabetic nephropathy.

In non-diabetic nephrotic syndrome we have previously demonstrated that abnormal hepatic synthesis rate of albumin is ameliorated by dietary protein restriction [10]. With regard to this, no study has evaluated the effects of LPD on albumin metabolism in type 2 diabetes mellitus. In the present study, we report that type 2 diabetic patients with macroalbuminuria are characterised by reduced plasma albumin concentration, which is not compensated by an increase in hepatic albumin synthesis. After LPD, their FSR and ASR of albumin, as well as serum albumin pool and concentration, increased significantly. Interestingly, LPD also resulted in a reduction of the elevated levels of two markers of low-grade inflammation (CRP and IL-6). Elevated IL-6 levels have been reported to be associated with reduced albumin concentration in end-stage renal disease [30]. Thus, it cannot be excluded that a low-grade inflammatory state may have lead to a reduced albumin hepatic response to macroalbuminuria, with consequent reduced plasma albumin levels. In agreement with this hypothesis, we found in the present study that the increment in hepatic albumin synthesis after LPD positively correlated with the decline of both plasma CRP ($r=0.653$, $p<0.05$) and IL-6 levels ($r=0.708$, $p<0.05$).

It has been previously reported that type 2 diabetic patients are characterised by elevated fibrinogen synthesis and plasma levels [5, 6]. Our data confirm this observation and demonstrate that LPD is able to reduce ASR of fibrinogen, although it did not significantly reduce fibrinogen FSR. This blunted effect of LPD on fibrinogen FSR is at variance to what we have previously observed in nephrotic syndrome [10]. The reason for this difference is unknown. However, in the present study, diabetic patients were all treated with ACE inhibitors and it has been reported that treatment with ACE inhibition or angiotensin receptor blocker may reduce plasma fibrinogen levels [31, 32]. Thus, it can be hypothesised that previous ACE-inhibition may have blunted the beneficial effect of LPD on fibrinogen metabolism. The beneficial effects of LPD on fibrinogen and inflammatory markers also seems to lower the cardiovascular risk, as CRP changed from a high value (>3 mg/l) to average risk (<3 mg/l) as defined by the American Heart Association statement on cardiovascular biomarkers [33]. In particular, the magnitude of reduction of CRP levels induced by LPD was similar to that seen after renin–angiotensin–aldosterone system blockade [34] or statin treatment [35].

In type 1 diabetic mellitus patients, concerns have been raised on the potential risk of protein malnutrition with a LPD, because this may be associated with enhanced protein breakdown induced by insulin deficiency [36]. No data are available on the mechanism of adaptation to moderate protein restriction in type 2 diabetic patients. In the present study, we observed that after LPD, type 2 diabetic patients had reduced ELF, an index of whole-body proteolysis, associated with a decrease in protein oxidation. This complex metabolic adaptation is somewhat similar to that observed in normal participants during hypoaminoacidaemia [37] and possibly prevents type 2 diabetic patients from developing protein malnutrition after a moderate protein restriction diet. In agreement with this hypothesis, we observed increased serum albumin levels and synthetic rates after LPD in type 2 diabetic patients.

Taken together, the present findings suggest that, in type 2 diabetic patients with macroalbuminuria, moderate dietary protein restriction (providing about 0.8 g kg^{-1} day^{-1}) may be useful in the management of diabetic nephropathy. In fact, LPD induced a significant reduction in proteinuria and low-grade inflammation, while ameliorating albumin synthesis with an increase in plasma albumin levels. In addition, these changes are associated with a decrease in protein oxidation and breakdown, suggesting an adaptive response to LPD, which probably prevents diabetic patients from developing protein malnutrition. However, the small number of patients may represent a limitation of this study

and additional data are needed to confirm the present findings.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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