Applied nutritional investigation

Long-term effects of moderate protein diet on renal function and low-grade inflammation in older adults with type 2 diabetes and chronic kidney disease

Mauro Giordano M.D., Ph.D. [a,*], Tiziana Ciarambino M.D. [a], Pietro Castellino M.D. [b], Alessandro Cataliotti M.D. [b], Lorenzo Malatino M.D. [b], Nicola Ferrara M.D. [c], Cecilia Politi M.D. [a], Giuseppe Paolisso M.D. [a]

[a] Department of Medical, Surgical, Neurologic, Metabolic and Geriatric Sciences, Second University of Naples, Naples, Italy
[b] Department of Internal Medicine, Faculty of Medicine, University of Catania, Catania, Italy
[c] Department of Translational Medical Sciences, University of Naples "Federico II", Naples, Italy

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ABSTRACT

Objectives: The aim of this study was to determine the long-term effects of a moderate protein diet (MPD) on renal function, low-grade inflammation, and oxidative stress in older adults with type 2 diabetes, which to date are unclear.
Methods: Seventy-four older adults with type 2 diabetes and chronic kidney disease (stage G3b–G4) were enrolled in the study. During the 4-wk baseline period (T0), all patients were asked to follow a normal protein diet regimen, providing 1.1 g/kg daily. Successively, all patients were asked to follow an MPD, for 36 mo, providing 0.7 g/kg daily, for only 6 d/wk. Patients who refused to follow an MPD treatment were included in the control (NPD [normal protein diet] group). During the 36 mo of the study, creatinine clearance, blood urea nitrogen, proteinuria, blood pressure, glycated hemoglobin (Hb)A$_{1c}$, fat-free mass, low-grade inflammation (interleukin-6 and C-reactive protein) were evaluated monthly and oxidative stress (urinary 8-epiprostaglandin [Epi-PG]F2α) was evaluated every 3 mo.
Results: During T0, mean creatinine clearance, proteinuria, blood urea nitrogen, blood pressure, HbA$_{1c}$, fat free mass, low-grade inflammation, and oxidative stress were similar in both groups. After 36 mo, a significant reduction in decline of renal function was observed in the MPD group but not in controls (2.4 $\pm$ 0.2 versus 5.7 $\pm$ 0.5 mL·min·y, respectively; $P < 0.05$ versus control). Similarly, a significant reduction in proteinuria, serum interleukin-6, serum C-reactive protein, and urinary 8-Epi-PGF2α excretion, was observed in the MPD group ($P < 0.05$ versus NPD).
Conclusion: In older adults with type 2 diabetes, long-term effects of an MPD regimen are associated with a significant decline of renal function, proteinuria, low-grade inflammation, and oxidative stress without a change in fat-free mass.

Introduction

Experimental animal models have demonstrated the beneficial effects of dietary protein restriction in delaying the progression of renal disease [1]. In fact, dietary protein restriction may retard the decline of renal function and alleviates uremic symptoms caused by the accumulation of phosphorus, sulphates, organic acids, and urea [2,3]. It has been suggested that dietary protein restriction also may delay the need for dialysis therapy

[4], and favors the correction of secondary hyperparathyroidism and metabolic acidosis [5]. However, data on dietary protein restriction are unclear. In a recent study with 112 patients with type 2 diabetes mellitus (T2DM) and overt nephropathy, it was reported that protein restriction was neither feasible nor efficacious. The authors reported that the prescribed protein intake in the low-protein diet group was not statistically different from the protein intake in the normal protein diet group. Thus, non-adherence to the prescribed dietary protein restriction may result in an underestimation of its beneficial effects, in this study [6]. In fact, adherence to dietary protein restriction also could affect depressive symptoms. We previously demonstrated that

* Corresponding author. Tel.: +39 081 566 6037.
E-mail address: mauro.giordano@unina2.it (M. Giordano).

the reduction of a dietary protein restriction regimen, from 7 to 6 d/wk was associated with an improvement in depressive symptoms and health-related quality of life in older patients with T2DM [7]. Furthermore, it has been reported that a reduced protein intake has an antioxidant effect in chronic kidney disease (CKD) in animals and humans [8,9]. In an animal study, dietary protein restriction appeared to ameliorate inflammation, suppress oxidative stress, and reduce proteinuria [10]. Additionally it has been reported that unsaturated lipids also might be linked to anti-inflammatory status [11]. In this regard, little data are available on the long-term effects of a moderate protein diet (MPD) restriction regimen in older adults. Thus, in this study, we evaluated the effects of an MPD regimen, after 36 mo, on the decline of renal function, low-grade inflammation, oxidative stress, proteinuria, and body composition in older patients with T2DM and CKD.

Methods

Patient population

In the study protocol, 74 older patients with T2DM participated. All patients were clinically assessed with a medical history, physical examination, and routine laboratory tests. Eligibility criteria for all patients included age >65 y; CKD (stage G3b–G4 defined according to the Kidney Disease: Improving Global Outcomes classification) [12] and T2DM (defined according to American Diabetes Association) [13] for at least 15 y and treated by diet plus insulin injection. All participants had arterial hypertension (defined according to Eighth Joint National Committee criteria) [14] and were being treated by diet plus oral Ras inhibitors. During the 4-wk baseline period (T0), all patients were requested to follow a normal protein diet regimen, providing 1.1 g/kg daily. Successively, all patients were asked to follow a restricted MPD for 36 mo, providing 0.7 g/kg daily, for 6 d/wk. Forty patients agreed to follow an MPD regimen (18 men; body mass index [BMI] 30.8 ± 3 kg/m^2; age 72 ± 3 y). Thirty-four patients (16 men; BMI 31.4 ± 2 kg/m^2; age 71 ± 2 y) refused to adhere to an MPD regimen and were included in the normal protein diet (NPD) group. Patient characteristics are reported in Table 1. The purpose of the study was explained to all patients and their voluntary written consent was obtained before starting. The study protocol was reviewed and approved by the Ethics Committee of our Institution (protocol N. 150114/514).

Experimental protocol

All patients were instructed to consume a weight maintenance diet providing about 27 to 30 kcal/kg daily and containing about 250 to 300 g of carbohydrates. During the 4-wk baseline period (T0), patients followed a normal protein intake regimen; dietary carbohydrates and lipids represented 50% and 25% of the total caloric intake. In the MPD group, lipid contribution to total calories was increased to 35% (increasing the unsaturated fat portion), phosphate and calcium intake were 872 ± 63 mg and 743 ± 79 mg, respectively. In the MPD group, more than 65% of the ingested protein was of high biological value. One day per week (usually on Sunday) patients were off their diet. At baseline, all patients were visited weekly. During the 36 mo of follow-up, both groups were visited monthly. To verify compliance with the diet, patients in the MPD group were asked to return to the clinical unit on a monthly basis for a 2-d dietary recording [15].

All participants were given instructions for 24-h urine samples, recording volume and time of collection at each visit. At each visit, two consecutive 24-h urine specimens were collected from the patients on the same day of the weighed dietary record to determine endogenous creatinine clearance (CrCl), urinary protein, and nitrogen excretion. CrCls were estimated using the Chronic Kidney Disease Epidemiology Collaboration formulas and expressed in mL·min·1.73 m^2 of surface area [16]. The 24-h urinary nitrogen output was considered the standard criteria for protein intake evaluation [15]. Urinary protein excretion was measured from 24-h urine samples using a modification of Coomassie Brilliant Blue method (Total Protein Test Kit, Bio-Rad Laboratories srl, Milan, Italy) [17]. Serum samples for interleukin (IL)-6 and C-reactive protein (CRP) were evaluated and done in duplicate using commercially available immunosorbent kits (human IL-6 enzyme-linked immunosorbent assay, Diaclone, France and CRP Ultra ABBOTT Diagnostics, Abbott Park, IL, USA). Food intake was analyzed by Dietosystem (DS Medica srl, 20125 Milano, Italy). Body composition was assessed with a multifrequency bioelectrical impedance meter using a Dietosystem Human IM Plus. Briefly, whole-body resistance and reactance were measured with four surface electrodes placed on the right wrist and ankle. An electrical current at the following frequencies: 1, 5, 10, 50 and 100 kHz

Table 1
Baseline clinical characteristics of patients in NPD and MPD groups

	NPD group (n = 34)	MPD group (n = 40)
Age (y)	71 ± 2	72 ± 3
BMI (kg/m^2)	31.4 ± 2	30.8 ± 3
SBP (mm Hg)	135 ± 3	136 ± 4
DBP (mm Hg)	81 ± 2	80 ± 2
FPG (mg/dL)	124 ± 8	127 ± 9
HbA$_{1c}$ (%)	6.7 ± 0.3	6.6 ± 0.4
Serum albumin (g/dL)	3.6 ± 0.2	3.5 ± 0.3
Serum creatinine (mg/dL)	2.0 ± 0.2	2.1 ± 0.2
Serum BUN (mg/dL)	40.4 ± 4	40.1 ± 3
FFM (%)	59 ± 2	58 ± 3
CRP (mg/L)	4.8 ± 0.5	4.9 ± 0.4
IL-6 (pg/mL)	5.6 ± 0.9	5.5 ± 0.9
8-epi-PGF2α (pmol/mmol Cr)	188 ± 39	201 ± 45
Urinary protein excretion (g/d)	2.3 ± 0.9	2.4 ± 0.5
CrCl (mL·min·1.73 m^2)	35.2 ± 0.5	33.8 ± 0.7

BMI, body mass index; BUN, blood urea nitrogen; CrCl, creatinine clearance; CRP, C-reactive protein; DBP, diastolic blood pressure; 8-epi-PGF2α, 8-epi-prostaglandin F2α; FFM, fat-free mass; FPG, fasting plasma glucose; HbA$_{1c}$, glycated hemoglobin; IL, interleukin; MPD, moderate protein diet; NPD, normal protein diet; SBP, systolic blood pressure
Values are mean ± SEM

was produced by a generator (Dietosystem Human IM Plus) and applied to the skin with adhesive electrodes (Mod. EL01 PG-500 28 × 34 mm), with the individual lying supine [18].

In all participants, urinary concentration of 8-isoprostane (iso)-prostaglandin (PG) F2α was determined at baseline and every 3 mo thereafter, by enzyme immunoassay for the quantitative determination 8-isoprostane (IBL Immuno-Biological Laboratories Hamburg, Germany). All samples were free of organic solvents before assay. Samples were assayed immediately after collection. Samples that could not be assayed immediately were stored at −80°C in the presence of 0.005% butylated hydroxytoluene to prevent oxidative formation of isoprostane. All samples were evaluated within 72 h. Urine samples were centrifuged briefly to remove sediment and proceed with the purification using the 8-isoprostane Affinity Purification Kit (DDT 58 Cod 516358, Italy Cabrus.a.s). Urine samples give an excellent correlation to gas chromatography-mass spectrometry after purified by solid-phase extraction and thin-layer chromatography before analyzed. Urinary concentrations of 8-iso-PGF2α (pmol/L) were corrected by urinary creatinine concentration (mmol/L) to account for differences in renal excretory function [19].

Statistical analysis

All data are expressed as mean ± SE. The data were analyzed by analysis of variance for repeated measures. Comparison between the two groups of patients was performed by unpaired t test. Comparison in the same group before and after of the treatments (T0 versus T36), were performed by paired t test. Differences at the P < 0.05 level of probability were considered significant. Statistical analysis was performed using SPSS software package (version 16.0, SPSS Inc, Chicago, IL, USA).

Results

At baseline (T0), BMI, fat-free mass (FFM,) systolic blood pressure (SBP), diastolic blood pressure (DBP), glycated hemoglobin (Hb)A$_{1c}$, serum creatinine, blood urea nitrogen (BUN), and urinary protein excretion were similar in both study groups and are reported in Table 1.

Renal function and blood pressure

After 36 mo, serum creatinine levels in the NPD group increased from 2.0 ± 0.2 mg/dL at baseline to 3.9 ± 0.3 mg/dL at T36 (P < 0.01); CrCl decreased from 35.2 ± 0.5 mL·min·1.73 m^2 at baseline to 18.1 ± 0.3 mL·min·1.73 m^2 at 36 mo (P < 0.01). During the same time period, BUN increased from 40.4 ± 4 mg/dL to 86.5 ± 2 mg/dL (P < 0.01). Mean urinary protein excretion increased from 2.3 ± 0.9 g/d but did not change after T36 (2.4 ± 0.8 g/d; P = ns).

After 36 mo, serum creatinine in the MPD group increased from 2.1 ± 0.2 mg/dL at T0 to 2.8 ± 0.6 mg/dL at T36 ($P < 0.05$); CrCl decreased from 33.8 ± 0.7 mL·min·1.73 m^2 at T0 to 26.6 ± 0.4 mL·min·1.73 m^2 at T36 ($P < 0.05$); and serum BUN increased from 40.1 ± 3 mg/dL at T0 to 50.2 ± 4 mg/dL at T36 ($P < 0.05$). Mean urinary protein excretion significantly decreased from 2.4 ± 0.5 g/d at T0 to 1.1 ± 0.2 g/d at T36 ($P < 0.05$).

After 36 mo, an annual decline was observed in CrCl of 5.7 ± 0.5 mL/min in NPD group, while it was significantly reduced to 2.4 ± 0.2 mL/min in the MPD group ($P < 0.05$ versus NPD).

In the NPD group, SBP and DBP at T0 were 135 ± 3 and 81 ± 2 mm Hg, respectively and did not change at T36 (137 ± 2 and 78 ± 2 mm Hg, respectively; $P = $ ns).

In the MPD group, SBP and DBP at T0 were 136 ± 4 and 80 ± 2 mm Hg, respectively and did not change at T36 (135 ± 3 and 79 ± 2 mm Hg, respectively; $P = $ ns). (See Fig. 1).

Nutritional parameters

In the NPD group, daily mean protein intake at T0 was 1.13 ± 0.2 g/kg and did not change during the 36 mo of treatment (i.e., at T36 it was 1.08 ± 0.2 g/kg; $P = $ ns). At T0, BMI was 31.4 ± 2 kg/m^2 and did not change at T36 (32.2 ± 1.0 kg/m^2; $P = $ ns). FFM at T0 was 59.3% ± 2% and did not change at T36 (58.2% ± 3%; $P = $ ns). At T0, serum albumin was 3.6 ± 0.2 g/dL and did not change at T36 (3.4 ± 0.2 g/dL; $P = $ ns). At T0, fasting plasma glucose was 124 ± 8 mg/dL and did not change at T36 (128 ± 7 mg/dL; $P = $ ns). HbA$_{1c}$ at T0 was 6.7% ± 0.3% and did not change at T36 (6.8% ± 0.1%; $P = $ ns).

In the MPD group, daily mean protein intake at T0 was 1.15 ± 0.2 g/kg and was significantly reduced during the 36 mo of treatment; at T36 it was 0.76 ± 0.1 g/kg ($P < 0.05$ versus T0). BMI at T0 was 30.8 ± 3 kg/m^2 and did not change at T36 (31.5 ± 1.1 kg/m^2; $P = $ ns); . FFM at T0 was 58.7% ± 3% and did not change at T36 (57.5% ± 4%; $P = $ ns). Serum albumin at T0 was 3.5 ± 0.3 g/dL and did not change at T36 (3.4 ± 0.3 g/dL; $P = $ ns). Fasting plasma glucose at T0 was 127 ± 9 mg/dL and did not change at T36 (126 ± 8 mg/dL; $P = $ ns); HbA$_{1c}$ at T0 was 6.6% ± 0.4% and did not change at T36 (6.5% ± 0.2%; $P = $ ns).

Low-grade inflammation and oxidative stress

In the NPD group, CRP levels at T0 were 4.8 ± 0.5 mg/L and did not change at T36 (4.6 ± 0.5 mg/L; $P = $ ns). IL-6 levels at T0 were 5.6 ± 0.9 pg/mL and did not change at T36 (5.8 ± 0.8 mg/L; $P = $ ns).

In the MPD group, CRP levels were reduced from 4.9 ± 0.4 mg/L at T0 to 2.8 ± 0.4 mg/L at T36 ($P < 0.05$). Similarly, IL-6 levels were reduced from 5.5 ± 0.9 pg/mL at T0 to 4.2 ± 0.7 pg/mL at T36 ($P < 0.05$).

In the NPD group, urinary 8-Epi-PGF2α excretions at T0 were 188 ± 39 pmol/mmol of creatinine and did not change significantly at T36 (197 ± 43 pmol/mmol of creatinine; $P = $ ns). In the MPD group, urinary 8-Epi-PGF2α excretions were reduced from 201 ± 45 pmol/mmol of creatinine at T0 to 115 ± 29 pmol/mmol of creatinine at T36 ($P < 0.05$). A positive correlation was observed between protein intake and urinary 8-iso-PGF2α excretion ($r = 0.70$; $P < 0.01$). (See Figs. 2 and 3.)

Discussion

In this study, we reported that, an MPD regimen (6 d/wk) in older patients with T2DM significantly reduced renal failure by
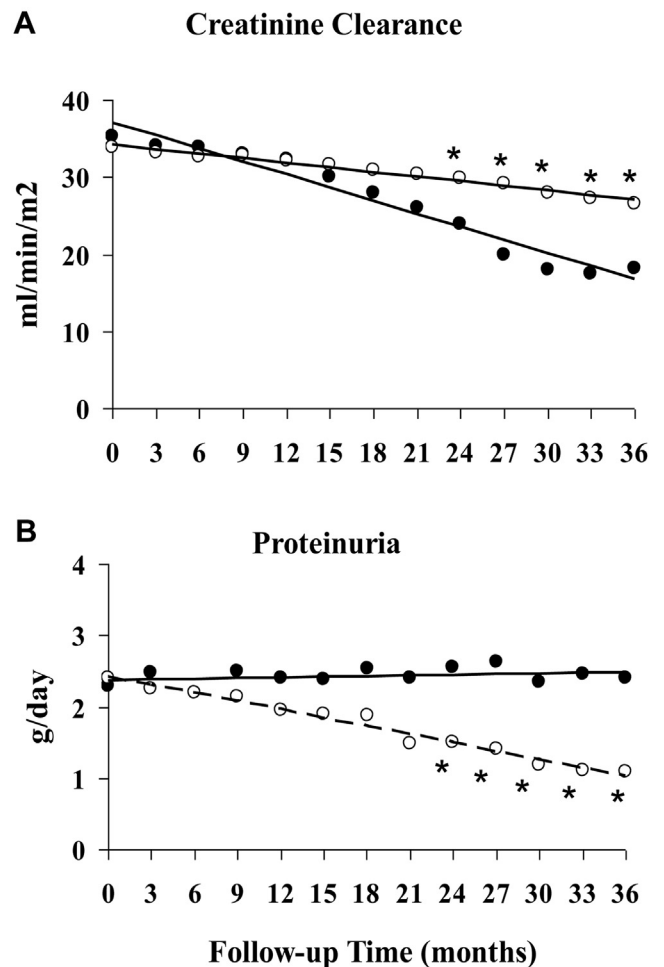

Fig. 1. Changes in (A) creatinine clearance and (B) 24-h proteinuria in older patients with diabetes on a moderate protein diet (MPD) (white) or normal protein diet (NPD) (black). *$P < 0.05$ versus NPD.

42% (5.7 versus 2.4 mL/min annually; $P < 0.001$). This beneficial effect is also associated with an improvement in low-grade inflammation, oxidative stress, and proteinuria. Additionally, the MPD did not affect either glycemic control or nutritional status, demonstrating its sustainability long-term.

The role of an MPD regimen in older adults is not clear. Dietary protein restriction has been proposed for many years to delay the progression of nephropathy. However, little data are available on the benefits of an MPD regimen in the management of diabetic nephropathy in the older population. Our data are in agreement with a previous large study that demonstrated similar positive effects of dietary protein restriction [20]. Similarly, results from another study reported the beneficial effects of a very-low-protein diet and a low-protein diet, both supplemented with ketoanalog [21]. However, in a recent study with patients who had diabetic nephropathy, it was reported that dietary protein restriction was neither feasible nor efficacious. That study also reported that the prescribed protein intake in the low-protein diet group resulted in a daily mean protein intake of about 1 g/kg, which was not statistically different from the normal protein diet group. Thus, the non-adherence to the prescribed dietary protein restriction may have underestimated its beneficial effects in this study [7]. Adherence to dietary protein
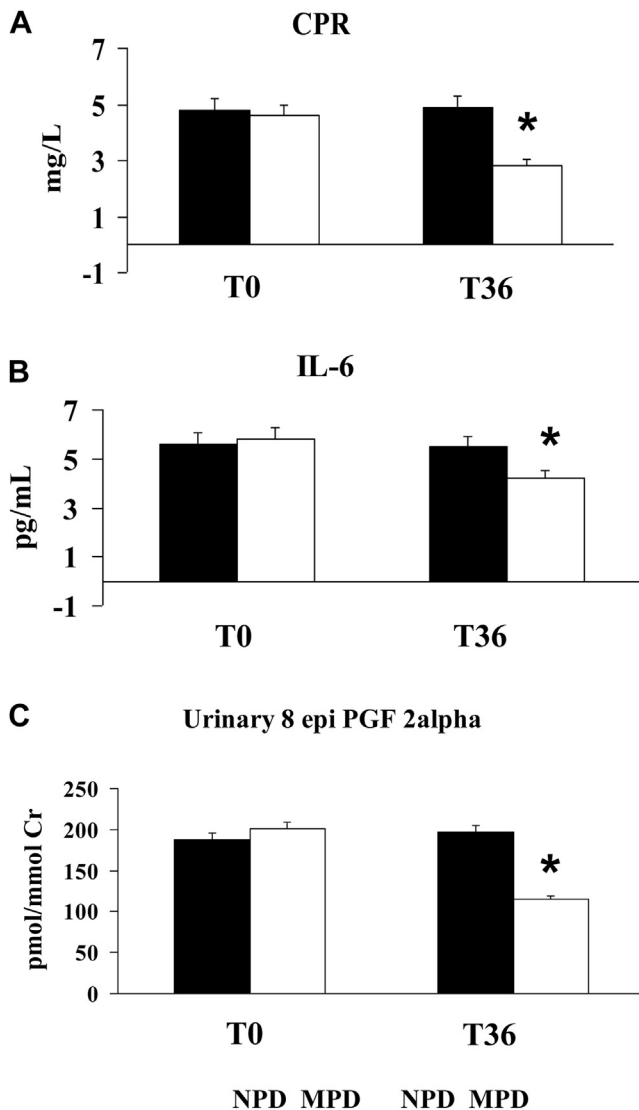
A

CPR

B

IL-6

C

Urinary 8 epi PGF 2alpha

NPD MPD   NPD MPD

Fig. 2. Changes in C-reactive protein (CRP), interleukin-6 (IL-6) and urinary 8-epiprostaglandin (Epi-PG)F2α in older patients with diabetes as the mean value at baseline (T0) and the mean value every 3 mo during the treatment follow-up (T36) in normal protein diet (NPD) (black) and moderate protein diet (MPD) (white) groups. Values are mean ± SEM. $^*P < 0.05$ versus NPD.

Fig. 3. Relationship between the urinary 8-epiprostaglandin (epi-PG)F2α and protein intake in the moderate protein diet (MPD) group ($r = 0.70$, $P < 0.01$).

restriction is probably a crucial point that could explain, at least in part, the different results observed in the long term.

We do not know the exact mechanisms by which the MPD improved the renal failure in our patients. It is well known that the persistence of heavy proteinuria is associated with an intraglomerular hemodynamic change that induces glomerulo-sclerosis and tubulointerstitial damage [22]. In this regard, we reported a significant reduction in proteinuria after an MPD regimen. This antiproteinuric effect of dietary protein restriction in diabetic nephropathy has been previously demonstrated [23] and it seems to be independent from angiotensin-converting enzyme inhibition therapy [24].

Additionally, the MPD group had a 10% of increase of unsaturated fat portion. Thus, it cannot be excluded that this may have contributed at least in part to a lower inflammation low-grade observed in this group. In fact, it has been reported that the unsaturated lipids may have been linked to anti-inflammatory
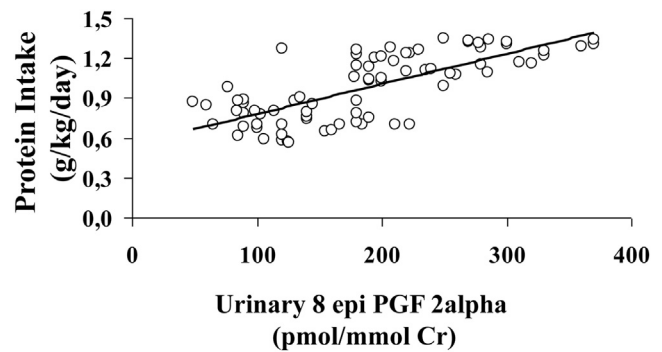
status [11]. It is also possible that the reduced inflammation observed in the present study, after an MPD diet, is associated with lower proteinuria that in turn could positively affect the renal failure. In this regard, it recently has been reported that moderate protein restriction improves oxidative stress, inflammation, and proteinuria in the remnant kidney model [11]. Furthermore, it has been reported that treatment with dietary protein restriction has an antioxidant effect both in humans and animals with CKD [10,25,26]. These data may help explain the mechanisms that underline the benefit of dietary protein restriction on decline of renal function. Thus, it is possible that an MPD regimen in our patients could suppress oxidative stress and inflammation with a consequent reduction in proteinuria. However, long-term dietary protein restriction may expose older individuals to inadequate protein-energy and micronutrient intakes, which may cause deterioration in nutritional status, with the developing of sarcopenia. Following an MPD 6 d/wk regimen, we did not observe changes in FFM. This observation is in agreement with previous data that reported the metabolic changes induced by a reduced protein intake associated with a decrease in protein oxidation and breakdown. This suggested an adaptive response to dietary protein restriction, which probably prevents patients with diabetes from developing protein malnutrition [27]. Additionally, other studies have reported that an MPD (providing sufficient intake of calories and high biological value of protein) does not lead to malnutrition and ameliorates metabolic acidosis [2]. However, the frustration of further oral needs observed during dietary protein restriction frequently leads to chronic depression, withdrawal, and poor quality of life [28]. In this regard, we demonstrated that for older individuals with T2DM, following dietary protein restriction for only 6 d/wk reduces the risk for developing depressive symptoms without worsening health-related quality of life [8]. We also observed that an MPD did not affect glycemia control in these patients. Moreover, these data are in agreement with a previous observation, which reported no changes in glucose metabolism after dietary protein restriction in both animals and humans [29–31].

Conclusions

The present findings suggest that, in older individuals with T2DM, an MPD restriction (providing about 0.7 g/kg per day, 6 d/wk) may be useful in the management of diabetic nephropathy. In fact, the MPD induced a significant reduction in decline of renal function, proteinuria, low-grade inflammation, and oxidative stress. Additionally, these results were not associated with

changes in FFM or glycemic control. A limitation of the present study is represented by the sample size and larger, future studies are needed to confirm the present findings.

Acknowledgments

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