Renal, Metabolic, and Hormonal Responses to Proteins of Different Origin in Normotensive, Nonproteinuric Type I Diabetic Patients

PANAYOTIS S. KONTESSIS, MD
IRINI BOSSINAKOU, MD
LEDA SARIKA, PHD
ELSA ILIOPOULOU, MD
ANTONIS PAPANTONIOU, MD
ROBERTO TREVISAN, MD
DEMETRA ROUSSI, MD
KATHERINE STIPSANELLI, MD
STELIOS GRIGORAKIS, MD
ATHANASIOS SOUVATZOGLOU, MD

OBJECTIVE — Whether the differences in renal function found in vegetarian compared with omnivorous subjects are related to quantity or quality of the protein is unknown. We have studied the renal function of nine normotensive, nonproteinuric type I diabetic patients who were fed in random order for 4 weeks either an animal protein diet (APD) (protein intake 1.1 $g \cdot kg^{-1} \cdot day^{-1}$) or a vegetable protein diet (VPD) (protein intake 0.95 $g \cdot kg^{-1} \cdot day^{-1}$). The two diets were isocaloric.

RESEARCH DESIGN AND METHODS — In a crossover study, we measured glomerular filtration rate (GFR) (inulin clearance), renal plasma flow (RPF) (*p*-aminohippurate clearance), plasma amino acids, growth hormone, glucagon, insulin-like growth factor I (IGF-I), and microalbuminuria.

RESULTS — GFR and RPF were lower with the VPD than with the APD (89.9 ± 4.1 vs. 105.6 ± 5.1 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$, $P < 0.05$, and 425.7 ± 22.2 vs. 477.8 ± 32.2 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$, $P < 0.05$, respectively). Renal vascular resistance (RVR) was higher with the VPD than with the APD (101 ± 25 vs. 91 ± 10 $mmHg \cdot min^{-1} \cdot ml^{-1}$, $P < 0.05$). Filtration fraction (FF) remained unchanged after either diet. Fractional clearance of albumin fell with the VPD to 2.0 ± 0.65 from 3.4 ± 1.15 × 10^{-6} ($P < 0.05$). At the end of the APD and VPD, the plasma levels of growth hormone and glucagon did not differ significantly. Plasma levels of IGF-1 were higher with the APD than with the VPD (1.1 ± 0.6 vs. 0.9 ± 0.13 U/ml, $P < 0.05$). Plasma concentrations of valine and lysine were significantly higher with the APD than with the VPD (234.6 ± 30.3 vs. 164.5 ± 25.4 mmol/l, $P < 0.05$, and 565 ± 45.1 vs. 430 ± 56.1 mmol/l, $P < 0.05$, respectively), whereas plasma valine was strongly correlated to the GFR ($r = 0.832$, $P < 0.01$). No differences were found in other amino acids.

CONCLUSIONS — A VPD has significantly different renal effects from an APD equal in protein intake in normotensive, nonproteinuric type I diabetic patients. This could be explained partly by differences in plasma concentrations of amino acids and IGF-I.

From the Renal Unit and 1st Endocrine Section, Alexandra General Hospital, Athens, Greece.
Address correspondence and reprint requests to Panayotis Kontessis, MD, Renal Unit, Alexandra General Hospital, 80 Vas. Sofias Ave., GR-115 28 Athens, Greece.
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APD, animal protein diet; FF, filtration fraction; GFR, glomerular filtration rate; HPLC, high-pressure liquid chromatography; IGF-I, insulin-like growth factor I; MDRD, Modification of Diet in Renal Disease Study; PAH, *p*-aminohippurate; P:S, polyunsaturated fat:saturated fat ratio; RIA, radioimmunoassay; RPF, renal plasma flow; RVR, renal vascular resistance; VPD, vegetable protein diet.

Protein intake has a profound effect on renal hemodynamics and excretory function. High protein intake may have deleterious effects on the kidney, particularly in patients with preexisting kidney disease (1–3). The restriction of dietary protein limits further damage both in animal models of renal disease and in human nephropathy (4–6). However, recently the data of the Modification of Diet in Renal Disease Study (MDRD) suggest that the effect of a protein-restricted diet in nondiabetic patients with moderate renal insufficiency is minimal (52). Micropuncture studies performed in rats subjected to subtotal nephrectomy or made diabetic with streptozotocin have demonstrated that the restriction of dietary protein reduced the elevated glomerular capillary pressure and filtration in both models of renal disease. Dietary protein restriction also reduced urinary protein and prevented progressive glomerular destruction (7–9). In renal studies, dietary protein restriction can retard the progression of renal failure (10) and maintain the functional renal reserve in patients with diabetic nephropathy (11). It is clear, however, that not all proteins are equal in relation to their renal effects. Vegan and lactovegetarian individuals have a lower level of glomerular filtration rate (GFR) compared with omnivorous subjects (12,13). In these studies, the protein intake was lower in the vegan and vegetarian groups. In the subtotal nephrectomized rat model, animals on a vegetable protein diet (VPD) had less proteinuria and less renal histological damage than those on an animal protein diet (APD) (14). A recent study in healthy humans has strongly suggested that this type of protein is important in regulating renal function (15). These results, however, have come from an experimental model of nephropathy or from healthy subjects. In this study, we describe the results of a crossover study undertaken to explore in type I diabetic patients the renal effects of a 4-week VPD or APD and to investigate their metabolic and hormonal mediators.

Table 1—*Clinical features of nine normotensive, nonproteinuric, insulin-dependent diabetic patients*

	IDDM patients
Patients (*n*)	9
Sex (M/F)	2/7
Age (years)	32 (20–48)
Body mass index (kg/m^2)	23.8 (20.6–27.8)
HbA_{1c} (%)	6.7 (5.1–8.4)
Mean blood pressure (mmHg)	93 (80.3–95.1)
GFR (^{51}Cr-EDTA) (ml · min^{-1} · 1.73 m^{-2})	110 (88–129)

Data are medians (ranges).

RESEARCH DESIGN AND METHODS

Study population

Nine nonproteinuric type I diabetic patients were recruited from our cohort of insulin-dependent patients. All subjects were between 20 and 48 years of age and were within 20% of ideal body weight with normal blood pressure (<140/90 mmHg). No patient was treated with antihypertensive treatment or, especially, with angiotensin-converting enzyme inhibitors. The entry requirements were type I diabetes with onset before the age of 30 years, normal arterial blood pressure, and absence of proteinuria and other causes of renal disease. All patients had initial GFRs between 88 and 129 ml · min^{-1} · (1.73 m^2)$^{-1}$, as determined by measurement of ^{51}Cr-EDTA. Their clinical features and baseline data are given in Table 1. Patients were randomly allocated to a 4-week period on an APD or on a VPD equal in protein intake. At the end of this period, they crossed over to the alternate diet, after at least 1 week on their normal diet to avoid carryover effects. During each dietary period, patients performed two 24-h urine collections for measurement of urea, creatinine, electrolytes, phosphate, total protein, and glucose. The means of the two measurements were used for calculation.

Dietary prescription and assessment

None of the patients were consuming a low-protein diet at the time of recruitment. The two diets were designed to be isocaloric and contained 1 g protein · kg body wt^{-1} · day^{-1}. The VPD contained vegetable protein exclusively, although supplements of animal fats were used to maintain the polyunsaturated fat:saturated fat ratio (P:S), as in the APD. The APD contained ~70% animal protein and 30% vegetable protein to enable an adequate carbohydrate and fiber intake. Calcium and phosphate tablets were prescribed for patients when on the VPD.

Dietary assessments were carried out by a nutritionist (D.R.) using a 3-day weight food record system. Patients weighed and recorded all of their food, using Soenhle digital scales, on 2 weekdays and 1 weekend day when on each diet.

Protein intake was also calculated from the urinary urea nitrogen (UUN) and an estimated nonurea nitrogen (NUN) excretion of 29 mg N · kg^{-1} · day^{-1} as follows (16)

$$UUN + NUN = IN$$

$$IN \times 6.25 = \text{protein intake (g/day)}$$

where IN is nitrogen intake.

Renal function study

At the end of each diet period, patients were admitted while fasting to a metabolic ward for clearance studies. A Teflon cannula (Venflon, Viggo, Helsingborg, Sweden) was inserted into an antecubital vein for low-dose insulin infusion (~1.0 U/h). The infusion was started early in the morning (7:00 A.M.), and the usual morning insulin dose was omitted. Blood glucose was monitored hourly, and the rate of infusion was adjusted to obtain plasma glucose levels between 63 mg/dl (3.5 mmol/l) and 108 mg/dl (6.0 mmol/l), which were maintained throughout the study. A second Teflon cannula was inserted into an antecubital vein of the contralateral arm for blood sampling. Experiments were performed during a steady state of water diuresis as described previously (17). When a steady state of diuresis was achieved, priming doses of polyfructosan (Inutest, Laevosan-Gesellschaft, Linz, Austria), 3.5 g in 35 ml water, and sodium *p*-aminohippurate (PAH) (Merck, Hoddesdon, U.K.), 0.6 g in 3 ml water, were administered intravenously as a slow bolus. These were followed by a constant infusion at a rate adjusted to obtain stable plasma concentrations of inulin and PAH as reported previously (18,19).

After 60 min of equilibration, four exactly-timed urine collection periods of 20 min each were made. At the midpoint of each urine collection period, pulse rate and blood pressure (phase IV) were taken by a single observer (A.P.), and blood samples were drawn for measurement of PAH, polyfructosan, glucose, urea, creatinine, total plasma protein, hematocrit, electrolytes, albumin, and IgG. Urines were aliquoted into tubes for measurements of PAH, polyfructosan, electrolytes, albumin, and β_2-microglobulin concentration.

Measurements and calculations

Plasma and urinary inulin were measured after perchloric acid hydrolysis using a centrifugal analyzer (Cobas Mira, Roche, Welwyn Carden City, U.K.) as described previously (20). Plasma and urinary PAH were measured using the method of Bratton and Marshall (21). Sodium, potassium, phosphate, urea, and creatinine were measured in urine and plasma using a multichannel autoanalyzer (RA 1000, Technicon, Tarrytown, NY). Glucose was measured by a glucose-oxidase method, hematocrit using routine Coulter counter

Table 2—*Dietary intakes during APD and VPD*

	APD	VPD
Energy (kcal · kg^{-1} · day^{-1})	23.3 ± 3.7	22.8 ± 3.8
Total protein (g · kg^{-1} · day^{-1})	1.1 ± 0.27	0.95 ± 0.28
Carbohydrate (g · kg^{-1} · day^{-1})	2.36 ± 0.65	2.8 ± 0.68
Fat (g · kg^{-1} · day^{-1})	0.95 ± 0.11	0.86 ± 0.12
Fiber (g · kg^{-1} · day^{-1})	0.11 ± 0.10	0.18 ± 0.03*
P:S	0.57 ± 0.04	0.70 ± 0.05*

Data are means ± SE. *P < 0.05 VPD vs. APD.

and total plasma protein by refractometry. Plasma albumin and IgG were measured by nephelometry (Nephelometer QM 300, Kalleztad, Austin, TX), urinary albumin by radioimmunoassay (RIA) (Diagnostic, Los Angeles, CA), and urinary β_2-microglobulin by RIA (Phadebas, β_2-Microtest, Pharmacia, Uppsala, Sweden). HbA_{1c} was measured using high-performance liquid chromatography (HPLC) (Hi-Auto A_{1c}, HA-1821, Daiichi Kagaku, Kyoto, Japan). Amino acid concentrations were measured in serum using a HPLC technique (Perkin Elmer, Padova, Italy). Plasma glucagon was measured by RIA (Diagnostic), growth hormone by an immunoradiometric assay (Farmos, Turku, Finland) and insulin-like growth factor (IGF-I) by RIA (Nichols Institute, San Juan Capistano, CA).

GFR and renal plasma flow (RPF) were calculated as the clearances of polyfructosan and PAH, respectively, using the standard formula. Fractional clearance of albumin was calculated by dividing its clearance by the GFR. Filtration fraction (FF) was calculated as GFR/RPF. Renal vascular resistance (RVR) was calculated as

$$RVR = MBP \times (1 - Hct)/RPF$$

where MBP is mean blood pressure (diastolic blood pressure + 1/3 pulse pressure) and Hct is hematocrit. Results represent the mean of the four measurements taken during the four 20-min experimental periods.

Statistical analysis

Data for fractional clearance of albumin (θAlb) was transformed logarithmically and analyzed with Student's paired t test. Differences between dietary periods were tested for difference using the Wilcoxon's signed-rank test. Correlations were tested by univariate regression analysis (Spearman's correlation was used for nonparametrically distributed data). $P < 0.05$ was considered statistically significant. Results are expressed as means ± SE unless otherwise stated.

RESULTS

Diet

Total energy intake was not significantly different in the two diets. Protein intake assessed by the 3-day weighed food records (APD 1.1 ± 0.27 g · kg^{-1} · day^{-1}; VPD 0.95 ± 0.28 g · kg^{-1} · day^{-1}) was similar to that calculated from urinary urea excretion (APD 1.0 ± 0.31 g · kg^{-1} · day^{-1}; VPD 0.90 ± 0.25 g · kg^{-1} · day^{-1}) and did not differ significantly between the two diets. Carbohydrate intake was higher with the VPD, but fat intake was comparable with the two diets. Fiber intake and P:S were higher with the VPD (Table 2).

Blood glucose and arterial pressure. Mean capillary glucose levels were similar during the two diet periods (APD 110 ± 0.15 mg/dl; VPD 92 ± 0.10 mg/dl), and HbA_{1c} was not significantly different at the end of the two diets (APD 6.7 ± 0.7%; VPD 6.4 ± 0.4%). During the clearance studies, there was no difference in plasma glucose concentrations that were maintained in the euglycemic range, and patients were aglycosuric. Mean blood pressure recorded at the end of the two diets was not significantly different (APD 89 ± 3.1 mmHg; VPD 87.5 ± 1.8 mmHg).

Renal function studies

GFR and RPF were significantly lower with the VPD than with the APD (GFR 89.9 ± 4.1 vs. 105.6 ± 5.1 ml · min^{-1} · 1.73 m^{-2}, $P < 0.05$; RPF 425.7 ± 22.2 vs. 477.8 ± 32.2 ml · min^{-1} · 1.73 m^{-2}, $P < 0.05$). Renal vascular resistance was higher with the VPD than with the APD (RVR 101 ± 25 vs. 91 ± 10 mmHg · min · ml^{-1}, $P < 0.05$), whereas FF remained similar on the two diets (Fig. 1).

The urinary albumin excretion rate (median [range]) was lower with the VPD (17.1 [4.1–44.5] vs. 10.4 [1.2–22.5] mg/24 h, $P < 0.01$), and the fractional clearance of albumin (θalbumin) was significantly lower with the VPD (APD 3.4 ± 1.15 × 10^{-6}; VPD 2.0 ± 0.65 × 10^{-6}, $P < 0.05$). The excretion of β_2-microglobulin was not different (APD 2.28 ± 0.27 mg/24 h; VPD 2.23 ± 0.31 mg/24 h).

Plasma and urinary solutes and electrolytes

In all nine patients, no differences were found between the APD and VPD in plasma concentrations of urea (44 ± 0.04 vs. 34 ± 0.02 mg/dl), creatinine (0.96 ± 0.05 vs. 1.03 ± 0.04 mg/dl), total protein (6.98 ± 0.32 vs. 6.83 ± 0.16 g/dl), albumin (4.13 ± 0.12 vs. 4.14 ± 0.05 g/dl), IgG (1,088 ± 56 vs. 990 ± 50 mg/dl), sodium (140 ± 0.96 vs. 139 ± 1.77 mmol/l), potassium (3.9 ± 0.12 vs. 4.0 ± 0.14 mmol/l), calcium (8.9 ± 0.24 vs. 9.0 ± 0.11 mg/dl), and phosphate (3.8 ± 0.16 vs. 3.9 ± 0.22 mg/dl). Similarly, no significant differences were found in urinary excretion of urea (23.4 ± 3.2 vs. 17.9 ± 2.9 g/24 h), creatinine (1.27 ± 0.08 vs. 1.11 ± 0.09 g/24 h), calcium (137 ± 29 vs. 110 ± 24 mg/24 h), and phosphate (687 ± 119 vs. 604 ± 99 mg/24 h). Fractional excretion of sodium was similar with the APD and VPD (APD 1.20 ± 0.12%; VPD 1.29 ± 0.14%).

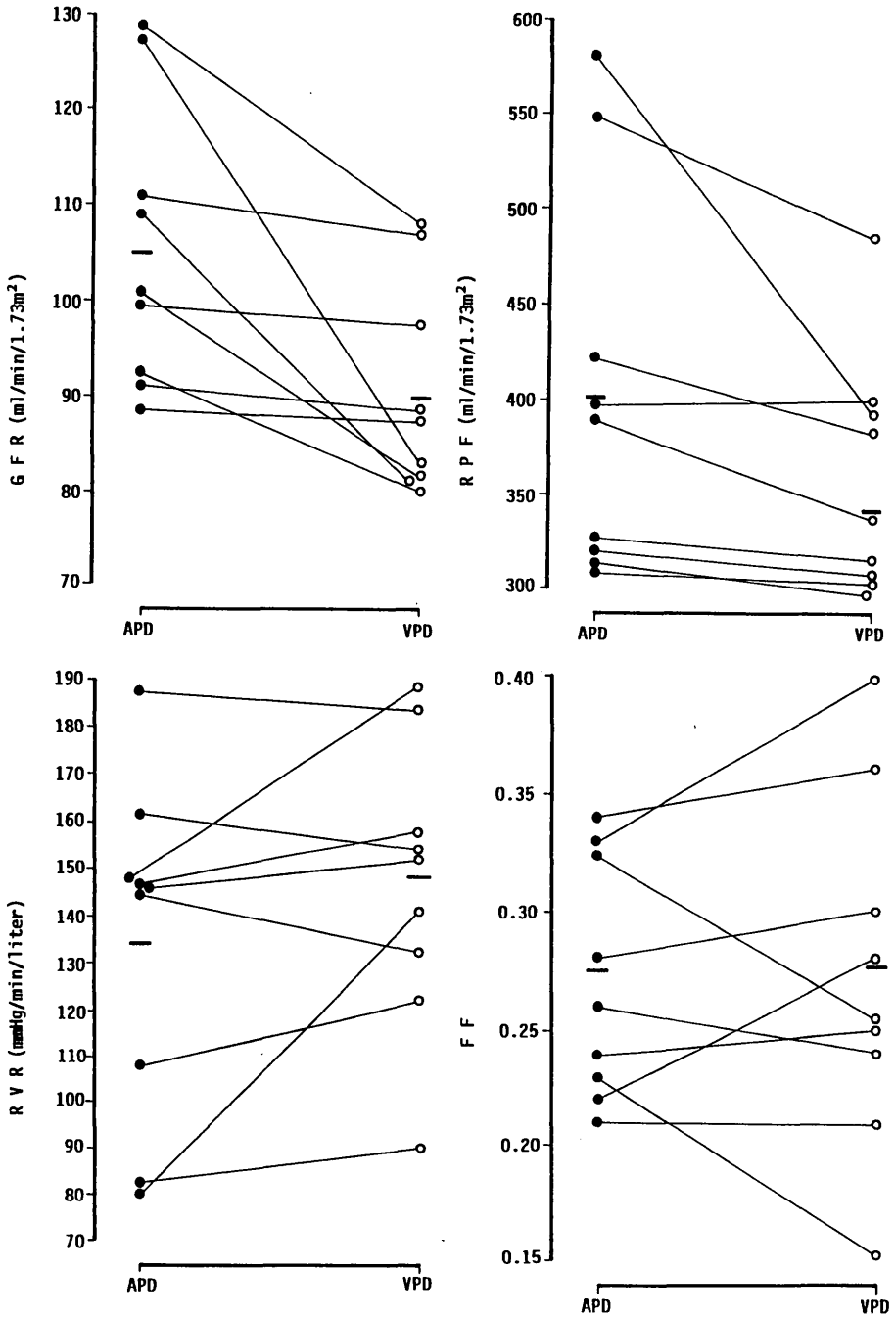

Figure 1—*GFR, RPF, RVR, and FF after 4 weeks on an APD (●) or a VPD (○) in nine normotensive, nonproteinuric type I diabetic patients. $P < 0.05$, APD vs. VPD.*

Plasma amino acid levels and hormones

From the plasma levels of the 10 amino acids measured, valine and lysine were significantly higher with the APD than with the VPD (234.6 ± 30.3 vs. 164.5 ± 25.4 mmol/l, $P < 0.05$, and 565 ± 45.1 vs. 430 ± 56.1 mmol/l, $P < 0.05$, respectively). The plasma levels of the rest were similar with both diets (Fig. 2). Plasma valine level was correlated to the GFR with the APD ($r = 0.832$, $P < 0.01$).

Plasma levels of IGF-I were higher with the APD than with the VPD (1.1 ± 0.6 vs. 0.9 ± 0.13 U/ml, $P < 0.05$), whereas the plasma levels of growth hormone and glucagon were similar at the end of both diets (APD vs. VPD: growth hormone, 5.1 ± 5.9 vs. 6.1 ± 2.2 ng/ml; glucagon, 224.3 ± 30 vs. 199.5 ± 12.9 pg/ml).

CONCLUSIONS— Restricted dietary protein intake has been reported to decrease albuminuria and to reduce the rate of deterioration in various forms of renal disease (1,4,5,22,23). Despite several studies to the contrary, the results of the MDRD (52) suggest that the progression of nondiabetic renal diseases in humans is slowed only minimally by dietary protein restriction. It is possible that dietary therapy would have been more effective if it had been started earlier in the natural history of the various nephropathies. It has been reported that dietary protein restriction prevents the progression of established diabetic nephropathy (10,24–26). However, a low-protein diet may cause hypoproteinemia, muscle wasting, or problems of compliance (27,28). It would be very important to explore alternatives of dietary protein modification that, while maintaining a normal protein intake, afford the same renal-sparing effect as a low-protein diet. Previously, it has been reported that vegan subjects have lower GFR and urinary albumin excretion than omnivorous subjects; however, they also eat a smaller quantity of protein (12,13). In humans and in an animal model of renal disease, it has been shown that the quality of protein may be important for renal function (14,29). Recently, we have demonstrated that independently of quantities of protein, vegetable protein has renal effects significantly different from those of animal protein in normal humans (15).

Our results indicate that a period of consuming a vegetable protein diet produces significant changes in renal function independently of the daily amount of protein in normotensive, non-

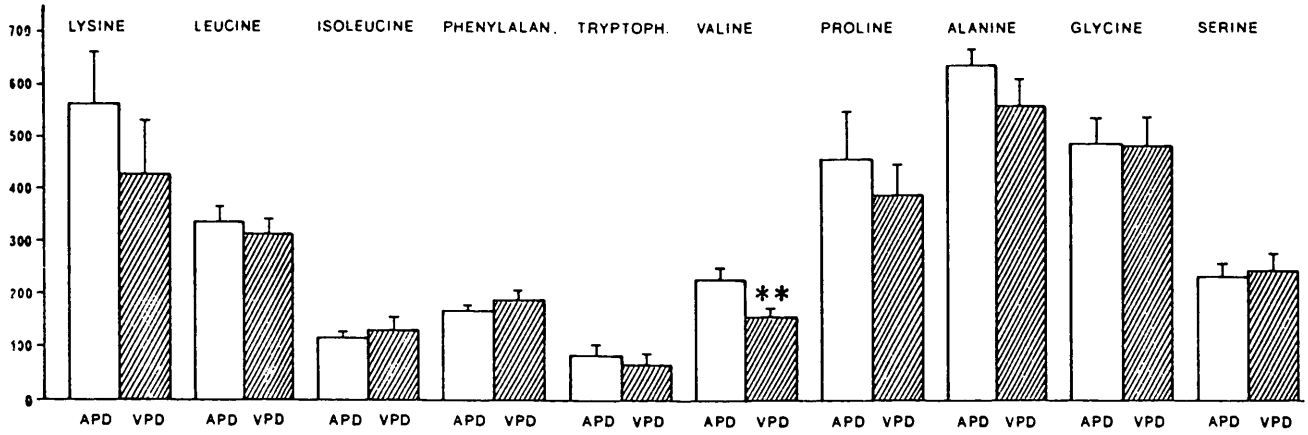

Figure 2—*Plasma concentration (mmol/l, mean ± SE) of 10 amino acids after 4 weeks on an APD (□) or a VPD (▨) in nine normotensive, nonproteinuric type I diabetic patients. **P < 0.05, APD vs. VPD.*

proteinuric diabetic patients. Patients who were consuming a vegetable protein diet had significantly lower GFR, RPF, and fractional clearance of albumin compared with those consuming the animal protein diet. The RVR was higher with the VPD, indicating that the renal vasodilatory effect of chronic meat ingestion was abolished by vegetable protein feeding. There is much evidence suggesting that the type of protein is responsible for this effect. The amount of protein intake was comparable between the two diets, whereas the fat and energy intakes were also similar with the APD and VPD. The expected higher amount of fiber with the VPD and the difference in the P:S did not alter the renal hemodynamics or the protein excretion in healthy humans (15). It has been shown that fiber supplementation to the APD did not have any effect on the renal variables measured that were indistinguishable from the APD (15). The nonsignificant difference in carbohydrate intake between APD and VPD is unlikely to be responsible for the renal changes because carbohydrates do not have any direct effect on renal function (30).

Consuming a VPD for 4 weeks induced a significant fall in the fractional clearance of albumin as reported previously by us in a group of healthy individuals (15). The fall in fractional clearance of albumin with the VPD could be the result of a lower glomerular filtration or an increased tubular reabsorption. These changes in albumin clearance seem to be glomerular in origin because the urinary excretion of β_2-microglobulin remained unchanged with the VPD. These findings are consistent with studies in diabetic and nondiabetic renal patients in whom a period of consumption of a low-protein diet induced a significant fall in the fractional clearance of albumin without any changes in the tubular handling of protein (31,32). It is possible that in diabetes, a condition in which baseline Δp is already significantly raised above normal (33), the consumption of a VPD for a period of 4 weeks could decrease Δp and affect the glomerular barrier permselectivity, as reported previously in studies with a low-protein diet (32,34). It is unlikely that differences in food absorption were implicated because the 24-h urinary urea excretion was similar with the two diets, and plasma protein concentration and body weight did not change.

Another hypothesis suggested for the higher GFR with an APD compared with a VPD is a rise in the tubuloglomerular feedback that triggers the rise in GFR because of enhanced reabsorption of amino acids in proximal tubuli linked to increased sodium reabsorption (44). However, we found no change in fractional excretion of sodium with the APD to support this hypothesis.

Attempting to explain the mechanisms of the renal effect of the VPD, we suggest that a difference in plasma amino acid levels has a contribution. In previous studies in healthy volunteers and diabetic patients, the plasma levels of alanine, glycine, and arginine increased significantly only after ingestion of tuna fish, which caused a significant increase in GFR. In contrast, ingestion of egg white or bean curd did not produce increased plasma levels of these amino acids or glomerular hyperfiltration (35,36). Pecis et al. (53), in a crossover study in hyperfiltering type I diabetic patients, observed a reduction in GFR after they consumed a diet with white meat (chicken and fish) and observed changes in the amino acid composition. Our data suggest that differences in plasma levels of valine and lysine could partly explain the different renal responses to an APD or a VPD diet. There is some evidence that the absorption kinetics of amino acids from vegetable and animal protein differ (37), and this difference may be responsible for the different renal effects. Although the plasma valine level was strongly correlated with the GFR, it is unlikely to have a direct renal effect because the infusion of a complete mixture of amino acids had no effect per se on renal hemodynamics (38,39). On

the other hand, in patients with well-controlled type I diabetes, it has been shown that valine and isoleucine are strongly correlated to the increase in GFR after consumption of a high-protein diet (40). A recent study has shown that the non-branched-chain amino acids and some branched-chain amino acids like valine increase GFR without affecting the kidney tissue and plasma levels of angiotensin II (41).

The different renal effects of an APD or a VPD are more likely to be mediated by different hormonal responses. Several studies proposed some hormonal mediators such as prostaglandins, glucagon, growth hormone, and insulin (39,42,43). This hypothesis is supported by studies in which exogenous somatostatin limits or abolishes the renal response to a high-protein diet or to amino acid infusion (38,39). In this study, prostaglandins were not measured, but in our previous study the renal vasodilatory prostaglandin response seen after meat ingestion was blunted significantly after a vegetable protein meal (15). In our study, plasma levels of glucagon and growth hormone did not differ significantly between the two diets. The nonsignificant changes in the secretion of these hormones, which potentially could affect renal vascular responses (54), fail to explain the difference between the APD and VPD. These findings are in accord with earlier results from a study in healthy volunteers in whom a period on a VPD did not affect the plasma levels of glucagon and growth hormone compared with the APD (15). Plasma glucagon concentration seems to be increased only after an acute intervention such as meal load or amino acid infusion (15,54).

Our data suggest that the difference in plasma concentrations of IGF-I contributed to the different renal responses to the APD or VPD. IGF-I is partly regulated by calorie and protein intake with decreased levels after lower calorie and protein intake (45,46). The renal synthesis of IGF-I is reported to be independent of changes in growth hormone production (47). Glomerular hyperplasia and mesangial sclerosis seen in an experimental diabetic kidney are associated with an increase in renal IGF-I (48). Few data are available concerning the relationship between IGF-I and GFR in humans. IGF-I induced a progressive rise up to 35% in GFR and RPF with no change in FF (49,50). Poor metabolic control in diabetes is associated with low plasma levels of IGF-I (51). However, in our study this could not explain the difference in IGF-I levels between the APD and VPD since metabolic control was similar. Our findings provide an alternative approach to managing microalbuminuria in diabetes. The type of protein ingested is crucial to the pattern of the renal response elicited. A VPD has significantly different renal effects from an APD with equal protein intake in normotensive, nonproteinuric type I diabetic patients. These effects seem to be comparable with those obtained by reducing protein intake. The renal changes induced by vegetable protein could be explained partly by differences in plasma concentrations of amino acids and IGF-I. Protein-modified, rather than protein-restricted, diets may prove advantageous in the long-term treatment of chronic renal failure. The short-term efficacy of the protein-modified diet in our nine diabetic patients should encourage more extensive investigations into its long-term effect on renal function and structure.

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