Renal Response to Restricted Protein Intake in Diabetic Nephropathy

JEREMY J. BENDING, ROSEMARY A. DODDS, H. KEEN, AND GIAN CARLO VIBERTI

Proteinuria in diabetes is associated with progressive glomerular damage. We studied the effects of 3-wk dietary protein restriction on proteinuria and renal function in 10 insulin-dependent diabetic men with diabetic nephropathy. Patients were randomly assigned by a crossover design to 40-g low-protein diet (LPD) or usual-protein diet (UPD). Glomerular filtration rate and renal plasma flow were measured by inulin and *p*-aminohippurate clearance at the end of each period under conditions of sustained euglycemia. Total calorie intake, body weight, serum albumin and total protein concentrations, hematocrit, blood pressure, and glucose control were similar during the two diets. Achieved protein intake was 46 ± 3 g/day during LPD and 81 ± 4 g/day during UPD ($P < .001$). Urinary urea appearance and plasma urea were significantly lower on LPD. Median total urinary protein was reduced from 3.9 g/day (range 0.5–12.3) on UPD to 2.4 (range 0.2–9.0) on LPD ($P < .006$), and there was a significant fall in the median fractional clearance of albumin from 2.0×10^{-4} (range 0.1–90.9) on UPD to 1.0×10^{-4} (range 0.1–51.4) on LPD and IgG from 2.1×10^{-5} (range 0.2–238) to 1.5×10^{-5} (range 0.1–77) ($P < .006$ and $P < .02$, respectively). The reabsorption rate of β_2-microglobulin was similar on the two diets and glomerular filtration rate, renal plasma flow, and filtration fraction remained unchanged. Thus, short-term dietary protein restriction reduces diabetic proteinuria independently of blood glucose or systemic blood pressure changes by improving glomerular permselectivity. *Diabetes* 37:1641–46, 1988

Restricted dietary protein intake has been reported to reduce proteinuria, affect the progression of chronic renal failure in animals and humans (1–8), and greatly lessen the severity of renal structural damage in virtually all models of experimental renal disease (8–14). The mechanisms by which dietary protein restriction ameliorates proteinuria and renal dysfunction have been explored in the remnant kidney model in the rat (15) and in nephrotic humans (16). Recent reports suggest that the renal response to protein ingestion may be different between diabetic and nondiabetic renal diseases (17). It is therefore important to investigate the mechanism of the renal response to a low-protein diet (LPD) in diabetic nephropathy. We studied the effects of short-term dietary protein restriction on the glomerular filtration rate (GFR), renal plasma flow rate (RPF), and proteinuria in a group of clinically proteinuric insulin-dependent diabetes mellitus (IDDM) patients and explored the determinants of the putative renal changes.

SUBJECTS AND METHODS

Subjects. We selected 10 IDDM patients for the study from our cohort of ~60 proteinuric IDDM patients to represent a wide range of GFRs and urinary protein excretion. In consideration of the experimental protocol, only male subjects were recruited. The diagnosis of diabetic nephropathy was made if persistent clinical proteinuria (i.e., total urinary protein excretion ≥0.5 g/day) was present in a patient with a diabetes duration >10 yr, with concomitant diabetic retinopathy but without clinical or biochemical evidence of other renal disease or cardiac failure. Renal biopsy performed in 5 patients showed diabetic changes only.

The clinical features of patients are given in Table 1. All patients were within 20% of ideal body weight (Metropolitan Life Insurance Tables, 1959) and had been treated with insulin since diagnosis; all had preproliferative or proliferative retinopathy and signs of neuropathy. Eight patients were receiving antihypertensive treatment, including β-blocking agents (metoprolol and atenolol), diuretics (thiazides and furosemide), and vasodilators (prazosin and hydralazine).

From the Unit for Metabolic Medicine, Division of Medicine, United Medical and Dental Schools, Guy's Campus, London.

Address correspondence and reprint requests to Professor G.C. Viberti, Unit for Metabolic Medicine, 4th Floor Hunt's House, Guy's Hospital, London SE1 9RT.

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TABLE 1
Clinical characteristics of male IDDM with clinical nephropathy

Patient	Age (yr)	Duration of diabetes (yr)	Insulin dose (U/24 h)	Mean blood pressure* (mmHg)	Duration of proteinuria (yr)
1	35	22	32	98	8
2	48	42	40	74	5
3	54	25	37	99	6
4	29	26	28	87	7
5	30	16	30	101	3
6	37	22	45	101	4
7	59	32	42	102	10
8	47	15	72	120	9
9	19	16	80	93	5
10	33	16	48	101	4
Mean ± SE	39.1 ± 3.9	23.2 ± 2.7	45.4 ± 5.5	98 ± 3.7	6.1 ± 0.7

*Diastolic pressure ± ⅓ pulse pressure.

Three patients were taking β-blockers only, three were on β-blockers and diuretics, and two were on β-blockers, diuretics, and vasodilators. No patient was taking angiotensin-converting enzyme inhibitors.

Patients were randomly allocated to take in turn an LPD or to continue with their usual diabetic diet (usual protein diet, UPD) for 3 wk. At least a week on UPD elapsed between the two experimental periods to avoid carryover effects. Measurements were made at the end of each diet period. Patients gave signed informed consent to the study, which was approved by the Guy's Hospital Ethics Committee.

Diet prescription and assessment. Diet was assessed at entry into the study in all patients on their standard diet and at weekly intervals on LPD and UPD. The prescribed LPD was designed to be isocaloric with the UPD and provided 40 g protein/day, half from animal sources and half from vegetable sources. Approximately 35% of energy came from fat and 57–60% from carbohydrate and alcohol via special low-protein high-carbohydrate products (G.F. Dietary, New Barnet, UK). In patients with nephrotic proteinuria (i.e., total urinary protein >3.0 g/day), LPD was supplemented with 1.6 g of protein for each gram of protein lost in the urine. Similar fiber intake on the two diets was maintained by encouraging patients to eat low-protein sources of vegetable fiber during their period of LPD. No restriction on salt intake was applied, but on LPD calcium and phosphate intakes were supplemented with calcium carbonate and phosphate tablets to an equivalent level of UPD.

A diet history and 3-day food record on 2 weekdays and 1 weekend day were chosen for assessing each individual's intake. Records were checked with each subject and then coded and analyzed with the DIET program of the University of London Computer at Queen Mary College, London.

Each subject performed a 24-h urine collection at home for determination of urea excretion before study at the end of each diet period. Subjects were weighed without shoes in indoor clothing, and plasma samples for urea determination were taken at the beginning and end of each 24-h urine collection to calculate urea appearance (UA) as an objective assessment of protein intake. UA was calculated as

$$UA = UUN + (PUN_f \times BW - PUN_i \times BW) \times 0.6$$

where UUN = urinary urea nitrogen, PUN = plasma urea nitrogen, BW = body weight, f = final, and i = initial. Assuming nitrogen balance over 24-h urine collection, nitrogen intake was the sum of UA plus an estimated nonurea nitrogen of 31 mg $N \cdot kg^{-1} \cdot day^{-1}$ (18). Protein intake (g/day) was calculated by multiplying the nitrogen intake by 6.25. Creatinine, sodium, and total protein concentration were also measured on each urine collection.

Blood glucose control and blood pressure measurement. Blood glucose control during both diets was assessed by patients' self-monitored capillary blood glucose records (BM test strips, Boehringer Mannheim, Bracknell, UK); a 7-point profile (before and 90 min after each meal and at bedtime) was performed weekly for 3 wk during each diet period. For each individual, the mean of all values on either diet was used for comparison. Serum fructosamine, an index of medium-term blood glucose control, was measured at the end of each diet period. Right brachial supine pressure (phase I/V) was also recorded by the same observer (J.J.B.) before each study with a standard sphygmomanometer.

Renal function determination. At the end of each diet period, subjects were admitted to a metabolic ward on the evening before the test. A Teflon cannula (Venflon, Viggo, Helsingborg, Sweden) was inserted under local anesthesia into a forearm vein and a low-dose infusion of insulin (~1.0 U/h i.v.) commenced at ~1800, before the evening meal. The usual evening insulin injection was omitted. To remove the possible confounding influence of differences in intra- and interindividual levels of glycemia, plasma glucose was clamped at a level between 3.5 and 5.5 mM overnight, by a modification of the technique described by DeFronzo et al. (19). Patients remained fasting; food, tea, coffee, and smoking, but not tap water, were prohibited from 2200 the previous night. Antihypertensive drugs were taken at the usual times.

Tests were performed the next morning starting at 0600, under euglycemic clamp and during a steady state of water diuresis. A high urine flow rate was obtained by infusing normal saline (0.154 mM) at 500 ml/h and in 8 patients by a further addition of 40 mg/L furosemide. In each patient, the same procedure was followed in both experiments. An injection of bolus doses of polyfructosan (Inutest, Boehringer Mannheim, Zurich, Switzerland), 3.5 g in 35 ml of water, and

sodium *p*-aminohippurate (PAH; Merck Sharpe & Dohme, Hertfordshire, UK), 600 mg in 3 ml of water, were followed by a constant infusion to maintain plasma concentrations of ~35 and ~2 mg/dl of polyfructosan and PAH, respectively.

After 60-min equilibration, four exactly timed urine-collection periods of ~20 min each were made. Subjects remained semisupine throughout the study, standing only to void. At the midpoint of each urine-collection period, blood samples were drawn from an indwelling Teflon cannula placed in the arm opposite to that used for infusion, and pulse rate and blood pressure were recorded by the same observer (J.J.B.). Urine volume was measured immediately, and aliquots taken into plain tubes. A 3.5-ml aliquot was also taken into a tube containing 20 μl gelatin (10 g/dl) and 20 μl 4 M NaOH for measurements of albumin, IgG, and β_2-microglobulin concentration, which were then made simultaneously with the hemodynamic measurements. All samples were frozen and stored at −20°C until assay.

Polyfructosan is handled by the kidney in an identical fashion to inulin, and has been validated for the determination of GFR (20).

Measurements and calculations. Polyfructosan was measured by the method of Heyrovsky (21) and PAH by the method of Bratton and Marshall (22). Inter- and intra-assay coefficient of variation were 3.6 and 3.1%, respectively, for Inutest and 4.0 and 1.1% for PAH. Sodium, potassium, urea, and creatinine were measured in urine and plasma using a multichannel autoanalyzer (Vickers, York, UK). Glucose was measured by a glucose oxidase method (Yellow Springs Analyzer, Yellow Springs, OH), serum fructosamine by a colorimetric method (23), hematocrit (Hct) by routine Coulter Counter, and total plasma protein by refractometry. Urinary total protein was measured by the Biuret method adapted to the Cobas-Bio (Roche Diagnostics) (24).

Plasma and urinary concentration of albumin (25) and β_2-microglobulin (β_2 Micro RIA, Pharmacia Diagnostics AB, Uppsala, Sweden) were measured by radioimmunoassay and IgG by a sensitive ELISA technique (26). GFR and RPF were calculated as the clearances of polyfructosan and PAH, respectively, with the standard formula, and values were corrected to 1.73-m² body surface area. Filtration fraction (FF) represents the ratio of GFR/RPF. Albumin and IgG excretion rates were calculated as concentration times urine flow rate. The fractional clearance of albumin and IgG were calculated by dividing their renal clearance by the polyfructosan clearance. Renal vascular resistance was calculated as MBP × (1 − Hct)/RPF, where MBP = mean blood pressure, calculated as diastolic plus one-third of the pulse pressure. Results represent the mean of four 20-min periods.

Statistics. Data were analyzed with parametric and nonparametric tests for paired data as appropriate to the distribution of the variable considered. Results are means ± SE or median and range for normally and nonnormally distributed values respectively. Differences were considered statistically significant if $P < .05$.

RESULTS

Diet. There was a significant reduction in total dietary protein intake during LPD (46 ± 3 g/day) compared with UPD (81 ± 4 g/day) ($P < .01$; Table 2). This was achieved mainly by a reduction in animal protein intake. The results obtained from the 3-day weighed-food records were confirmed by the significant reduction in both the urinary UA and plasma urea concentration. These also suggest good patient compliance. The protein intake calculated from UA was 47 ± 7 g/day on LPD and 84 ± 8 g/day on UPD, not different from that derived from food records. Total energy intake was slightly but not significantly lower on LPD, and carbohydrate intake was higher. Fiber intake remained unchanged, but fat intake fell. Plasma albumin and body weight remained stable on the two diets. Intake of calcium (UPD, 956 ± 80 mg/day; LPD, 938 ± 83 mg/day) and phosphate (UPD, 1424 ± 91 mg/day; LPD, 1436 ± 113 mg/day) were similar on the two diets, and 24-h urinary sodium excretion did not differ between UPD (165 ± 18 mmol/day) and LPD (177 ± 25 mmol/day). Median (range) plasma creatinine concentration fell from 124 mM (93–474) on UPD to 118 mM (79–375) on LPD ($P < .03$); urinary excretion of creatinine similarly fell in 8 of 10 patients, but the difference did not achieve statistical significance [UPD, 12.3 mmol/day (8.3–14.3); LPD, 11.4 (4.0–14.8)].

Glycemia and arterial pressure. Glycemic control, as assessed by mean capillary blood glucose levels and serum fructosamine concentration, was similar during each diet (mean blood glucose: UPD, 9.0 ± 1.2 mM; LPD, 8.9 ± 0.9 mM, NS; serum fructosamine: UPD, 3.0 ± 0.2 mM; LPD, 2.7 ± 0.1 mM, NS). There was also no significant difference in mean blood pressure recorded at the end of the two dietary periods (UPD, 139 ± 7/88 ± 3 mmHg; LPD, 141 ± 8/90 ± 3 mmHg).

Renal hemodynamics. Three weeks of LPD produced no measurable changes in either GFR or RPF (Table 3). Con-

TABLE 2
Dietary changes and body weight during prescription of usual protein diet or protein-restricted diet in 10 male IDDM patients with diabetic nephropathy

	Usual-protein diet	Low-protein diet	*P*
Total energy intake (cal)	2106 ± 157	1909 ± 100	NS
Total protein intake (g/day)	81 ± 4.3	46 ± 3.2	<.01
Dietary fat intake (g/day)	100 ± 11	73 ± 6	<.02
Carbohydrate intake (g/day)	210 ± 17	248 ± 18	NS
Urea appearance (g N/day)	11.2 ± 1.2	5.3 ± 1.0	<.01
Plasma urea (mM)	11.2 ± 2.0	7.0 ± 1.3	<.01
Plasma albumin (g/L)	38.8 ± 1.7	38.7 ± 1.7	NS
Body weight (kg)	72.0 ± 2.3	71.9 ± 2.3	NS

Results are means ± SE.

TABLE 3
Glomerular filtration rate, renal plasma flow, and filtration fraction in male IDDM patients with diabetic nephropathy on usual- (UPD) and low-protein (LPD) diets

	Glomerular filtration rate (ml · min^{-1} · 1.73 m^{-2})		Renal plasma flow (ml · min^{-1} · 1.73 m^{-2})		Filtration fraction	
Patient	UPD	LPD	UPD	LPD	UPD	LPD
1	80.7	74.8	276.9	251.7	0.29	0.30
2	126.6	131.5	488.5	516.9	0.26	0.25
3	58.3	57.6	223.3	228.5	0.26	0.25
4	38.4	43.6	179.8	222.3	0.21	0.20
5	7.5	16.1	54.2	107.7	0.14	0.15
6	52.7	79.2	299.4	454.1	0.18	0.17
7	65.9	44.1	392.3	308.2	0.17	0.14
8	22.1	24.6	135.9	158.8	0.16	0.15
9	118.0	104.5	527.7	530.2	0.22	0.20
10	68.2	68.6	335.5	343.7	0.20	0.20
Mean ± SE	63.8 ± 12.0	64.5 ± 11.2	291.3 ± 47.6	312.2 ± 46.5	0.21 ± 0.01	0.20 ± 0.02

Comparisons between UPD and LPD were not statistically significant.

sequently, filtration fraction remained unchanged. During the clearance studies, there were no significant differences in plasma glucose (UPD, 4.4 ± 0.1 mM; LPD, 4.5 ± 0.1 mM), mean blood pressure (UPD, 104 ± 4 mmHg; LPD, 107 ± 4 mmHg), (UPD, 40 ± 1%; LPD, 41 ± 2%) or plasma protein concentrations (UPD, 67 ± 1.6 g/L; LPD, 66.7 ± 1.3 g/L). Renal vascular resistance was 354 ± 123 mmHg · min^{-1} · L^{-1} on UPD and 273 ± 62 mmHg · min^{-1} · L^{-1} on LPD (NS). Plasma concentration and urinary excretion of sodium and potassium were also similar on the two diets (data not shown).

Proteinuria. Three weeks of LPD induced a significant ($P < .006$) reduction in total urinary protein excretion from a median of 3.9 g/day (range 0.5–12.3) on UPD to 2.4 g/day (range 0.2–9.0) (Fig. 1). Median urinary albumin excretion during the clearance studies was significantly lower on LPD (373 μg/min, range 13–3790) than on UPD (570 μg/min, range 22–3761) ($P < .01$).* Table 4 shows the individual values of urinary albumin excretion during the clearance studies on UPD and LPD. Similarly, median urinary IgG excretion fell to 12.7 μg/min (range 1.5–192) on LPD from 17.5 μg/min (range 1.9–286) on UPD ($P < .02$). Fractional clearance of albumin and IgG also fell significantly on LPD (Table 5). The median (range) excretion of β_2-microglobulin was unchanged after restriction of dietary protein [UPD, 3.5 μg/min (1.4–16.6); LPD, 3.2 μg/min (1.0–39.7)], the absolute reabsorption rate of β_2-microglobulin being similar in UPD at 152 ± 22 μg/min and LPD at 138 ± 18 μg/min.

DISCUSSION

This study demonstrates that a protein-restricted diet leads to a significant fall in diabetic proteinuria by reducing the fractional clearance of both albumin and IgG. That this effect is likely to be mediated by the reduction in protein in the diet is supported by considerable experimental evidence (6,8,13). Mineral intake was kept constant on the two diets and urinary sodium excretion did not change. The potential contributory effect of the reduction in fat intake must remain speculative at present in humans (27). The amelioration of glomerular permselectivity was obtained in the absence of changes in ambient glycemia, systemic arterial pressure, and renal vascular resistance. Moreover, clearance studies were performed under euglycemic conditions to exclude the potentially confounding acute effects of different blood glucose levels on renal hemodynamics (28,29). Modifications in diabetic control and systemic arterial pressure are therefore unlikely to account for the reduction of proteinuria in these diabetic patients. Similarly, changes in plasma protein concentration and body weight are unlikely to be responsible

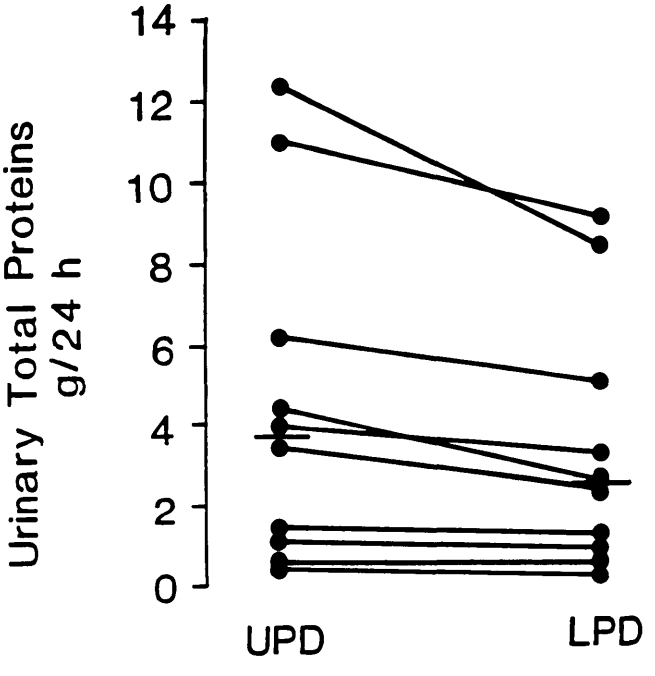

FIG. 1. Urinary total protein excretion (g/24 h) in 10 male IDDM patients with diabetic nephropathy on usual- (UPD) and low-protein (LPD) diet. *Horizontal bars* indicate median values. $P < .006$.

*One patient, with the lowest total urinary protein excretion, had a normal urinary albumin excretion of 13 μg/min on LPD and 22 μg/min on UPD during the acute clearance studies. This phenomenon has been reported in nephropathic patients on antihypertensive treatment (Parving A-H, Hommel E, Mathiesen E, Skøtt P, Edsberg B, Bahnsen M, Lauritzen E: Prevalence of microalbuminuria, arterial hypertension, retinopathy, and neuropathy in patients with insulin-dependent diabetes. *Br Med J* 296:156–60, 1988)

TABLE 4
Urinary albumin excretion rates during clearance studies under a steady state of water diuresis in male IDDM patients with diabetic nephropathy on usual- and low-protein diets

Patient	Albumin excretion rate (μg/min)	
	Usual-protein diet	Low-protein diet
1	1108	931
2	83	49
3	67	33
4	248	175
5	2387	2126
6	490	233
7	22	13
8	3761	3790
9	793	513
10	651	527

as these variables remained stable. The glomerular filtration of plasma proteins is dependent on several factors, including the hemodynamic forces operating across the glomerular capillary wall, as well as the glomerular membrane charge and size-selective properties (30). As the proteinuria of diabetic nephropathy progresses in severity, it changes from a highly selective proteinuria, in which anionic albumin is preferentially lost, to a low-selectivity proteinuria characterized by the loss of relatively greater amounts of the larger essentially neutral IgG (31,32). This late phase is characterized by the development of discrete size-selective defects in the glomerular membrane that are probably additional to the existing charge-selective defects (31). The reduction in the fractional clearance of IgG suggests that the LPD affects the size-selectivity properties of the diabetic glomerular capillary wall. This view is supported by findings in a recent study of nephrotic patients in whom the fractional clearance of high-molecular-weight neutral dextrans was reduced by 11 days of LPD (16). The reduction in the albumin clearance may also result from the improvement in glomerular membrane size selectivity because the filtration of this protein is restricted both by size and charge. We cannot exclude, however, that restriction of dietary protein intake may have altered the charge-selectivity properties of the glomerular wall. Moreover, in spite of the relative constancy during LPD intake of GFR and RPF, an LPD-mediated fall in intraglomerular pressure cannot be ruled out in these diabetic patients. Although human studies do not permit direct measurements, alteration in intraglomerular pressure could be the cause of both the decrease in fractional clearance of albumin and IgG. This interpretation finds support in experiments in the rat remnant-kidney model with established renal injury, in which a period of 2 wk of dietary protein restriction significantly lowered glomerular capillary hydraulic pressure and reduced proteinuria. Single-nephron GFR and RPF remained unchanged in these studies (15). Constancy of GFR and RPF during LPD feeding has also been reported in rats with adriamycin-induced nephrosis (33). A restriction in dietary protein similar to that applied in this study produces a significant fall in the GFR, although not in the RPF, of normoalbuminuric and microalbuminuric diabetic patients (34,35). The reason for the difference between these groups of diabetic patients may reside in the severity of the renal disease. In numerous experimental renal diseases, the renal microcirculation in the surviving nephrons is characterized by increased GFR, RPF, and glomerular capillary pressure (1). Moreover, proteinuric diabetic patients have been reported to show no change in GFR in response to a meat meal (17). It is possible that in the setting of severe renal damage, maximal perfusion of residual renal tissue occurs, and dietary protein changes are unable to modify it.

A decrease in the excretion of albumin and IgG could have occurred because of an enhanced tubular reabsorption of these proteins. However, this is unlikely because tubular reabsorption was probably saturated in these persistently proteinuric patients (36), and the reabsorption of a freely filtered low-molecular-weight protein such as β_2-microglobulin, an index of tubular reabsorptive function, was unchanged by the LPD. Although the use of β_2-microglobulin to estimate tubular reabsorption of other proteins has limitations because of the structural differences between proteins that may affect their uptake by proximal tubular cells, our findings are consistent with an effect of LPD primarily on glomerular permeability (37).

The mediators of the beneficial effect of LPD on glomerular membrane function were not explored by our study, but protein intake has been known to influence the urinary excretion of prostaglandins (38) as well as the renin-angiotensin system (39), factors that could be involved in the pathogenesis of proteinuria (40).

Proteinuria may not just reflect the severity of the renal damage but contribute to glomerular histological lesions by deposition in glomerular structures as well as through stimulation of mesangial matrix production and mesangial cell proliferation (41,42). In diabetes, subclinical increases of albumin excretion are measurable in patients at risk of clinical nephropathy when renal function is still unimpaired (43). An increase in the clearance of albumin and IgG is accompanied by progressive impairment of the renal function (44). The induction of improvements in the barrier function of the glomerular membrane may be relevant to the current attempt to retard the progression of diabetic renal failure with dietary protein restriction in the long term (45).

TABLE 5
Fractional clearance of albumin and IgG during clearance studies on usual (UPD)- and low-protein (LPD) diets in male IDDM patients with diabetic nephropathy

Patient	Fractional clearance of albumin ($\times 10^{-4}$)		Fractional clearance of IgG ($\times 10^{-5}$)	
	UPD	LPD	UPD	LPD
1	3.8	3.6	8.7	4.7
2	0.2	0.1	0.2	0.1
3	0.3	0.2	0.3	0.2
4	1.3	0.9	1.5	1.6
5	90.9	34.8	238.0	77.0
6	2.4	0.8	2.7	1.8
7	0.1	0.1	0.2	0.3
8	56.7	51.4	117.0	71.3
9	1.5	1.0	1.1	1.0
10	2.5	2.0	5.7	1.3
Median	1.95	0.95	2.1	1.45
Range	0.1–90.9	0.1–51.4	0.2–238.0	0.1–77.0
P	−.006		−.02	

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