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A SHORT-TERM LOW-PROTEIN DIET REDUCES GLOMERULAR FILTRATION RATE IN INSULIN-DEPENDENT DIABETES MELLITUS PATIENTS

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1. The effect of a 7-day low-protein diet on renal function was studied in 17 normotensive, normoalbuminuric, insulin-dependent diabetes mellitus (IDDM) patients. Glomerular filtration rate (GFR) and urinary albumin excretion (UAE) were measured after 7 days on an isocaloric low-protein diet (0.5 g protein/kg per day).

2. Compliance was confirmed by 24-h urinary urea levels. GFR was measured after a single injection of ^{51}Cr-EDTA and UAE by radioimmunoassay.

3. GFR was reduced by 13.8% on the low-protein diet (139.9 $\pm$27.7 *vs* 120.4 $\pm$25.1 ml min^{-1} (1.73 m^2)$^{-1}$) ($P<0.05$). This effect was of the same magnitude as that obtained by others after long-term strict metabolic control. No changes were observed in UAE (5.6 $\pm$ 6.4 *vs* 5.7 $\pm$ 6.8 μg/min) during the study. The patients were classified as hyperfiltering (N = 9; GFR = 160.3 $\pm$16.6 ml min^{-1} (1.73 m^2)$^{-1}$) or normofiltering (N = 8; GFR = 117.1 $\pm$17.6 ml min^{-1} (1.73 m^2)$^{-1}$) on the basis of GFR, and no difference in the reduction of GFR was observed in either group.

4. The reduction in GFR is probably caused primarily by the reduction of protein intake since other factors that might influence the GFR such as glucose control and blood pressure did not change during the study.

Key words: insulin-dependent diabetes mellitus, low-protein diet, hyperfiltration, normoalbuminuria.

Introduction

Hyperfiltration and microalbuminuria, early functional alterations of diabetic disease, are considered to be predictive of diabetic nephropathy (Viberti and Wiseman, 1986). The increased transglomerular pressure has been suggested to be a common determinant of increases in both glomerular filtration rate (GFR) and urinary albumin excretion (UAE) (Zats et al., 1985). The pathogenetic role of hyperfiltration in renal diabetic disease was determined in studies on animals with experimental diabetes mellitus. The administration of angiotensin converting enzyme inhibitors to diabetic rats prevents hyperfiltration and albumin hyperexcretion (Zats et al., 1986). In experimentally induced diabetes in rats, the adoption of a low-protein diet can attenuate hyperfiltration (Zats et al., 1985) as well as the histological renal aspects of diabetes (Wen et al., 1975; Zats et al., 1985).

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The influence of a protein diet on GFR has been known for many years. Pullman et al. (1954) demonstrated that a high-protein diet can increase GFR by about 30% and that a low-protein diet can reduce it. Vegetarians have GFR and UAE below levels observed in omnivorous subjects who eat more protein (Wiseman et al., 1987b). In a few clinical studies on IDDM patients, hyperfiltration was reduced by low-protein diets (Cohen et al., 1987; Wiseman et al., 1987a; Rudberg et al., 1988). This effect of a low-protein diet on renal function was observed after long periods of protein restriction in vegetarians (Wiseman et al., 1987b) or, usually after 3 weeks in IDDM patients (Cohen et al., 1987; Wiseman et al., 1987a), although a significant rise in GFR was observed 2 h after a protein meal (Viberti et al., 1987).

It has been suggested that the reduction of GFR in response to a low-protein diet is greater in hyperfiltering patients when compared to normofiltering IDDM patients (Rudberg et al., 1988) but these observations have not yet been confirmed.

The objective of the present study was to test the hypothesis that short periods on low-protein diets (7 days) can reduce the GFR and UAE of IDDM patients without nephropathy and to determine whether this effect is greater in hyperfiltering than in normofiltering subjects.

Subjects and Methods

Seventeen patients with IDDM (8 women and 9 men) aged 30.6 ± 6.0 years (range 22 to 43), body mass index of 21.6 ± 2.8 kg/m^2 (range 17.8 to 27.8) and duration of diabetes of 5.8 ± 5.2 years (range 1.0 to 24.8) were studied. These patients were selected from IDDM patients who were seen in our outpatient clinic from December 1985 to July 1986, in order to observe prospectively the evolution of early abnormalities of the kidney in diabetes. Inclusion criteria were: over 18 years of age, more than one year of duration of diabetes, without obesity (body mass index < 30 kg/m^2), no history or clinical evidence of autonomic neuropathy, hypertension (blood pressure below 160/95 mmHg), cardiovascular disease, urinary infection, or other renal disease and 24-h urine protein below 0.5 g. Blood pressure was measured on two occasions, with a standard clinical sphygmomanometer (25 x 12 cm cuff) on the right arm, in the seated position after 5 min of rest by the same observer. Diastolic blood pressure was recorded at the Korotkoff sound phase IV. Blood pressure was the mean of 2 measurements. During the pre-experiment equilibration period (6 months), GFR was measured two or three times in each patient and variations ranged from 2.8 to 9.7%. All subjects were conventionally treated with subcutaneous insulin, NPH plus regular insulin, administered by multiple daily injections (2 or 3). No other medication was used. The Valsalva ratio was used as an index of cardiovascular autonomic function (2.0 ± 0.4; N = 17) as described previously (Kruter et al., 1982). Six patients who showed reduction in the vibratory perception threshold, as measured with a tuning fork applied to the external malleolus, were considered to have symmetrical sensory polyneuropathy. Fundoscopy was abnormal in two patients. One had background retinopathy and the other proliferative retinopathy.

Measurements were made on the day before the beginning of the study and after 7 days on a low-protein diet. During the study the usual dose of insulin was maintained for all patients.

Dietary assessment

To analyze the usual diet of the subjects a 3-day food record was chosen as a quantitative method (Eppright et al., 1952). Two weekdays and one weekend day were included in the evaluation to make the information as representative as possible. Records were checked with each patient to clarify terms, weights and recipes. Ingested foods were recorded up to 2 weeks before the beginning of the study with the aid of a nutritionist. The low-protein diet was designed to be isocaloric with the patient's usual diet (a minimum of 30 kcal kg^{-1} day^{-1}). The protein content of this diet was 0.5 g/kg body weight (vegetable sources only). Dietary compliance was determined by the measurement of 24-h urinary urea and only patients whose urea excretion was lower during the low-protein period than during the usual diet were included in the analysis of the results. No patient was excluded due to low compliance to the diet.

Kidney function

GFR was measured using the technique of a single injection of ^{51}Cr-EDTA (Chantler and Barrat, 1972). The normal value for 20 healthy volunteers matched for age, sex and body mass index with the patients was 114.9 ± 9.9 ml min^{-1} (1.73 m^2)$^{-1}$ (range: 102.6 to 136.8 ml min^{-1} (1.73 m^2)$^{-1}$). The pooled coefficient of variation of the method was 11.7%.

UAE was measured on 24-h urine by radioimmunoassay (DPC, Los Angeles, CA). The lowest value detected with this kit was 0.9 μg/ml. UAE was measured in ten normal individuals matched for age, sex and body mass index. The normal range was 1.5 to 13.0 μg/min, with a pooled coefficient of variation of 29.8%.

Fractional albumin clearance was calculated by the urinary albumin clearance/GFR ratio.

Other measurements

The metabolic control before and after the low-protein diet was assessed by measuring overnight fasting plasma glucose and 24-h urinary glucose with a Centrifi-Chem System Autoanalyzer using the glucose-oxidase method. Plasma cholesterol, triglycerides, creatinine, sodium, calcium and phosphorus were measured after an overnight fast, and urea, creatinine, sodium, calcium and phosphorus were measured in 24-h urine by routine methods on the day before and on the day after the study period.

Statistical analysis

Data are reported as means ± SD and were analyzed by nonparametric tests (Wilcoxon and Wilcoxon-Mann-Whitney tests) except for the blood pressure results which

were analyzed by the two-tailed paired Student *t*-test. Correlation studies were performed using the Speerman method. The level of significance was set at 5%.

Results

The dietary data for IDDM patients on a usual diet at the beginning of the study, and on a low-protein diet are reported in Table 1. The total energy of the low-protein diet was higher and there was a significant 64.2% reduction in protein intake (based on the diet reported by the patients). This information was supported by a significant 52.8% reduction in urinary urea excretion. Carbohydrate intake increased and fat intake did not change on the low-protein diet. Urinary sodium excretion was also unchanged. Urinary calcium and phosphorus excretion decreased on the low-protein diet, as expected, because the protein source was only vegetable. No changes in body mass index or plasma albumin were observed during the study.

Table 1 - *Data for 17 insulin-dependent diabetes mellitus patients on their usual diet and after 7 days on a low-protein diet.*

Data are reported as means ± SD. *P<0.05 (Wilcoxon test).

	Usual diet	Low-protein diet
Energy (kcal kg^{-1} day^{-1})	27.8 ± 7.2	31.7 ± 3.3*
Protein (g kg^{-1} day^{-1})	1.4 ± 0.4	0.5 ± 0.1*
Protein (% energy)	20.7 ± 4.8	6.6 ± 0.8*
Carbohydrate (% energy)	45.6 ± 8.8	61.1 ± 3.8*
Fat (% energy)	34.2 ± 5.6	31.5 ± 4.4
Urinary sodium (mEq/24 h)	215.4 ± 116.2	209.3 ± 116.8
Urinary calcium (mg/24 h)	121.7 ± 94.0	65.5 ± 36.0*
Urinary phosphorus (mg/24 h)	731.0 ± 477.0	419.0 ± 272.0*
Urinary urea (g/day)	23.5 ± 11.0	11.1 ± 4.1*
Body mass index (kg/m^2)	21.5 ± 2.5	21.6 ± 2.4
Plasma albumin (g/dl)	4.2 ± 0.5	4.5 ± 0.3

The effects of a low-protein diet on renal function are shown in Table 2. GFR decreased by about 13.8% and UAE was unchanged even when fractional albumin clearance was calculated (9.4 ± 11.1 x 10^{-8} at the beginning of the study and 11.0 ± 14.2 x 10^{-8} at the end; P>0.05, Wilcoxon test).

There was no correlation between percent reduction in GFR and percent reduction of urinary urea excretion (Speerman correlation, r = 0.03; P>0.05).

When the IDDM patients were classified as hyperfiltering (H) or normofiltering (N) according to the mean + 2 SD GFR value determined for 20 healthy control individuals a reduction in GFR was observed after the low-protein diet, about 15.5% among H IDDM

Table 2 - *Glomerular filtration rate and urinary albumin excretion in 17 IDDM patients on their usual diet and after 7 days on a low-protein diet.*

*P<0.05 (Wilcoxon test); + not detectable by the method used.

Patients	GFR (ml min^{-1}) (1.73 m^2)$^{-1}$		UAE (µg/min)	
	Usual diet	Low-protein diet	Usual diet	Low-protein diet
AS	193.2	174.5	17.5	17.0
AMRS	156.5	132.7	1.6	1.4
JLB	159.3	136.6	+	1.1
SR	147.7	138.4	3.8	1.6
DOP	162.4	121.0	0.5	5.8
RS	171.7	134.4	12.8	3.7
CS	169.1	135.9	3.9	0.3
JCT	138.8	107.9	0.5	2.7
CP	143.7	135.9	0.2	1.9
MFR	119.0	83.8	0.5	5.8
RNA	122.0	106.6	21.5	9.0
MEP	124.2	106.0	5.1	1.9
EEB	131.6	121.0	2.2	0.3
AN	94.6	104.3	5.6	3.2
MCP	128.6	132.9	5.1	24.5
TMC	84.9	62.2	9.0	14.8
NU	131.7	111.9	0.5	2.4
Mean ± SD	139.9 ± 27.7	120.4 ± 25.1*	5.6 ± 6.4	5.7 ± 6.8

patients (GFR = 160.3 ± 16.6 to 135.3 ± 17.7 ml min^{-1} (1.73 m^2)$^{-1}$; N = 9) and 11.8% among N IDDM patients (GFR = 117.1 ± 17.6 to 103.6 ± 21.9 ml min^{-1} (1.73 m^2)$^{-1}$; N = 8). Although the reduction was significant in both groups (P<0.05, Wilcoxon test), there was no difference between them (P>0.05, Wilcoxon-Mann-Whitney test).

Blood pressure was the same at the beginning and at the end of the low-protein diet. Systolic blood pressure was 115.5 ± 10.5 mmHg for patients on their usual diet and 116.6 ± 14.6 mmHg for patients on the low-protein diet. The corresponding values for diastolic pressure were 73.2 ± 8.5 and 71.9 ± 8.8 mmHg (P>0.05, paired Student *t*-test).

Seven days on a low-protein diet did not cause a significant change in the metabolic parameters measured. The results for patients on their usual diet and for patients on a low-protein diet were as follows: fasting plasma glucose, 9.5 ± 6.3 and 7.1 ± 4.1 mM; urinary glucose, 22.8 ± 18.2 and 11.6 ± 18.2 g/24 h; cholesterol, 5.1 ± 1.4 and 4.5 ± 1.1 mM, and triglycerides, 79.1 ± 29.3 and 74.2 ± 25.2 mg/dl, respectively. Eleven patients measured glucose at home 3 times weekly before breakfast using reagent strips (Haemoglucotest). Again, no significant difference was observed between patients on their usual diet (7.3 ± 1.9 mM) and patients on a low-protein diet (5.9 ± 1.8 mM) (P>0.05, Wilcoxon test).

Discussion

This study demonstrates that the reduction of protein intake for a short period of time (7 days) decreases GFR in IDDM patients. This effect is observed both in hyperfiltering and in normofiltering subjects, with no difference in GFR responses between these two groups.

To our knowledge, this is the shortest period of time for which an effect of a low-protein diet on GFR has been demonstrated. In other studies on normal or diabetic subjects, the duration of protein restriction was 2 or 3 weeks (Pullman et al., 1954; Cohen et al., 1987; Viberti et al., 1987; Wiseman et al., 1987a). The only study involving a seven-day period reported the effect on GFR of a change from high to moderate protein intake (Kupin et al., 1987). A recent study on IDDM patients involved a 10-day period of a low-protein diet (Rudberg et al., 1988), but the protein intake (10% of total energy) was higher than the intake used in our study which was about 7% of total energy. The mean reduction in protein intake in relation to the usual diet was greater in our study, about 64.2% as opposed to about 50% reported in other studies (Cohen et al., 1987; Wiseman et al., 1987a; Rudberg et al., 1988).

Compliance with the prescribed diet in our study was confirmed by a significant mean reduction of 52.8% in urinary urea excretion and supported by the reduction of urinary calcium and phosphorus.

GFR was reduced by about 13.8% within seven days. A similar reduction was obtained by Wiseman et al. (1985) only after 12 months by strict metabolic control using subcutaneous insulin infusion pumps. Furthermore, of the 9 hyperfiltering subjects in the present study, 3 showed normal GFR and the other 4 almost normal values ($\leq$134 ml min^{-1} (1.73 m^2)$^{-1}$). The highest GFR observed after the low-protein diet was 138.4 ml min^{-1} (1.73 m^2)$^{-1}$).

Although the reduction in GFR after a low-protein diet tended to be more marked in hyperfiltering than in normofiltering patients, this difference was not significant. These data are in contrast to those reported by Rudberg et al. (1988). This difference may be explained by the absence of high UAE levels in our patients. The fact that patients with incipient nephropathy, who may show a reduced response to a low-protein diet, were included in the normofiltering group by Rudberg may be responsible for the smaller response observed in this group.

No changes in UAE were observed during the present study. A possible explanation for this is that no patient showed UAE values indicating a risk for diabetic nephropathy (higher than 30 μg/min) (Wiseman et al., 1984). Indeed, only two patients, one in the hyperfiltering group (UAE = 17.5 μg/min) and the other in the normofiltering group (UAE = 21.6 μg/min), had baseline values above the upper limit of our normal range (13 μg/min). Our patients probably were studied earlier in the course of diabetic renal disease when compared to patients studied by others in low-protein diet studies in which a reduction of UAE was demonstrated. The mean duration of diabetic disease was 5.8 $\pm$ 5.2 years for our patients and ranged from 10.5 to 19.6 years in the other studies (Cohen et al., 1987; Wiseman et al., 1987a).

In the present study the reduction of protein intake probably acted as a unique factor in reducing GFR since no changes were observed in blood glucose or blood pressure which also influence GFR. Even if the low-protein diet had promoted an improved control of diabetes metabolism, not detected by the parameters analyzed, the GFR reduction of 13.8% observed after the low-protein was only achieved after a long period of time (12 months) of strict metabolic control (Wiseman et al., 1985).

The evidence for the pathogenetic mechanisms of hyperfiltration in the absence of microalbuminuria in the development of diabetic nephropathy is not yet conclusive. Therefore, the reduction of protein intake, even before microalbuminuria begins, may represent an effective and easy therapeutic measure for preventing diabetic renal disease. Long-term studies of these patients will be needed to ascertain the effectiveness of low-protein diets.

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