

ORIGINAL COMMUNICATION

Protein restriction, glomerular filtration rate and albuminuria in patients with type 2 diabetes mellitus: a randomized trial

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Objective: Protein restriction delays the progression of non-diabetic and type 1 diabetic renal disorders. This study assessed whether protein restriction delays the onset or early progression of renal disorders in type 2 diabetes.

Design: Randomized controlled trial. Outcomes were albuminuria (mg/24 h) and, as an estimate of the glomerular filtration rate, cimetidine-influenced creatinine clearance.

Setting: Primary care.

Subjects: Patients with type 2 diabetes and microalbuminuria or at least detectable albuminuria, or a diabetes duration > 5 y.

Interventions: The experimental group received dietary counselling on protein restriction ($n = 63$); a control group ($n = 68$) received the usual dietary advice. The duration of intervention and follow-up was 28 ± 7 months.

Results: After 6 months, protein intake differed only by 0.08 g/kg/day between the study groups. Subsequently, this difference decreased and eventually disappeared. An initial effect of protein restriction on albuminuria in favor of the experimental group was not sustained, and the glomerular filtration rate decreased in the experimental group at a 1.6 ± 2.2 ml/min/1.73 m² y lower rate than in the control group ($P = 0.5$). Comparison of patients in the experimental group with a decrease in protein intake of at least 0.20 g/kg/day, with controls with no decrease, indicated a similarly small and insignificant effect on glomerular filtration rate.

Conclusions: It is concluded that, in the longer term prevention or delay of renal damage in patients with type 2 diabetes, protein restriction is neither feasible nor efficacious.

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Introduction

The number of type 2 diabetic patients with end-stage renal failure is increasing, and imposes a similar, or even greater

burden than end-stage renal disease due to type 1 diabetes (US Renal Data System, 1994; Humphrey *et al*, 1989; Pugh *et al*, 1995; Raine, 1995; Ritz & Stefanski, 1996; Parving, 1996,1998). Type 2 diabetes is said to be the major single cause of end-stage renal disease (US Renal Data System, 1994; Humphrey *et al*, 1989; Pugh *et al*, 1995). Evidence of the beneficial effects of protein restriction on non-diabetic and type 1 diabetic renal disorders is ample and consistent (Fouque *et al*, 1992; Pedrini *et al*, 1996; Waugh & Robertson, 1997; Kasiske *et al*, 1998). The most recent meta-analysis suggests that the effect of protein restriction on renal function is greater in type 1 diabetic patients than in non-diabetic patients (Kasiske *et al*, 1998). There is little doubt

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that the evolution of nephropathy is similar in most respects in both type 1 and type 2 diabetes (Ritz & Stefanski, 1996; Parving, 1996,1998). In type 2 diabetes, however, the effects of protein restriction have not yet been studied (Pedrini *et al*, 1996; Waugh & Robertson, 1997; Kasiske *et al*, 1998).

The idea that prevention of *early* renal damage may be more effective than attempts to slow down or reverse the progression of manifest renal disease has seldom been taken into account in the design of trials (Maschio *et al*, 1991; Dullaart *et al*, 1993; Maschio, 1995). The present study addressed the question of whether protein restriction can play a role in the prevention or delay of renal disorders in type 2 diabetes. In a primary care setting, patients with type 2 diabetes were selected if they had no clinically obvious nephropathy, but were at a relatively high risk for progressive albuminuria. The findings, after 12 months of intervention, suggested a beneficial effect on albuminuria (Pijls *et al*, 1999). The present paper addresses the question of whether, in the longer term, protein restriction influences the intermediate end-point albuminuria and the 'harder' end-point glomerular filtration rate (GFR).

Methods

Design

The present study is a randomized, physician-blinded trial with, on average, 28 months of follow-up and 6-monthly measurements. It compares a protein-restricted diet with the usual dietary guidelines for patients with diabetes (DNSG, 2000; Position Statement, 1996). The reversible, short-term GFR-lowering effect of protein restriction on GFR is opposite in direction compared to its long-term, irreversible and GFR-retaining effect (Levey *et al*, 1996a). A similar pattern has been observed for antihypertensive treatment (Apperloo *et al*, 1997). Therefore, rates of change in GFR are analyzed after exclusion of the initial 6 months of follow-up (Klahr, 1997). The same approach was applied with regard to change rates of albuminuria. In this way we sought to assess structural rather than reversible hemodynamic effects (Van Guldener & Donker, 1997). The study protocol was approved by the Medical Ethics Committee of the Vrije Universiteit in Amsterdam.

Patient selection: the qualification period

Forty-six general practitioners selected all their patients with type 2 diabetes (WHO, 1985) who were younger than 79 y of age, not in a period of recovery from severe morbidity, and with no protein-losing enteropathy, venous leg ulcer, pressure ulcer, malignancy, or psychiatric or serious psychosocial problems ($n = 658$). Of these patients, 351 were both willing and able to collect two 24 h urine samples. Subsequently, we selected those patients who had no clinically obvious nephropathy, but within this group were at a relatively high risk for progressive albuminuria. For this purpose, the following criteria were applied: (a) microalbuminuria (30–

300 mg/24 h, mean of two samples), relatively high albuminuria within the normo-albuminuric range (albuminuria > 20 mg/24 h in at least one sample, or detectable urinary albumin, ie albumin concentration > 6.5 mg/l, in two samples) (Parving *et al*, 1996); or (b) known diabetes duration > 5 y (Niskanen *et al*, 1996; Smulders *et al*, 1997).

In order to enhance the feasibility of protein restriction, patients were excluded if their baseline protein intake was lower than 0.80 g/kg/day, if compliance in keeping pre-study appointments was low, if they were reluctant to try to change their diet, or if they did not seem to be able, according to the dietician, to understand the diet information. Of the 204 patients found to be eligible in the qualification period, informed consent with regard to participation in the randomized trial was given by 160 patients.

Randomization

With the aid of a computer program in QBasic, one of the authors (LP) prepared random allocation schedules, stratifying for the use of ACE inhibitors and in blocks of eight patients. In order of receipt of informed consent, an administrative assistant assigned patients to the experimental ($n = 81$) or to the control group ($n = 79$). The study group allocation was not known to the general practitioner or to the laboratory personnel.

Intervention and control conditions

Patients in both study groups received counselling from a dietician on the usual dietary guidelines, with restriction of saturated fat intake as the main topic (DNSG, 2000; Position Statement, 1996). In the experimental group only, the additional aim of the dietary guidance was to decrease protein intake to 0.8 g/kg/day by partially replacing it, isocalorically, by unsaturated fat and carbohydrates in combination with water-soluble, non-digestible carbohydrates (ie a type of dietary fiber; DNSG, 2000; Position Statement, 1996). Patients visited the dietician again after 1 and 3 months, and subsequently every 3 months; consultations lasted for approximately 30 min. Dieticians gave feedback based on repeated dietary interviews and on the protein intake estimated from 3-monthly measurements of 24 h urinary urea excretion. Patients were not aware of the association between protein intake and urea excretion. The patients received, based on their habitual diet and as a basis for variation, individually designed daily menus. This was supported by variation lists, indicating foods that contain equivalent amounts of protein, and a food composition table and recipes. For the study groups separately, plenary education meetings were held with the dietician and researchers. Both groups received the same amount of attention. The general practitioners continued to be responsible for the treatment of the diabetes, and continued the pre-study medication.

Outcomes

The degree of over-estimation of glomerular filtration rate (GFR) by creatinine clearance decreases when tubular creatinine secretion is blocked by cimetidine. Van Acker *et al* developed a protocol to measure cimetidine-influenced creatinine clearance (Van Acker *et al*, 1992). As described below, we adapted this protocol in consultation with Van Acker and colleagues.

During the qualification period, patients had collected two 24 h urine samples. Just before randomization, a third 24 h sample was collected, for which patients took six times 400 mg of cimetidine. A 400 mg tablet was taken twice on the evening of the day before, and four times on the collection day itself. The last dose was taken before going to bed. The following morning, non-fasting venous blood samples were taken. Subsequently, duplicate 24 h urine samples were collected 6-monthly, with a 1 day interval between the two collections. Only the second urine sample was cimetidine-influenced, and again non-fasting venous blood samples were obtained the following morning.

Creatinine clearance, which was used as an estimate of glomerular filtration rate (GFR), was computed as the ratio between the urinary creatinine excretion rate and serum creatinine. Both at baseline and during follow-up, there appeared to be no systematic difference in the creatinine excretion between the non-cimetidine-influenced and the cimetidine-influenced urine samples. For this reason, we computed baseline creatinine excretion as the average in the total of three 24 h urine samples collected at baseline, and creatinine excretion during follow-up as the average in the duplicate samples. Both urinary and serum concentrations of creatinine were determined by means of the Jaffé method (BM/Hitachi 747/737, Boeringer Mannheim).

In the same urine samples in which creatinine was analyzed, we determined concentrations of albumin (nephelometrically, by means of Beckman Instruments GmbH, Munich, order no. 441450) and of urea (BM/Hitachi 747/737, Boeringer Mannheim). If the urinary albumin concentration was lower than the detection level of 6.5 mg/l, it was set at 3.3 mg/l. The albumin concentration was multiplied by the volume of the 24 h samples, thus expressing albuminuria as albumin excretion rate in mg/24 h. Based on patient records of the time at start and completion of the urine collections, adjustments were made for deviations from the instructed 24 h collection period.

Other variables

Based on the 24 h excretion of urinary urea, protein intake was estimated by applying the Maroni formula (Maroni *et al*, 1985). As described previously (Pijls *et al*, 1999), both at baseline and at the 6-monthly follow-up measurements, freshly voided random urine samples were assessed for signs of urinary tract infections. At the right arm of the patient, who was seated, the trained dietician measured systolic and diastolic blood pressure three times at Korotkoff

I and V, respectively, using a Hawksley random-zero meter. The means of the second and the third reading are reported here. A previous report describes the methods of assessment of use of medication, HbA1c, date of diabetes diagnosis and ethnic origin (Pijls *et al*, 1999).

Data analysis

Calculations were made in SPSS version 5.02. The mean differences in baseline characteristics between the two study groups were subjected to a two-sided *t*-test. Differences in categorical variables were tested with χ^2 -test. The repeated measurements during the intervention period were aggregated to a single figure indicating an individual's rate of change in GFR, and to a figure indicating the rate of change in albuminuria. For this purpose, betas were calculated per individual by means of linear regression analysis on all individually available follow-up measurements (Kleinbaum *et al*, 1988). For albuminuria we calculated both the absolute (mg/24 h y) and, based on ln-transformed data, the relative (%/year) change rates.

We first applied intention-to-treat analysis by comparing the rates of change in GFR and albuminuria between the two study groups. Within the scope of this analysis, as many subjects as possible were included: all patients with at least two follow-up measurements. This implied a follow-up period of, from the randomization and the start of the intervention, at least 12 months. Effect modification was analyzed for age and gender, and for baseline measurements of GFR, albuminuria, and the use of ACE inhibitors.

Subsequently, the above-mentioned analyses were adjusted for baseline differences in gender and GFR. By adjusting for changes in blood pressure, it was assessed whether the effects estimated by the intention-to-treat analysis were mediated through these changes.

Best-case analyses were then applied. Patients in the experimental group were included only if they had had, on average during the intervention period, a protein intake that was at least 0.20 g/kg/day lower than at baseline (Fouque *et al*, 1992); patients in the control group were included if their average protein intake during the study was not lower than at baseline. It was also assessed, finally, whether a dose-response relationship was present between the mean level of protein intake during the intervention period, and the rates of change in GFR (Levey *et al*, 1996b) and AER (Dullaart *et al*, 1993).

Results

Follow-up

Table 1 presents a trial profile. Of the 160 patients included in the trial, 131 patients (82%) completed a follow-up of at least 12 months. Loss to follow-up resulted mainly from comorbidity and patient-related logistic problems, eg with the collection of urine. Diet problems, as a reason for loss to follow-up, were reported by three patients only (Table 1). In

the initial, randomly selected experimental and control groups of 81 and 79 patients, respectively, baseline serum creatinine concentration was 105 ± 18 and $99 \pm 19 \mu\text{mol/l}$ ($P = 0.04$), and GFR was 79 ± 18 and $84 \pm 22 \text{ ml/min/1.73 m}^2$ ($P > 0.20$). The percentage of men were 58 and 52%, respectively. Table 2 shows that in patients who completed at least 12 months of follow-up, the differences in sex-ratio between the experimental and control group were somewhat more pronounced, and differences in GFR were less pronounced. Table 3 presents the distribution of the length of follow-up, which was 28 ± 7 months on average.

Changes in protein intake

At baseline in the experimental and control group, respectively, protein intake was 1.18 ± 0.24 and

Table 1 Loss to follow-up in a randomized trial on protein restriction and the course of glomerular filtration rate in patients with type 2 diabetes

	Experimental	n	Control
Informed consent		160	
Random allocation	81		79
Died	- 1		- 2
Incident comorbidity	- 7		- 4
Problems with diet	- 3		0
Patient-related logistic problems	- 7		- 5
At least 12 months of follow-up	63		68

Table 2 Baseline characteristics of 131 patients with at least two follow-up measurements^a

	Protein-restricted	Control
n	63	68
Age (y)	63 ± 8	65 ± 8
Gender (% men) ^b	63	53
Non-Caucasian (n)	2	2
Known diabetes duration (y)	6.7 ± 4.1	7.2 ± 4.8
Height (m)	1.72 ± 0.09	1.70 ± 0.07
Body weight (kg)	81 ± 14	81 ± 14
Body Mass Index (kg/m ²)	27.4 ± 4.1	28.2 ± 4.8
Present smoker (n)	12	10
Diabetes treatment (n)		
diet only	14	16
oral medication	48	49
insulin	1	2
HbA _{1c} (%)	7.7 ± 1.4	7.7 ± 1.5
Systolic blood pressure (mmHg)	139 ± 19	137 ± 18
Diastolic blood pressure (mmHg)	80 ± 11	79 ± 10
Use ACE inhibitor (n)	7	7
Protein intake (g/kg/day)	1.18 ± 0.24	1.15 ± 0.26
Glomerular filtration rate (ml/min/1.73 m ²)	82 ± 19	85 ± 23
Albuminuria (mg/24 h)	21.2 (10,41)	20.5 (8,42)
Microalbuminuria (n with 30–300 mg/24 h)	19	22
Normoalbuminuria (n with 0–30 mg/24 h)	44	46
with diabetes duration > 5 y	27	33

^aMean \pm s.d., or geometric mean with 25th and 75th percentiles or n.

^b $P = 0.19$ (protein-restricted vs control group).

Table 3 Follow-up of patients with at least 12 months of follow-up

	Protein-restricted	Control
n	63	68
Mean follow-up (months)	29 ± 6^a	28 ± 7
Follow-up to (months):		
12	3 ^b	6
18	4	4
24	9	11
27	7	5
30	16	15
33	21	22
36	2	3
42	1	2

^aMean \pm s.d.

^bn.

$1.15 \pm 0.26 \text{ g/kg/day}$. After the first 6 months, it had decreased in the experimental group to 1.11 ± 0.20 , and increased in the control group to $1.19 \pm 0.29 \text{ g/kg/day}$ ($P_{\text{change in experimental versus change in control group}} = 0.08$). On average during the intervention period the total protein intake was, in the experimental and control group, respectively, 1.10 ± 0.18 and $1.14 \pm 0.24 \text{ g/kg/day}$ ($P = 0.4$). Figure 1 presents the non-aggregated data on 44 patients in the experimental group and 50 patients in the control group who completed 24 months of follow-up, and for whom data at 0, 6, 12, 18 and 24 months were available. Figure 1 shows that, after the first 6 months, protein intake remained fairly stable in the experimental group, but started to decrease in the control group. As a result, after 12 months of follow-up and subsequently, protein intake in the experimental group was no longer lower than in the control group ($n = 94$).

Effect on GFR

At 6 months GFR had decreased in the experimental group by 2.9 ± 17 , and in the control group by $1.3 \pm 15 \text{ ml/min/1.73 m}^2$. After the first 6 months the annual decline in GFR in the experimental group amounted to $4.8 \pm 12 \text{ ml/}$

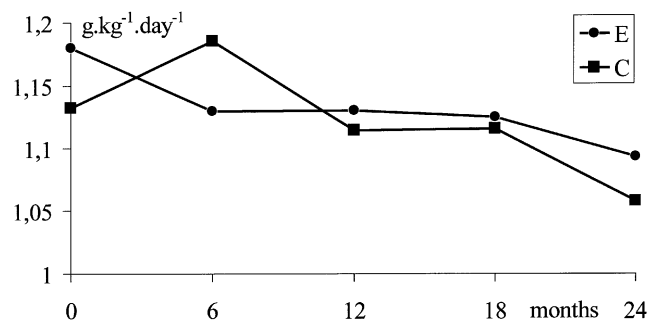


Figure 1 Protein intake. Data on patients with complete 24-month follow-up ($n = 94$). P -value > 0.2 for the experimental vs control group at 0, 6, 12, 18 and 24 months.

Table 4 Change rates (per year) of GFR and albuminuria in patients with at least 12 months of follow-up

	Protein-restricted (n=63)	Control (n=68)
Glomerular filtration rate (ml/min/1.73 m ²) ^a	- 4.8 ± 12	- 6.4 ± 14
Albuminuria (mg/24 h) ^b	+ 1.2 (- 0.8, 10.8)	+ 0.1 (- 2.5, 6.1)
Albuminuria (%) ^c	+ 16 (- 4, 28)	+ 3 (- 13, 21)

^aMean ± s.d., $P=0.5$ (t-test).

^bMedian with 25th and 75th percentiles, $P=0.09$ (Wilcoxon).

^cGeometric mean with 25th and 75th percentiles, $P=0.11$ (t-test on ln-transformed data).

min/1.73 m² (Table 4). In the control group, the decline was 1.6 ± 2.2 ml/min/1.73 m²/y greater than in the experimental group. This small difference in rate of decline was far from statistically significant ($P=0.5$). The effect estimate was not substantially influenced by adjustment for baseline levels of GFR, albuminuria, systolic or diastolic blood pressure (Dullaart *et al*, 1993, or for age, gender, the use of ACE inhibitors or duration of follow-up. In the experimental and control group, respectively, a rate of decline exceeding 1.2 ml/min/1.73 m²/y (Pedrini *et al*, 1996) occurred in 71 and 79% of the patients ($P=0.3$). Figure 2 presents data that have not been aggregated over time.

As described in the methods section, estimation of GFR was based on creatinine excretion in both cimetidine-influenced and non-influenced urine samples. Additional analysis that only included creatinine excretion in cimetidine-influenced urine samples did not indicate a substantial intervention effect either. The same applied to an analysis in which GFR was estimated according to the formula of Cockcroft and Gault (1976), which excludes the possibility of errors in urine collection influencing the findings. According to this formula, the rates of decline in GFR in the experimental and control group were - 2.1 ± 3.6 and - 1.8 ± 5.9 ml/min/1.73 m²/y, respectively ($P=0.7$).

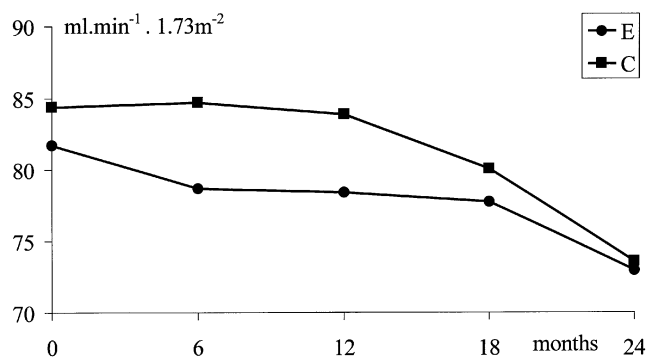


Figure 2 Glomerular filtration rate. Data on patients with complete 24-month follow-up ($n=94$). P -value > 0.2 for the experimental vs control group at 0, 18 and 24 months > 0.5; $P=0.19$ at 6 and 12 months.

The overall absence of an effect may have resulted from opposite effects in different sub-groups. Analysis of effect modification addresses this issue. Among patients with a baseline GFR lower than 81 ml/min/1.73 m², ie the median value, the rate of GFR decline was 3.8 ± 2.4 ml/min/1.73 m²/y lower in the experimental group than in the control group ($n=64$, $P=0.12$). Among patients with a higher baseline GFR, the rate of decline was not slower in the experimental than in the control group; in the experimental group the estimated decline rate even tended to be somewhat faster than in the control group (0.8 ± 3.8 ml/min/1.73 m²/y, $n=67$, $P=0.8$; $P_{\text{effect modification}}=0.26$). The effect estimate was similar for baseline normoalbuminuric and microalbuminuric patients, and was not modified by gender, age, or use of ACE inhibitors (all $P > 0.6$).

The best-case analysis implied confinement of the analysis to patients in the experimental group with at least 0.20 g/kg/day decrease in protein intake (Fouque *et al*, 1992), and those in the control group with no decrease. This analysis on small numbers of patients did not indicate a slower rate of decline in GFR in the experimental than in the control group either; in the experimental group ($n=14$) the decline was even estimated to be 2.5 ± 5.8 ml/min/1.73 m²/y faster than in the control group ($n=30$; $P=0.7$).

No dose-response relationship was observed between individual average protein intake during the intervention, and the coinciding course of the GFR. Both within the experimental group as well as in the combination of both study groups, Pearson's r was lower than 0.06 ($P > 0.6$). The absence of this association can probably not be ascribed to a too small range of the change in protein restriction; 5 and 95 percentiles of mean protein intake during the intervention were 0.81 and 1.53 g/kg/day, respectively.

Effect on albuminuria

After the first 6 months, albuminuria had decreased in the experimental group by 12% and increased in the control group by 16% ($P=0.02_{\text{experimental versus control group}}$). During the period after the first 6 months of follow-up, the mean rate of increase in albuminuria was *higher* and thus less favorable in the experimental group than in the control group: +18 and +3%/y, respectively ($P=0.11$; Table 4). The estimate of the difference between the study groups in albuminuria change rate was not altered by adjustment for baseline levels of GFR, albuminuria, systolic and diastolic blood pressure, or for age, gender, use of ACE-inhibitors and duration of follow-up. Gross progression of albuminuria, defined as an annual increase rate of 10% or more (Pedrini *et al*, 1996), was observed in 49 and 40% of the subjects ($P=0.15$) in the experimental and control group, respectively.

Urinary tract infections can result in spuriously high estimates of albuminuria. We therefore assessed whether the effect estimate was altered by exclusion of data on albuminuria if such infection was observed in combination

with an estimate of albuminuria greater than 20 mg/24 h. This appeared not to be the case (data not shown). In addition, an analysis was applied in which albuminuria was expressed as albumin-creatinine ratio (mg/mmol) instead of albumin excretion rate (mg/24 h). Such analysis precludes the influence of errors in urine collection; it did not alter the effect estimate either. Figure 3 presents data that have not been aggregated over time.

Analysis of effect modification revealed that the apparently adverse intervention effect was stronger in baseline microalbuminuric patients than in baseline normoalbuminuric patients ($P_{\text{effect modification}}=0.04$). Among normoalbuminuric patients, annual increases in the experimental and control group, respectively, were 14 and 13% ($P=0.97$). In microalbuminuric patients, however, change rates were +22 and -17%/y in the experimental and control group, respectively ($P=0.04$). The effect estimate was not modified by baseline GFR, gender, age or the use of ACE inhibitors (all $P>0.4$).

Adjusted analyses indicated that the apparent effect was not mediated by changes in systolic and diastolic blood pressure. Best-case analysis indicated a somewhat stronger effect than the intention-to-treat analysis; it indicated albuminuria increase rates of +20%/y in the experimental group ($n=14$) and +3%/y in the control group ($n=30$; $P=0.24$). A tendency towards a dose-response relationship was observed with albuminuria change rates in relative terms (%/y), but not if expressed in absolute terms (mg/24 h/y). Linear regression analysis in the combined study groups indicated, adjusted for baseline intake, that a 0.10 g/kg/day lower protein intake tended to be associated with a 4% higher albuminuria change rate (Pearson's $r=-0.13$, $P=0.13$). Confinement of the analysis to the experimental group indicated a similarly interpretable 5% (Pearson's $r=-0.19$, $P=0.14$).

Discussion

The issue of adherence, including an analysis of its determinants, is discussed in detail elsewhere (Pijls *et al*, 2000). The

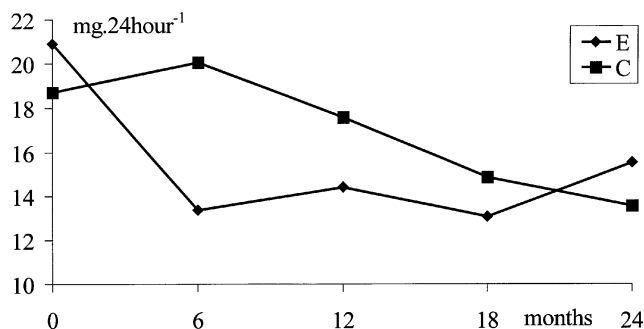


Figure 3 Albumin excretion rate. Median values; data on patients with complete 24-month follow-up ($n=94$). P -value >0.5 for the experimental vs control group at 0, 6, 12, 18 and 24 months.

same applies to cross-sectional determinants of the baseline level of albuminuria (Pijls *et al*, 2001).

Effect on GFR

In an intention-to-treat analysis of a randomized controlled trial, the efficacy of protein restriction can be demonstrated only if an actual decrease in protein intake has been established. In the present trial no substantial protein restriction was achieved and, as was to be expected, intention-to-treat analysis showed no clear effect on GFR. This finding does not necessarily imply, however, that protein restriction is not efficacious, but implies only that the study question remains unanswered. Therefore, additional attempts were made to go at least some way towards an answer to the study question, ie best-case analysis and assessment of the presence of a dose-response relationship (Dullaart *et al*, 1993; Levey *et al*, 1996b). However, an effect was not suggested either. It is thus very unlikely that, had a more substantial protein restriction been achieved and maintained, an effect on renal function would have been observed.

A slight tendency towards a GFR-retaining effect of the intervention was observed in patients with a lower baseline GFR. In those with a higher GFR at baseline, the effect tended to be the opposite, ie lowering GFR and thus, one could postulate, correcting hyperfiltration to a minor extent (Nowack *et al*, 1992; Nelson *et al*, 1996). Within the latter sub-group, however, no dose-response relationship was observed (data not shown).

Several meta-analyses concerning non-diabetic (Fouque *et al*, 1992; Pedrini *et al*, 1996; Waugh & Robertson, 1997; Kasiske *et al*, 1998) and type 1 diabetic patient (Pedrini *et al*, 1996; Waugh & Robertson, 1997; Kasiske *et al*, 1998) have addressed the effect of protein restriction on macroalbuminuria, on the rate of decline in GFR, and on the course of end-stage renal disease. The most recent meta-analysis of randomized trials among type 1 diabetic patients, with 2 y of follow-up, suggested that the rate of decline in GFR was slowed down by 5.8 ml/min/y (Kasiske *et al*, 1998). This is a lesser effect than the estimate of 8.4 ml/min/y made by Waugh and Robertson, based on trials with an average follow-up of 16 months (Waugh & Robertson, 1997). Another finding in the most recent meta-analysis was that the smaller trials more often reported positive effects, suggesting publication bias in favor of an effect (Kasiske *et al*, 1998).

Validity of cimetidine-influenced creatinine clearance as an estimate of GFR

Direct measurement of GFR implies the use of radioactive pharmaceuticals and/or the administration of substances by infusion. Creatinine clearance, which can be measured with less difficulty, also provides an estimate of GFR. However, since creatinine is not only filtered glomerularly but also secreted tubularly, creatinine clearance over-estimates GFR.

Van Acker *et al* have demonstrated that the lower the GFR, the greater the relative contribution of tubular creatinine secretion to the total creatinine filtration and secretion (Van Acker *et al*, 1992). The result is that decreases in GFR are under-estimated by creatinine clearance. In order to prevent this under-estimation, a protocol was applied to measure clearance while blocking tubular creatinine secretion by means of cimetidine. In consultation with the investigators (Van Acker *et al*, 1992), a protocol based on a 3 h clearance was modified into a protocol with 24 h urine collection, which included a sufficiently high (Van Acker *et al*, 1992) and safe (Richter *et al*, 1989) dose of cimetidine.

Compared to the annual decrease of 1.2 ml/min/1.73 m² reported in another group of type 2 diabetic patients with no macroalbuminuria (Nielsen *et al*, 1997), the observed annual decrease of 5–6 ml/min/1.73 m² was rather rapid. The latter was, however, mainly due to a decrease in urinary excretion of creatinine after 12 months of follow-up. This decrease was much larger than could be explained by the rather small changes in body weight (data not shown). Applying the Cockcroft and Gault formula (Cockcroft & Gault, 1976) indicated an average GFR decline rate 1.7 ± 5 ml/min/1.73 m², which is much closer to the previously reported 1.2 ml/min/1.73 m² (Nielsen *et al*, 1997).

Effect on albuminuria

As was the case with the GFR, the hypothesis was tested that protein restriction beneficially influences albuminuria. During the first 6 months, albuminuria decreased in keeping with protein intake in the experimental group, and increased in keeping with protein intake in the control group (Pijls *et al*, 1999). However, these short-term changes may, to some extent, be a reflection of haemodynamic rather than structural changes (Van Guldener *et al*, 1997). Subsequently, the difference in albuminuria between the study groups disappeared. Since the difference in protein intake also disappeared, no effect would be expected after the first 6–12 months. Nevertheless, intention-to-treat analysis showed, surprisingly and intriguingly, a tendency towards an adverse intervention effect on the course of albuminuria. As described, the analysis of the albumin–creatinine ratios, the best-case analysis and the dose–response analysis supported this suggestion.

The conclusion that protein restriction *adversely* influences albuminuria would be discordant with the results of other intervention studies (Pedrini *et al*, 1996; Waugh & Robertson, 1997). Nevertheless, in cross-sectional studies involving both subjects with type 1 (Kalk *et al*, 1992; Riley & Dwyer, 1998) and subjects with type 2 diabetes (Jameel *et al*, 1992), respectively, 11, 4 and 9% lower protein intakes were reported in microalbuminuric subjects than in normoalbuminuric subjects. However, in other cross-sectional studies on type 1 (Watts *et al*, 1988) and type 2 (Hoogeveen *et al*, 1998) diabetes, respectively, protein intake was 14 and 7% higher in microalbuminuric subjects, and none of the

differences observed in the latter five studies were statistically significant.

Riley and Dwyer applied an additional analysis to their data on type 1 diabetes (Riley & Dwyer, 1998). Depending on which factors this analysis was adjusted for, the prevalence of microalbuminuria in the highest quintile of protein intake (> 2% of energy intake) was only 10–38% of the prevalence in the lowest quintile. The authors speculated that a sustained high protein intake may have harmful effects, on the one hand, but on the other hand it might also provoke compensatory mechanisms that ameliorate these effects. However, no detailed mechanism could be identified or proposed.

The apparent effect found in the present study was not mediated through changes in blood pressure; it is therefore unlikely that the findings can be explained by changes in sodium intake. The result was not altered either by adjustment for changes in the use of ACE inhibitors, so there seems to be no obvious explanation. It is concluded that, in the range of normo- and microalbuminuria, the present findings do not suggest a beneficial effect of lower protein intake on the course of albuminuria.

Conclusions

In type 2 diabetic patients with no clinically obvious nephropathy, it appeared that protein restriction for an average period of 28 months was not feasible. Intention-to-treat analyses suggested no beneficial effect on the course of the GFR. Other approaches such as best-case analysis did not suggest an effect either; it is thus very unlikely that, had substantial protein restriction been achieved, an effect on renal function would have been observed. Furthermore, an initial effect of lowering albuminuria was not sustained. In the longer term prevention or delay of renal damage in patients with type 2 diabetes, protein restriction is probably neither feasible nor efficacious.

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References

- Apperloo AJ, De Zeeuw D & De Jong PE (1997): A short-term antihypertensive treatment-induced fall in glomerular filtration rate predicts long-term stability of renal function. *Kidney Int.* **51**, 793–797.
- Cockcroft DW & Gault MH (1976): Prediction of creatinine clearance from serum creatinine. *Nephron* **16**, 31–41.
- DNSG (2000): Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes Recommendations for the nutritional management of patients with diabetes mellitus. *Eur. J. Clin. Nutr.* **54**, 353–355.
- Dullaart RPF, Beusekamp BJ, Meijer S, Van Doormaal JJ & Sluiter WJ (1993): Long-term effects of protein-restricted diet on albuminuria and renal function in IDDM patients without clinical nephropathy and hypertension. *Diabetes Care* **16**, 483–492.

- Fouque D, Laville M, Boissel JP, Chifflet R, Labeeuw M & Zech PY (1992): Controlled low protein diets in chronic renal insufficiency: meta-analysis. *Br. Med. J.* **304**, 216–220.
- Hoogeveen EK, Kostense PJ, Jager A *et al* (1998): Serum homocysteine level and protein intake are related to the risk of microalbuminuria. The Hoorn Study. *Kidney Int.* **54**, 203–209.
- Humphrey LL, Ballard DJ, Frohnert PP, Chu C-P, O'Fallon M & Palumbo PJ (1989): Chronic renal failure in non-insulin-dependent diabetes mellitus. A population-based study in Rochester, Minnesota. *Ann. Intern. Med.* **111**, 788–796.
- Jameel N, Pugh JA, Mitchell BD & Stern MP (1992): Dietary protein is not correlated with clinical proteinuria in NIDDM. *Diabetes Care* **15**, 178–183.
- Kalk WJ, Osler C, Constable J, Kruger M & Panz V (1992): Influence of dietary protein on glomerular filtration and urinary albumin excretion in insulin-dependent diabetes. *Am. J. Clin. Nutr.* **56**, 169–173.
- Kasiske BL, Lakatua JDA, Ma JZ & Louis TA (1998): A meta-analysis of the effects of dietary protein restriction on the rate of decline in renal function. *Am. J. Kidney Dis.* **31**, 954–961.
- Klahr S (1997): Prevention of progression of nephropathy. *Nephrol. Dial. Transplant.* **12**(Suppl 2), 63–66.
- Kleinbaum DG, Kupper LL & Muller KE (1988): *Applied Regression Analysis and other Multivariable Methods*. Chapter 5, pp 41–79. Belmont, CA: Duxbury Press.
- Levey AS, Beck GJ, Bosch JP *et al* for the Modification of Diet in Renal Disease Study Group (1996a): Short-term effects of protein intake, blood pressure, and antihypertensive therapy on glomerular filtration rate in the Modification of Diet in Renal Disease Study. *J. Am. Soc. Nephrol.* **7**, 2097–2109.
- Levey AS, Adler S, Caggiula AW *et al* for the Modification of Diet in Renal Disease Study Group (1996b): Effects of dietary protein restriction on the progression of advanced renal disease in the Modification of Diet in Renal Disease Study. *Am. J. Kidney Dis.* **27**, 652–663.
- Maroni BJ, Steinman TI & Mitch WE (1985): A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int.* **27**, 58–65.
- Maschio G (1995): Low-protein diet and progression of renal disease: an endless story. *Nephrol. Dial. Transplant.* **10**, 1797–1800.
- Maschio G, Oldrizzi L & Ruggi C (1991): Is there a 'point of no return' in progressive renal disease? *J. Am. Soc. Nephrol.* **2**, 832–840.
- Nelson RG, Bennett PH, Beck GJ *et al* for the Diabetic Renal Disease Study Group (1996): Development and progression of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. *New Engl. J. Med.* **335**, 1636–1642.
- Nielsen S, Schmitz A, Rehling M & Mogensen CE (1997): The clinical course of renal function in NIDDM patients with normo- and microalbuminuria. *J. Intern. Med.* **241**, 133–134.
- Niskanen LK, Parviainen M, Penttila I & Uusitupa MIJ (1996): Evolution, risk factors, and prognostic implications of albuminuria in NIDDM. *Diabetes Care* **19**, 486–493.
- Nowack R, Raum E, Blum W, Ritz E (1992): Renal hemodynamics in recent-onset type II diabetes. *Am. J. Kidney Dis.* **10**, 342–347.
- Parving H-H (1996): Initiation and progression of diabetic nephropathy. *New Engl. J. Med.* **335**, 1682–1683.
- Parving H-H (1998): Renoprotection in diabetes: genetic and non-genetic risk factors and treatment. *Diabetologia* **41**, 745–749.
- Parving H-H, Osterby R, Anderson PW & Hsueh WA (1996): Diabetic nephropathy. In: *The Kidney*, 5th edn, ed. BM Brenner, Vol. 2, pp 1864–1892. Philadelphia, PA: WB Saunders.
- Pedrin MT, Levey AS, Lau J, Chalmers TC & Wang PH (1996): The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: a meta-analysis. *Ann. Intern. Med.* **124**, 627–632.
- Pijls LTJ, De Vries H, Donker AJM & Van Eijk JThM (1999): The effect of protein restriction on albuminuria in patients with type 2 diabetes; a randomized trial. *Nephrol. Dial. Transplant.* **14**, 1445–1453.
- Pijls LTJ, de Vries H, van Eijk JThM & Donker AJM (2000): Adherence to protein restriction in patients with type 2 diabetes mellitus: a randomized trial. *Eur. J. Clin. Nutr.* **54**, 347–352.
- Pijls LTJ, de Vries H, van Eijk JThM & Donker AJM (2001): Determinants of albuminuria in people with type 2 diabetes mellitus. *Diabetes Res. Clin. Pract.* **52**, 133–143.
- Position Statement (1996): Nutrition recommendations and principles for people with diabetes mellitus. *Diabetes Care* **19**, S16–S19.
- Pugh JA, Medina RA, Cornell JC & Basu S (1995): NIDDM is the major cause of diabetic end-stage renal disease. More evidence from a tri-ethnic community. *Diabetes* **44**, 1375–1380.
- Raine AEG. The rising tide of diabetic nephropathy—the warning before the flood? *Nephrol. Dial. Transplant.* **10**, 460–461.
- Richter JM, Colditz GA, Huse DM, Delea TE & Oster G (1989): Cimetidine and adverse reactions: a meta-analysis of randomized clinical trials of short-term therapy. *Am. J. Med.* **87**, 278–284.
- Riley MD & Dwyer T (1998): Microalbuminuria is positively associated with usual dietary saturated fat intake and negatively associated with usual dietary protein intake in people with insulin-dependent diabetes mellitus. *Am. J. Clin. Nutr.* **67**, 50–57.
- Ritz E & Stefanski A (1996): Diabetic nephropathy in type II diabetes. *Am. J. Kidney Dis.* **27**, 167–194.
- Smulders YM, Rakic M, Stehouwer CDA, Weijers RNM, Slaats EH & Silberbusch J (1997): Determinants of progression of microalbuminuria in patients with NIDDM. *Diabetes Care* **20**, 999–1005.
- US Renal Data System (1994): *US Renal Data System 1994 Annual Report*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Division of Kidney, Urologic, and Hematologic Diseases.
- Van Acker BAC, Koomen GCM, Koopman MG, De Waart DR & Arisz L (1992): Creatinine clearance with cimetidine for measurement of GFR. *Lancet* **341**, 1326–1329.
- Van Guldener C & Donker AJM (1997): Lisinopril and albumin excretion in diabetes. (Letter.) *Lancet* **350**, 662–663.
- Watts GF, Gregory L, Naoumova R, Kubal C & Shaw KM (1988): Nutrient intake in insulin-dependent diabetic patients with incipient nephropathy. *Eur. J. Clin. Nutr.* **42**, 697–702.
- Waugh NR & Robertson AM (1997): Protein restriction in diabetic renal disease. In: ed. R Williams, A Nicolucci, HMJ Krans & G Ramirez. *Diabetes Module of the Cochrane Database of Systematic Reviews* (updated 1 September 1997) The Cochrane Library. The Cochrane Collaboration, Issue 4. Oxford: Update Software.
- WHO (1985): *Diabetes mellitus*. WHO Technical Report Series 727. Geneva: WHO.