Dietary Protein Intake Is Not Correlated With Clinical Proteinuria in NIDDM

NAUMAN JAMEEL, MD, MPH
JACQUELINE A. PUGH, MD
BRAXTON D. MITCHELL, PHD
MICHAEL P. STERN, MD

OBJECTIVE— To determine whether dietary protein intake is correlated with clinical proteinuria in subjects with non-insulin-dependent diabetes mellitus (NIDDM).

RESEARCH DESIGN AND METHODS— Cross-sectional analysis of data obtained from the San Antonio Heart Study, a population-based survey of diabetes and cardiovascular risk factors. Subjects were enrolled in two phases: phase 1 between 1979 and 1982 and phase 2 between 1984 and 1988. This study was based on 376 NIDDM subjects who had both urinalysis and complete dietary protein intake information available. Dietary protein intake was measured by 24-h dietary recall in phase 1 and by food-frequency questionnaire in phase 2. An early-morning spot urine was obtained from study subjects. Clinical proteinuria was defined as ≥1 on Ames Albustix test.

RESULTS— In phase 1, the subjects with negative or trace proteinuria had a mean protein intake of 79.9 g/day compared with 72.1 g/day for subjects with ≥1 proteinuria. In phase 2, the mean protein intake was 72.2 g/day in the negative/trace group and 65.3 g/day in the ≥1 proteinuria group. In multivariate analysis, adjusting for age, sex, ethnicity, systolic blood pressure, and 2-h blood glucose, we were again unable to detect a significant correlation between dietary protein intake and clinical proteinuria.

CONCLUSIONS— These data do not support the hypothesis that high-protein intake is a risk factor for clinical proteinuria in NIDDM subjects. Therefore, any recommendation for protein restriction in the diets of NIDDM subjects, before the development of NIDDM-related nephropathy, must be made with caution.

FROM THE DEPARTMENTS OF CLINICAL EPIDEMIOLOGY AND GENERAL MEDICINE, UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT SAN ANTONIO; AND THE AUDIE L. MURPHY MEMORIAL VETERANS HOSPITAL, SAN ANTONIO, TEXAS.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO NAUMAN JAMEEL, MD, MPH, AMBULATORY CARE 11C, THE AUDIE L. MURPHY MEMORIAL VETERANS HOSPITAL, SAN ANTONIO, TX 78284.

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Interest in the role of protein intake in renal disease dates back to the early 1900s when investigators first reported that high dietary protein resulted in significant renal structural damage in animal models (1). The mechanism by which dietary protein caused kidney damage was unknown. Brenner et al. (2) hypothesized that a high-protein diet elevates the glomerular filtration rate, probably by increasing renal blood flow and intraglomerular pressure. In diabetic subjects, this increase in renal blood flow and intraglomerular pressure may increase the risk of diabetic nephropathy and other forms of chronic renal disease (2). Experiments with diabetic rats fed a high-protein diet support this hypothesis (3). Moreover, low-protein feedings delay the development of diabetic nephropathy in rats (3,4). However, corresponding data in humans implicating high-protein diets as a risk factor for diabetic nephropathy are less conclusive.

Several studies suggest that a protein-rich diet may aggravate susceptibility to nephropathy in insulin-dependent diabetes mellitus (IDDM) (5–7), but few, if any, studies have examined this relationship in non-insulin-dependent diabetes mellitus (NIDDM). Therefore, we hypothesized that dietary protein intake is correlated with clinical proteinuria in people with NIDDM. We tested our hypothesis by examining cross-sectional data from the San Antonio Heart Study (SAHS), a population-based study of diabetes and cardiovascular disease in Mexican Americans and non-Hispanic whites. A unique feature of this data base is the inclusion of Mexican Americans, a population with an unusually high incidence of NIDDM-related end-stage renal disease (8).

RESEARCH DESIGN AND METHODS

— Baseline data collection in the SAHS was carried out in two phases: phase 1 from October 1979 to

October 1982 and phase 2 from October 1984 to October 1988. Details of the study design, sampling procedures, and field methods have been reported previously (9,10). In both phases, households were randomly sampled from low-, middle-, and high-income neighborhoods. All 25- to 64-yr-old men and nonpregnant women residing in the selected households were eligible for the study. A total of 2217 Mexican Americans and non-Hispanic whites were examined in phase 1 of the study, and 2957 Mexican Americans and non-Hispanic whites were studied in phase 2. The overall response rate for the clinic visit for both phases combined was 65.3%.

A glucose tolerance test was performed in the morning on each participant with a 75-g glucose equivalent load (Koladex or Orangedex, Custom, Baltimore, MD). Individuals were instructed to fast 12–14 h before the test. After obtaining a fasting blood specimen, additional specimens were drawn 1 and 2 h after administration of the glucose load. Plasma glucose concentrations were measured with a bichromatic analyzer (Abbott, South Pasadena, CA).

Diabetes was diagnosed according to the criteria of the National Diabetes Data Group (NDDG; 11). Subjects who did not meet the NDDG plasma glucose criteria were also considered to be diabetic if they were taking an oral antidiabetic agent or insulin. Diabetic subjects not taking insulin were considered to have NIDDM. Diabetic patients taking insulin but with body mass index (BMI) of >30 kg/m^2 and an age at diagnosis of >40 yr were also considered to have NIDDM. NIDDM was diagnosed in 376 of 3302 (11.4%) Mexican Americans and 87 of 1872 (4.6%) non-Hispanic whites. Beginning in 1984, diabetic subjects from both phases were recontacted and asked to participate in a diabetes complications examination (retinopathy, proteinuria, peripheral vascular disease, and functional assessment). For phase 1 subjects, this examination took place between 2 and 6 yr after their initial SAHS examination. Phase 2 subjects were asked to participate in the diabetes complications examination as soon as their diabetic status had been confirmed. One non-Hispanic white and nine Mexican-American diabetic subjects from phase 1 had died before being invited to participate in the complications examination; thus, vital status was ascertained on 98.5% of these subjects. The response rates to the diabetes complications examination (both phases combined) were similar for Mexican Americans and non-Hispanic whites (86.4 and 80.2%, respectively). Clinical proteinuria was assessed in an early-morning spot urine at the time of the diabetes complications examination with Albustix (Ames, Elkhart, IN). This study is based on 147 phase 1 and 229 phase 2 subjects who had both urinalysis and complete dietary protein intake information available.

Nutritional data for all subjects were obtained at the time of their initial medical examination. Therefore, nutritional data were obtained 2–6 yr before measurement of proteinuria for phase 1 subjects and within a few months of proteinuria measurement for phase 2 subjects. For phase 1 subjects, nutritional data were obtained by 24-h dietary recall based on the nutritional assessment methods used in the Ten State Nutritional Survey (12) and recommended by the American Public Health Association (13). During the interview, participants were asked to recall foods consumed during the preceding 24-h period and to specify the portion size.

Protein intake of phase-2 subjects was assessed by a food-frequency questionnaire. Foods included in the questionnaire were those identified by an experienced dietitian as being both important sources of the nutrients of interest and those commonly consumed by our study population. From the food-frequency questionnaire, 20 food items were determined to be significant sources of protein for our study population by comparing the questionnaire with a listing of foods that account for 85% of the protein intake in SAHS phase 1 (24-h dietary recall data). For each food item on the questionnaire, participants were asked to specify their usual portion size and how often, on average, they consumed the food.

From both the dietary recall data (phase 1) and the food-frequency data (phase 2), grams of protein per day were calculated by multiplying the frequency of consumption of the food by the protein content of the specified usual portion size for each food item. Protein intake in grams per kilogram per day ($g \cdot kg^{-1} \cdot day^{-1}$) was also calculated by dividing the protein intake (in grams/day) value by the participant's weight in kilograms. The U.S. Department of Agriculture food composition table, handbook 456 was the source of information regarding the protein content of foods (14).

The data for proteinuria were dichotomized into negative or trace versus ≥1. This dichotomy has been previously used in another epidemiological study of diabetes complications (15). The data were also analyzed with a proteinuria cutoff point of negative versus trace or more. Because similar results were obtained with both cutoff points, only the analyses with the former cutoff point are presented. We also measured albuminuria (also known as "microalbuminuria") in a subset of our subject population (103 phase 1 and 131 phase 2 subjects). Albuminuria was determined by a quantitative immunoturbidimetric method (Ames, Slough, UK; 10). An albuminuria value of ≥30 mg/L was considered positive.

Statistical analyses were performed with the SAS statistical package (SAS, Cary, NC). Means for conventional risk factors for diabetic nephropathy were calculated and compared for statistical significance with Student's *t* test (Table 1). In both phases, the distribution of protein intake for the two proteinuria groups was consistent with

Table 1—*Summary of clinical features by proteinuria groups in San Antonio Heart Study phases 1 and 2 non-insulin-dependent diabetic subjects*

	Phase 1		Phase 2	
Proteinuria	Negative/ trace	Positive	Negative/ trace	Positive
N	125	22	193	36
Age (yr)	56.7	58.7	52.5	54.6
Duration of diabetes (yr)	7.5	10.4	4.7	9.4*
Systolic blood pressure (mmHg)	133.3	147.5*	129.7	135.7
Diastolic blood pressure (mmHg)	75.6	76.3	74.3	75
Fasting blood glucose (mM)	8.3	10.4	9.3	10.5
2-h blood glucose (mM)	15.3	16.9*	16.9	20*

*$P < 0.05$ between proteinuria groups.

the univariate test of normality. Student's *t* test was used to compare the mean protein intake between the proteinuria groups (Table 2). Within each phase of the study, the relationship between protein intake and proteinuria was evaluated with the multiple logistic regression model to control for the effects of age, sex, ethnicity, systolic blood pressure, and 2-h blood glucose (16).

RESULTS— The proportion of diabetic subjects with proteinuria was 15% in phase 1 and 15.7% in phase 2.

Table 1 compares clinical features between the proteinuria-negative/trace group and the proteinuria-positive group. In phase 1 NIDDM subjects, systolic blood pressure and 2-h plasma glucose were significantly higher in the proteinuria-positive group compared with the proteinuria-negative group. In phase 2 NIDDM subjects, duration of diabetes and 2-h plasma glucose were significantly different between the two proteinuria groups ($P < 0.05$ for all).

Table 2 shows that mean protein intake for the SAHS subjects with NIDDM compared with national survey data obtained from the Second National Health and Nutrition Examination Survey (NHANES II, conducted between 1976 and 1980). Note that mean protein intake in SAHS phases 1 and 2 is similar to NHANES II data.

Table 3 shows the mean protein intake (in g/day) by proteinuria groups for men and women in phases 1 and 2. Note that the mean protein intake is higher in men than women. In neither phase was there a significant difference in mean protein intake between subjects with and without proteinuria. The analyses were then repeated with protein intake in grams per kilogram per day as the independent variable (data not shown); no difference in results was observed when protein intake was expressed in these terms.

We also studied the dose-response relationship between protein intake and proteinuria by stratifying our study population into high-, moderate-, and low-protein intake groups on the basis of NHANES II data (17). High-protein intake in males and females is >130 and >86 g/day, respectively (90th percentile protein intake for males and females in age-group 45–74 yr in NHANES II data). Low-protein intake in men and women is <42 and <28 g/day, respectively (10th percentile protein intake for men and women in age-group 45–74 yr in NHANES II data). Moderate protein intake is the value between these extremes. Table 4 shows that the proportion of subjects with clinical proteinuria was not statistically different among the high-, moderate-, and low-protein intake groups ($\chi^2 = 0.492$, $P = 0.48$).

The relationship between mean protein intake and proteinuria was also evaluated with a multiple logistic regression model. The model controlled

Table 2—*Comparison of mean protein intake (g/day) in San Antonio Heart Study (SAHS) subjects with Second National Health and Nutrition Examination Survey (NHANES II) data*

	Age-group (yr)			
	25–44	45–54	55–64	65–74
Men				
SAHS phase 1*	117.6	84.5	98.5	87
SAHS phase 2†	116.7	82.6	82.9	
NHANES II*	101	93	84	73
Women				
SAHS phase 1*	52.4	66.1	61.6	68.7
SAHS phase 2†	76.6	68.4	56.9	
NHANES II*	63	58	55	51

Explanation of missing data in 65- to 74-yr age-group in SAHS phase 2. No subjects were >64 yr at time of dietary protein determination for SAHS phase 2.
*24-h dietary recall method.
†Food-frequency questionnaire.

Table 3—*Mean protein intake (g/day) by proteinuria groups in San Antonio Heart Study (SAHS) phase-1 and -2 non-insulin-dependent diabetic subjects*

	Proteinuria		
	Negative/trace	Positive	*P*
SAHS			
Phase 1			
Men	97.6 (57)	82.1 (13)	0.12
Women	65.1 (68)	57.7 (09)	0.50
Phase 2			
Men	82.8 (71)	81.6 (10)	0.90
Women	66 (122)	59 (26)	0.21

n given in parentheses.

for possible confounding effects of age, sex, ethnicity, systolic blood pressure, and 2-h blood glucose. In neither phase did adjustment for these factors change the results; i.e., in the multivariate analysis, there was no statistical association between dietary protein and proteinuria.

Finally, dietary protein intake was compared in NIDDM subjects with or without albuminuria (Table 5). In both phases and in both men and women, protein intake was lower in the albuminuria-positive group than in the albuminuria-negative group. The association achieved statistical significance for men in phase 1 and for women in phase 2.

CONCLUSIONS— Contrary to our hypothesis, we found no correlation between protein intake and proteinuria among NIDDM subjects. Therefore, our data do not support the hypothesis that high protein intake is a risk factor in the development of NIDDM-related proteinuria. This finding was consistent across both phases of the SAHS, with two different methods of nutritional assessment. Furthermore, the albuminuria and protein intake analysis on a subgroup of our study population strongly argues against a positive correlation between protein intake and nephropathy in NIDDM.

There are three areas of concern regarding the methodology of our study: *1*) the level of protein intake of our cohort, *2*) the cutoff level chosen for proteinuria, and *3*) the method by which proteinuria was measured. Regarding the level of protein intake, the relatively older age of our study subjects (83% >45 yr and 10% >65 yr old) explained why the mean protein intake in our study was lower than the mean protein intake in the average American diet (reported as 90–110 for men and 55–65 g/day for women in national surveys). Protein intake gradually declines with age in many national surveys, including NHANES II. For a given age-group, the mean protein intake in SAHS phases 1 and 2 was similar to NHANES II data (Table 2). The age of our subjects was also taken into consideration when we defined our proteinuria groups. We observed that 15–25% of diabetic subjects aged ≥60 yr had urinary albumin concentrations >30 μg/min (18). One possible explanation may be coexisting hypertension in these subjects because patients with hypertension have mildly elevated albumin excretion rates (19). Bennett (20) recommends the use of higher limits to define the abnormal range in older subjects. Therefore, subjects with negative and trace proteinuria have been grouped together. Regarding our use of Albustix, this method has a sensitivity limit of 250–300 mg/L (21). Due to the reasons discussed above, albuminuria detected by Albustix was not a drawback for our NIDDM patient population. To the contrary, it may be more appropriate, because it has been suggested that the use of higher limits to define clinical proteinuria in elderly diabetic patients may improve the sensitivity, specificity, and predictive value of the positive test (20).

The partially cross-sectional nature of the study leaves open the possibility that prospective studies might

Table 4—*Proportion of subjects with clinical proteinuria*

Protein intake	Proteinuria		Total	With proteinuria (%)
	Positive	Negative/trace		
High*	9	55	64	14.1
Moderate†	43	240	283	15.2
Low‡	6	23	29	20.7
Total	58	318	376	15.4

No significant difference between groups ($\chi^2 = 0.492$, $P = 0.48$).
*Mean protein intake >130 g/day (men) and >86 g/day (women).
†Mean protein intake 42–130 g/day (men) and 28–86 g/day (women).
‡Mean protein intake <42 g/day (men) and <28 g/day (women).

Table 5—*Mean protein intake (g/day) by albuminuria groups in subset of subjects from San Antonio Heart Study (SAHS) phases 1 and 2*

	Albuminuria		
	Negative	Positive	*P*
SAHS			
Phase 1			
Men	101.2 (43)	74.2 (10)	0.003
Women	66.7 (42)	45.9 (8)	0.128
Phase 2			
Men	84.4 (36)	72.9 (10)	0.326
Women	68.3 (59)	55.8 (26)	0.036

n given in parentheses.

find a relationship. Because proteinuria in these NIDDM subjects is generally "silent," it seems unlikely that proteinuric subjects deliberately altered their diet. The high-protein diet–renal disease hypothesis is not widely known by the lay public.

Indirect support for the hypothesis of Brenner et al. (2) has come from clinical trials on protein restriction in diabetic subjects with established nephropathy. A few clinical intervention studies have claimed beneficial effects of dietary protein restriction in patients with diabetic renal disease (22–24) but can be criticized for having too few subjects, short duration of follow-up, and/or lack of control for major confounding variables (25). Moreover, the effect of a low-protein diet may not be necessarily due to the reduction of dietary protein (25). Also important are other complex nutritional changes that occur concurrently when dietary protein is restricted (25). Furthermore, beneficial effects of dietary protein restriction in established nephropathy do not necessarily mean that such dietary maneuvers will help in the prevention of nephropathy in diabetic subjects in general.

The ideal proportion of carbohydrate, fat, and protein in the diabetic diet has been the subject of changing recommendations over the years (26). The recommendations have shifted from restriction of carbohydrate to restriction of total and saturated fat accompanied by a higher percentage of carbohydrate. In a diabetic diet, a delicate balance in proportion of carbohydrate, fat, and protein needs to be maintained. Without a higher level of evidence of benefit, any overzealous restriction in dietary protein for diabetic subjects may translate to an undesirable increase in carbohydrates or fat intake to maintain the recommended caloric intake. Dietary restriction of protein in diabetic subjects may increase the risk of malnutrition or infection. Furthermore, participants in clinical trials have found it difficult to comply with protein restriction (23).

In conclusion, this study did not find a positive correlation between protein intake and clinical proteinuria in NIDDM. Further prospective studies may shed additional light on this question. High-protein intake has not been proved as a risk factor for the development nephropathy in NIDDM; thus, any potential benefits of dietary protein restriction have to be weighed against possible adverse effects. Therefore, any recommendation for protein restriction in a diabetic diet, before the development of nephropathy in NIDDM, should be made with caution.

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