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Dietary flaxseed meal reduces proteinuria and ameliorates nephropathy in an animal model of type II diabetes mellitus

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Dietary flaxseed meal reduces proteinuria and ameliorates nephropathy in an animal model of type II diabetes mellitus.

Background. Evidence is emerging that varying the type or source of dietary protein intake can have beneficial effects on chronic renal disease. Consumption of soybean and soy-based food products, as the source of plant protein, can retard the development and progression of chronic renal disease. We studied the obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat, a model of obesity and type II diabetes mellitus that consistently develops nephropathy resembling diabetic nephropathy. We specifically sought to determine whether changing the source of protein intake from animal protein, casein, to plant protein in the form of either soy protein concentrate or flaxseed protein in the diet has a different impact on renal function and nephropathy in this model.

Methods. Male obese SHR/N-cp rats were randomly assigned to one of three diets containing either 20% casein, 20% soy protein concentrate, or 20% flaxseed meal. Except for the protein source, all three diets were identical and contained similar amounts of protein, fat, carbohydrates, minerals, and vitamins. All animals were maintained on these diets for 6 months. At the end of the study, blood sampling and 24-hour urine collections were performed for renal functional measurements, and the kidneys were harvested and examined for histologic evaluation.

Results. All three groups had similar amounts of food intake and body weight gain and exhibited fasting hyperglycemia and hyperinsulinemia. Plasma glucose levels did not differ among the three groups, but plasma insulin concentration was significantly lower in rats fed flaxseed meal than those fed either casein or soy protein concentrate. Mean plasma creatinine, creatinine clearance, and urinary urea excretion also did not differ significantly between the three groups. By contrast, urinary protein excretion was significantly lower ($P < 0.01$) in rats fed flaxseed than in rats fed either casein or soy protein concentrate. Morphologic analysis of renal structural lesions showed that the percentage of abnormal glomeruli with mesangial expansion and the tubulointerstitial score (an index of severity of tubulointerstitial damage) were significantly reduced in rats fed flaxmeal compared to those fed casein or soy protein concentrate.

Conclusion. We conclude that dietary protein substitution with flaxseed meal reduces proteinuria and glomerular and tubulointerstitial lesions in obese SHR/N-cp rats and that flaxseed meal is more effective than soy protein in reducing proteinuria and renal histologic abnormalities in this model. The reduction in proteinuria and renal injury was independent of the amount of protein intake and glycemic control. Which dietary component(s) present in flaxseed meal is (are) responsible for the renal protective effect remains to be determined.

Key words: type II diabetes mellitus, hyperglycemia, hyperinsulinemia, flaxseed meal, soy protein, proteinuria, diabetic nephropathy, glomerular disease, tubulointerstitium.

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There is abundant evidence that varying the amount of protein in the diet exerts a profound influence on renal function and the course of renal disease [1–5]. High protein intake has long been known to aggravate renal injury and accelerates the progression of chronic renal failure, whereas low protein intake produces the opposite effects. In addition, there is also evidence that changing the source or type of dietary protein may have a beneficial effect on renal function and renal disease. This is supported by nutritional intervention studies in animals and humans showing that replacement of animal protein with plant protein in the diet reduces proteinuria and preserves renal function [6–9]. Evidence is emerging that consumption of soybean and soy-based food products, as the source of plant protein, can retard the development and progression of chronic renal disease [10–19]. Besides soybean, other plant seeds, particularly flaxseed, have also received increasing attention for their potential beneficial role in chronic renal disease. For example, there are few reports suggesting that dietary flaxseed has protective effects on the kidney in some animal models of chronic renal disease [20–22] and in humans with lupus nephritis [23, 24]. As yet, there are no studies that have assessed the effect of dietary flaxseed on diabetic nephropathy.

The spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rat is a genetic animal model that exhibits obesity, type II diabetes mellitus, and mild hypertension [25–28]. The SHR/N-cp rat is a congenic strain and was originally derived from an initial mating of a male obese spontaneously hypertensive (Koletsky) rat which was heterozygous for the cp gene with a spontaneously hypertensive rat (SHR) of the Okamoto strain, followed by multiple cycles of backcrossing of the progeny to the SHR strain. A minimum of ten backcrosses was carried out to eliminate the non-cp genes of the Koletsky strain. Obese homozygotes (cp/cp), unlike their lean littermates, exhibit marked obesity, glucose intolerance, hyperinsulinemia, hyperlipidemia, and mild hypertension. Obese SHR/N-cp rats also consistently develop marked proteinuria and renal pathologic lesions characterized by renal hypertrophy, glomerular mesangial expansion, and tubulointerstitial lesions, which are features resembling diabetic nephropathy in humans [29, 30]. The role of two different dietary types of plant protein, namely soy protein and flaxseed protein, on nephropathy in this strain has not been examined. Therefore, we have studied the obese SHR/N-cp rat to examine the effects of dietary soy protein isolate and flaxseed meal on nephropathy in this model. We specifically sought to determine whether changing the source of protein intake from animal protein, casein, to either soy protein or flaxseed protein in the diet has a different impact on the proteinuria and renal pathologic abnormalities in this model.

METHODS

Animals

Male obese SHR/N-cp rats were obtained from the National Institutes of Health at approximately 5 to 6 weeks of age. At this age, obesity is already evident in SHR/N-corpulent (cp/cp) rats as indicated by higher body weight than their lean littermates and increased abdominal girth. The experimental protocol was approved by the Institutional Animal Care and Use Committees of the George Washington University, Washington, D.C., and by the Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. All animals were housed simultaneously in individual stainless steel wire cages with controlled temperature (22°C to 24°C) and relative humidity (40% to 50%) and maintained on a reverse 12-hour dark (9:00 a.m. to 9:00 p.m.) and light (9:00 p.m. to 9:00 a.m.) cycle.

Diets and experimental design

All animals were initially provided with Purina rat chow for 2 weeks and were maintained on this diet until 8 to 10 weeks of age. Food and water were consumed ad libitum. The rats were then randomly divided into three groups of 10 rats and fed American Institute of Nutrition (AIN)-93 diet [31] supplemented with casein, soy protein concentrate, or flaxseed meal, as the sole source of dietary protein. Group 1 rats received 20% casein, group 2 rats received 20% soy protein concentrate, and group 3 rats received 20% flaxseed meal. With the exception of the protein source, all three diets were identical and contain similar amounts of protein, fat, carbohydrates, minerals, and vitamins. To ensure uniformity of the protein source, all protein components (casein, soy protein concentrate, and flaxseed meal) used in this study were obtained from the same batch. The levels of isoflavones in soy protein concentrate and lignan content in flaxseed meal were analyzed by Archer Daniels Midland Company, Decatur, IL. Soy protein concentrate contained 234.9 mg/kg total isoflavones. Approximately 97% of the isoflavones are in glucoside form. The ratio of genistein:daidzein:glycitein (as aglycone equivalents) is 1.3:1:0.3. Flaxseed meal contained 17.0 mg/g secoisolariciresinol diglucoside (SDG). All diets contained (g/kg) dextrinized cornstarch, 155; sucrose, 100; corn oil, 40; cellulose, 50; mineral mix (AIN-93M-MX), 35; vitamin mix (AIN-93-VX), 10; L-cystine, 1.8; choline bitartrate, 2.5; and tert-butylhydroquinone, 0.0008. Amount of cornstarch was adjusted in each diet to provide 20% of energy from protein. Casein and L-cystine were purchased from Sigma Chemical Co., St. Louis, MO. Soy protein concentrate and flaxseed meal were obtained gratis from Protein Specialties Division, Archer Daniels Midland. Tert-butylhydroquinone was purchased from Aldrich Chemical Co., Milwaukee, WI. All other ingredients were obtained from Dyets, Inc., Bethlehem, PA. All animals were fed the experimental diets for 6 months, and body weight and food intake were measured biweekly throughout the study. At the end of the feeding period, animals were placed metabolic cages and timed-urine collections were made with the rats in the fasting state. After these collections, rats were weighed and sacrificed by decapitation under carbon dioxide anesthesia. Blood was collected in ethylenediaminetetraacetic acid (EDTA) and Trasylol (100 KU/L) and plasma was separated for subsequent biochemical analyses. Immediately after sacrifice, the abdomen was opened by a midline incision and the abdominal aorta was perfused with cold 0.9% normal saline using a Cole-Palmer peristaltic pump set to deliver perfusion solution at a rate of 10 mL/min. Following perfusion, the kidneys were dissected free of fat, quickly removed, and weighed. The kidney was trisected coronally and placed in 10% buffered formalin.

Metabolic and renal functional measurements

Plasma concentrations of glucose and plasma and urinary concentrations of urea and creatinine were measured using Alcyon analyzer and ATAC 8000 (Abbott

Laboratories, Abbott Park, IL, USA) and kits from Elan Diagnostics (Smithfield, RI, USA). Plasma insulin was measured by radioimmunoassay using rat insulin as standard. Plasma total protein and urinary protein excretion was measured by the Lowry method. Creatinine clearance, an estimate of glomerular filtration rate (GFR), was determined by standard clearance techniques. Urinary protein excretion was expressed both as the ratio of urine protein concentration and urine creatinine concentration (mg U protein/mg U creatinine), as well as the product of urine protein concentration and urine volume (V) (U protein V in mg/24 hours). Urinary urea excretion rate ($U_{UN}V$), an index of protein intake, was also determined as the product of urine urea concentration and V.

Histology

Glomerular morphology. Coronal sections of the in situ perfused kidney were embedded in paraffin. The sections were cut at 3 micra and stained with hematoxylin-eosin (H&E), periodic acid-Sciff (PAS), and Masson's trichrome stains. All cut sections were coded and evaluated blindly by two pathologists without knowledge of the experimental diets Concordance between the two pathologists was greater than 95%. The sections were reviewed and evaluated separately in the following manner. Slides were inserted into the micrometer stage of a Nikon Eclipse Model E600W microscope and scanned in passes at about 2 mm distance per pass at 200× magnification. For each specimen, point counting of 50 to 100 consecutive glomeruli within a single section was performed twice assessing and distinguishing the identity of glomeruli with normal morphology from those with abnormal lesions, namely mesangial expansion and glomerular sclerosis. The total number of abnormal glomeruli and glomeruli with mesangial expansion and with sclerosis were tabulated and expressed as percentages. Glomerular sclerosis was defined by the absence of cellular elements in the tuft, collapse of capillary loops or folding of the glomerular basement membrane with an accumulation of amorphous material, and adhesion of the obsolescent segment of Bowman's capsule. The PAS and Masson trichrome stains were used to assess the extent of mesangial expansion and confirm the presence or absence of glomerular sclerosis.

Tubulointerstitial morphology. The renal tubules and surrounding interstitium were evaluated in a semiquantitative manner using a grading system with a score of 0 to 4+, which assessed tubulointerstitial damage as follows: 0 represents normal histology with no pathologic lesions; 1+ indicates small focal lesion, with very minimal tubular atrophy and dilatation, and interstitial inflammatory cell infiltration involving less than 25% of the renal parenchyma and intertstitium; 2+ indicates increased tubular dilatation and atrophy, mild inflammatory cell infiltrate without interstitial fibrosis, affecting 25% to 50% of the parenchyma and intertstitium; 3+ indicates increased tubular atrophy and moderate inflammatory cell infiltrate with early interstitial fibrosis, involving 50% to 75% of parenchyma; and 4+ indicates severe extensive tubulointerstitial changes and fibrosis involving 75% or more of the parenchyma and interstitium. The concordance between the two-blinded pathologists was greater than 95% identical scoring.

Table 1. Body weight, food intake, and fasting plasma levels of glucose and insulin in obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rats fed a diet supplemented with either casein, soy protein concentrate, or flaxseed meal

Parameter	Casein	Soy protein	Flax meal
Body weight *g*	558 ± 22	547 ± 26	575 ± 19
Food intake *g/day*	14.6 ± 1.0	14.7 ± 0.9	12.9 ± 1.0
Serum glucose *mmol/L*	12.8 ± 2.1	13.3 ± 1.2	12.9 ± 1.6
Serum insulin *pmol/L*	3485 + 372[a]	3121 + 296[a]	2279 + 284[b]

Values are mean ± SEM of 10 rats.
Values with different superscripts within a row are significantly different at $P < 0.05$.

Statistical analysis

Results are expressed as mean + standard error of the mean (SEM). Comparisons between groups were made using one-way analysis of variance (ANOVA). When an effect was statistically significant ($P < 0.05$), mean comparisons were made. A Sidak adjusted significance level was used for the pair-wise comparisons of the means so that the overall significance level was 0.05. Differences between means were considered significant when the P value was less than 0.05.

For analysis of glomerular lesions, the percent of abnormal glomeruli was analyzed as a one-factor general linear model using PROC MIXED with diet as the factor. Further mean pair-wise comparisons were made with Sidak adjusted P values. The tubulointerstitial score data were analyzed by an exact version of the Kruskal-Wallis nonparametric rank test.

RESULTS

The results of experiments performed in obese SHR/N-cp rats fed the three different protein diets are summarized in Tables 1 and 2, respectively.

Food intake, weight gain, and metabolic parameters

The animals tolerated the diets and food intake did not differ significantly between the three diet groups. After 6 months of feeding, all rats gained weight and the mean final body weight was similar among the three groups. Obese SHR/N-cp rats consuming either of the three different diets exhibited both hyperglycemia and hyperinsulinemia with relatively higher fasting levels of plasma

Table 2. Renal functional measurements in obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rats fed a diet supplemented with either casein, soy protein concentrate, or flaxseed meal

Parameter	Casein	Soy protein	Flax meal
Plasma creatinine *umol/L*	27.5 ± 3.6	24.9 ± 2.9	27.9 ± 2.4
Creatinine clearance *mL/min*	1.44 ± 0.21	1.90 ± 0.35	2.16 ± 0.39
Urine urea excretion *mg/24 hours*	216 ± 18	195 ± 15	213 ± 8
Urine protein excretion *mg/mg creatinine*	3.18 ± 0.35[a]	2.92 ± 0.27[a]	1.70 ± 0.12[b]
mg/24 hours	21.2 ± 2.2[a]	19.9 ± 2.1[a]	14.5 ± 1.1[b]

Values are mean ± SEM of 10 rats.
Values with different superscripts within a row are significantly different at $P < 0.01$.

Table 3. Kidney weight and percentage of glomerular lesions in obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rats fed a diet supplemented with either casein, soy protein concentrate, and flaxseed meal

Phenotype	Casein	Soy protein	Flax meal
Kidney weight *g*			
Left	1.83 ± 0.09	1.91 ± 0.08	1.75 ± 0.05
Right	1.92 ± 0.07	1.93 ± 0.07	1.75 ± 0.05
Glomerular lesions %			
Total abnormal glomeruli	29.4 ± 3.2[a]	25.8 ± 3.2[a]	10.8 ± 3.2[b]
With mesangial expansion	27.6 ± 3.7[a]	24.4 ± 1.9[a]	10.0 ± 2.3[b]
With glomerular sclerosis	1.80 ± 0.81	1.40 ± 0.79	0.80 ± 0.55

Values are mean ± SEM of 10 rats.
Values with different superscripts within a row are significantly different at $P < 0.01$.

glucose and insulin when compared to lean counterparts, as has been reported in previous studies in which 10% casein and 10% lactalbumin were used as the sources of dietary protein [26, 27]. However, mean fasting levels of plasma glucose did not differ significantly between the three groups, regardless of the protein source. Plasma insulin concentration was slightly lower in rats fed soy protein concentrate than those fed casein, but the decrease was not statistically significant. In contrast, plasma insulin was significantly lower in rats fed flaxseed meal than those fed either casein or soy protein concentrate.

Renal functional measurements

Plasma concentration of creatinine in obese rats fed casein, which averaged 0.31 ± 0.04 mg/dL, was not significantly different from those observed in animals fed either soy protein concentrate (0.28 ± 0.03 mg/dL) or flaxseed meal (0.32 ± 0.03 mg/dL). However, mean creatinine clearance was higher in rats fed either soy protein concentrate or flaxseed meal than in rats fed casein with the highest values observed in the animals fed flaxseed meal. However, these differences between the three groups did not reach statistical significance. Similarly, mean $U_{UN}V$ did not differ among the animals fed the three diets. By contrast, urinary protein excretion, whether expressed as the ratio of U protein/U creatinine (mg/mg) or as the product of urine protein concentration and urine volume (U protein V) (mg/24 hours) was significantly lower ($P <$ 0.01) in rats fed flaxseed than in rats fed either casein or soy protein concentrate.

Kidney weight and renal histology

Kidney weight of obese rats fed casein averaged 1.91 ± 0.09 g (Table 3). Mean kidney weight of obese rats fed soy protein concentrate was 1.92 ± 0.09 and nearly the same as that of rats fed casein. Mean kidney weight was slightly lower in rats fed flaxseed meal (1.75 ± 0.05) than in animals fed casein or soy protein concentrate, but the differences were not stastistically significant.

On light microscopy, the typical renal lesions of obese SHR/N-cp rats are shown in Figure 1. The glomeruli of rats fed casein shows segmental or diffuse mesangial expansion (as evidenced by increased PAS staining of the mesangium) with or without increased proliferation of mesangial cells (Fig. 1A and D). The majority of abnormal glomeruli have expanded mesangium but without increased cellularity, whereas only a few glomeruli show segmental or global sclerosis. The tubulointerstitium shows local and focal areas of tubular atrophy and dilatation often filled with proteinaceous casts in lumina, mononuclear cell infiltration, and, rarely, interstitial fibrosis. Abnormal glomeruli are frequently seen adjacent to foci of tubulointerstitial lesions. Glomerular and tubulointerstitial lesions were less pronounced in kidneys of rats fed soy protein concentrate (Fig. 1B and E). By contrast, most of the glomeruli and tubules, and the surrounding interstitium are preserved in kidneys of animals fed flaxseed meal (Fig. 1C and F).

The percentages of total abnormal glomeruli and with mesangial expansion and with glomerular sclerosis are summarized in Table 3. Of the three groups, rats fed casein had the greatest number of abnormal glomeruli with mesangial expansion (27.6% ± 3.7%) and sclerosis (1.80% + 0.81%). Rats fed soy protein showed slightly less number of abnormal glomeruli with mesangial expansion (24.4% ± 1.9%) and sclerosis (1.40% ± 0.79%) than those fed casein but these differences were not statistically significant. Rats fed flaxseed meal, however, showed the lowest percentage of abnormal glomeruli with expanded mesangium (10.0% ± 2.3%) that were significantly different from those fed casein or soy protein concentrate ($P < 0.01$). Rats fed flaxseed meal also had the lowest percentage of abnormal glomeruli with sclerosis (0.80% ± 0.55%) but this decrease did not reach statistical significance.

The data for tubulointerstitial score from each animal fed the three different diets are shown in Table 4. The Kruskal-Wallis nonparametric rank test show that overall there is a significant difference among the three diet groups with $P = 0.03$. There was a statistically lower

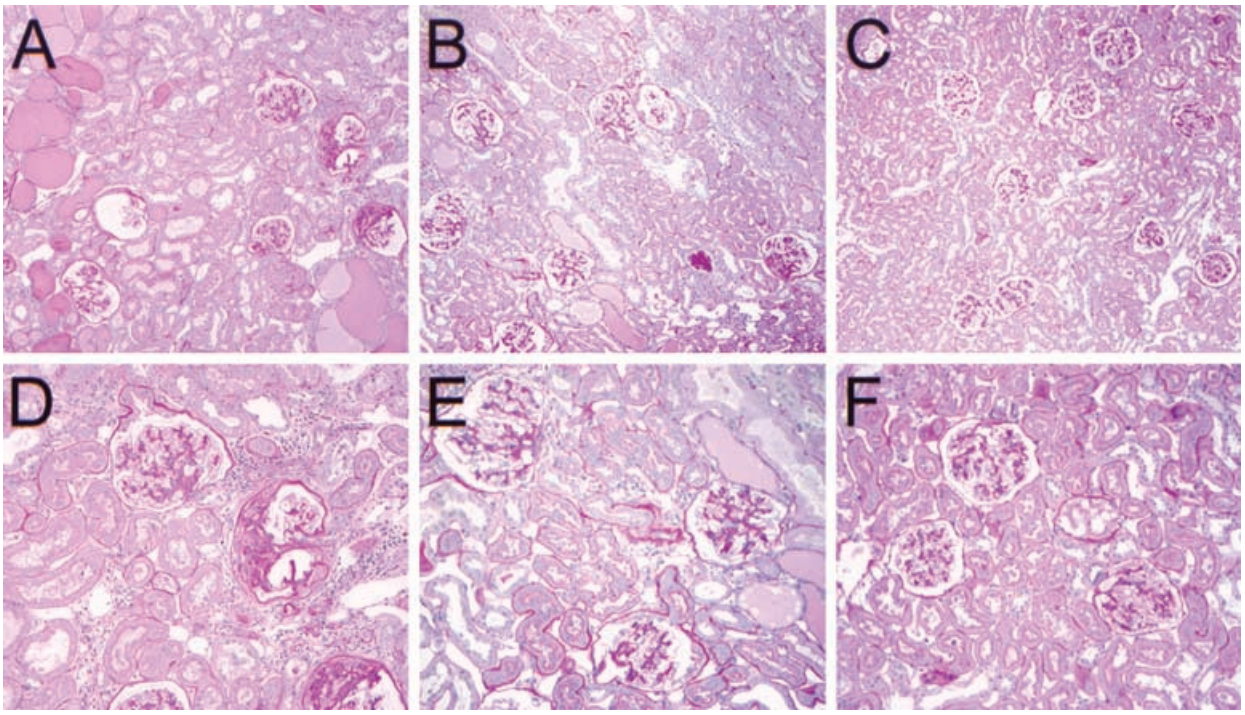

Fig. 1. Representative light micrographs periodic acid-Schiff (PAS)-stained kidney sections from obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rats fed with either casein (*A*), soy protein concentrate (*B*), or flaxseed meal (*C*) (×40). (A) Some glomeruli show increased PAS staining, whereas others are sclerotic. The tubulointerstitium shows foci of atrophic or dilated tubules, some filled with proteinaceous casts inside lumina and surrounded by mononuclear cell infiltrate, and, rarely, interstitial fibrosis. (B) Glomerular and tubulointerstitial lesions appear to be less prominent with soy protein concentrate feeding. (C) In contrast, glomerular and tubulointerstitial lesions are markedly reduced by flaxseed meal supplementation. Higher magnification (×200) shows essentially similar pattern of severity of lesions with casein (*D*), soy protein concentrate (*E*), or flaxseed meal (*F*).

TI score for flaxseed meal diet compared to casein diet ($P = 0.015$). However, differences between soy protein and casein and between flaxseed and soy protein were not significant, with $P = 0.48$ and $P = 0.08$, respectively.

DISCUSSION

To our knowledge, the present study is the first to demonstrate that substituting flaxseed meal for casein in the diet significantly reduces urinary protein excretion and renal glomerular and tubulointerstitial lesions in obese SHR/N-cp rats with hyperglycemia and hyperinsulinemia. Soy protein substitution also reduced proteinuria and renal pathologic lesions in these animals but these reductions were only minimal and did not reach statistical significance. Flaxseed meal or soy protein supplementation was not associated with significant changes in plasma glucose levels in obese animals, indicating that the observed renal protective effects of flaxseed meal or soy protein were independent of glycemic control.

It should also be noted that, except for the protein source, the diets used in the present study were similar in the total amounts of protein, fat, carbohydrate, minerals, sodium, and vitamins. In addition, there were no significant differences between the food intake and final body weights among the three diet groups. Moreover, urinary urea excretion also did not differ significantly among the three groups, which further suggests that the total amount of protein intake of the animals was similar. Therefore, it seems highly unlikely that the different effects of flaxseed meal and soy protein on proteinuria and nephropathy can be ascribed to differences in protein, energy or fat intake.

Several groups of investigators have shown a beneficial renal effect of soy protein in other animal models of chronic renal disease. Williams and Walls [6] were the first to show that in rats with subtotal nephrectomy substituting soybean for casein in the diet resulted in less proteinuria, less hypertrophy of the remnant kidney, less glomerular sclerosis and tubular atrophy, as well as lower mortality. Iwasaki et al [10] also showed that replacing dietary casein with soy protein isolate for 18 months or longer in the Fischer 344 (F344) rat, which develop age-related chronic nephropathy, slowed the progression of renal pathologic lesions and improved their survival. Other groups have shown similar benefits of soy protein feeding in rodent models of inherited polycystic kidney disease (PKD), namely, the DBA/2FG-*pcy* (*pcy*) mouse [11], CDI-*pcy/pcy* mouse [12], and the Han:SPRD-cy rat [13]. In all these PKD models, soy protein substitution was found to be effective not only in slowing cyst

Table 4. Tubulointerstitial score in obese spontaneously hypertensive/NIH-corpulent (SHR/N-cp) rats fed a diet supplemented with either casein, soy protein concentrate, and flaxseed meal

	Tubulointerstitial score[a]					
Diet	0	1+	2+	3+	4+	
	Number of rats					Total
Casein	0	4	3	3	0	10
Soy protein	2	4	1	3	0	10
Flaxmeal	7	1	0	2	0	10

[a]Grading was based on semiquantitative analysis of the severity of tubulointerstitial damage using a score of 0 to 4+.

growth but also in reducing noncystic pathologic lesions in the tubules and the renal intertstitium. More recently, Maddox et al [19] showed that feeding a diet containing 25% soy protein isolate as compared to casein-containing diet was also effective in slowing the development of proteinuria and glomerular injury in the obese Zucker rat. In the present study, however, we found only minor and insignificant reductions in proteinuria and renal lesions with soy protein replacement. Because soy protein feeding per se did not have any significant effect on hyperglycemia and hyperinsulinemia, it is possible that the persistence of the diabetic and insulin resistant state in obese SHR/N-cp rats during the study might have limited renal protective effects of soy protein ingestion. In one clinical study of male patients with type II diabetes mellitus, obesity, hypertension, and proteinuria, consumption of a soy-based diet (that provided soy protein as half of the daily protein intake) for 8 weeks had no significant effects on GFR, proteinuria, and glycohemoglobin levels in these subjects [32].

Our results are complementary to previous reports by other groups showing a renal protective effect of dietary flaxseed in animals and humans with chronic renal disease. Hall et al [20] first reported a renal protective effect of dietary flaxseed in the MRL/*lpr* mouse, a murine model of lupus nephritis. These investigators found that feeding 15% flaxseed diet as opposed to a standard rodent diet without flaxseed for 14 weeks in female MRL/*lpr* mice delayed the onset of proteinuria and preserved GFR in these animals. Subsequently, the same group of investigators applied dietary flaxseed to patients with lupus nephritis and found that flaxseed supplementation given sequentially at 4 weekly intervals in these patients reduced proteinuria and increased creatinine clearance in proportion to increasing dose of flaxseed [23]. Similar beneficial effects of dietary flaxseed have also been observed in the rat 5/6 renal ablation model [21] and in the Han:SPRD-cy rat model of PKD [22]. Our study indicates that dietary flaxseed supplementation is also protective against chronic renal disease in a model of obesity and type II diabetes mellitus and that flaxseed meal is more effective than soy protein in reducing proteinuria and glomerular and tubulointerstitial lesions in this model. Our data further show that this greater renal beneficial effect of dietary flaxseed is independent of the quantity of protein intake and the hyperglycemic state of the animals. However, we observed that flaxseed meal supplementation as compared to casein and soy protein concentrate was associated with lower plasma insulin levels in obese rats, suggesting an improvement of hyperinsulinemia and insulin-resistance in these animals. Since insulin itself is one of the hormonal factors implicated in the pathogenesis of mesangial matrix accumulation in diabetic nephropathy [33, 34], and since reduction of insulin levels in vivo delays or reduces glomerulosclerosis and tubular and interstitial lesions in obese hyperinsulinemic Zucker rats [35], it is possible that the reduction of hyperisulinemia by flaxseed meal in the present study may have contributed in part to the improvement of proteinuria and renal lesions in obese SHR/N-cp rats. Taken together, these studies and our present results indicate that dietary flaxseed can be protective against renal injury in various types of chronic renal disease suggesting that this benefit extends to renal disease associated with type II diabetes mellitus.

The present study did not determine which component(s) in flaxseed meal is (are) responsible for its salutary effects on proteinuria and nephropathy. Previous studies showing a protective effect of dietary flaxseed on the kidney have used either whole flaxseed or flaxseed oil. Whole flaxseed contains approximately 41% fat and 21% protein of the seed weight [36, 37]. The fat in the intact seed is particularly rich in α-linolenic acid, which accounts for approximately 57% of the total fatty acid in flaxseed oil. Because α-linolenic acid has intrinsic lipid-lowering and anti-inflammatory properties [38–42], and because α-linolenic acid content was found to be increased in renal tissues of subtotally nephrectomized rats fed flaxseed and flax oil [21], this component of flaxseed has been suggested to be partly responsible for the beneficial renal effects of dietary flaxseed observed in earlier studies [20–23]. However, the flaxseed meal used in our study, unlike that in earlier studies, has been fully defatted and does not contain α-linolenic acid. This excludes the possibility that this fatty acid played a role in the reduction of proteinuria and renal pathologic lesions afforded by flaxseed meal in the present study and suggests that other components of flaxseed may be involved. Which constituent(s) of defatted flaxseed is (are) responsible for its renoprotective effect needs further investigation.

It is known that flaxseed is also the richest source of lignans [43], which is one of the major classes of phytoestrogens, a group of plant-derived diphenolic compounds structurally related to endogenous estrogen that possess a wide range of biologic activities [44]. For example, plant lignans have been shown to induce specific reversible and competitive antagonism of platelet-activating factor

(PAF), a major factor involved in inflammation [45]. Since serum levels and urinary excretion of PAF are increased in patients with systemic lupus erythematosus and in mice with lupus nephritis [46, 47], inhibitory action of flaxseed-derived lignans on PAF could conceivably explain the beneficial effects of dietary flaxseed in lupus nephritis, as was suggested by Hall et al [20] in their studies of the MRL/*lpr* mouse. Lignans are also known to possess antiproliferative properties [48, 49], which may also contribute to the inhibitory effects of flaxseed on glomerular or renal epithelial cell proliferation seen in PKD and proliferative glomerular diseases. One of the major lignan precursors derived from flaxseed, SDG, has been shown to have antioxidant activity in animals [50–52]. In addition, Clark et al have shown that chronic oral administration of SDG (the lignan precursor also extracted from flax) for 22 weeks in MRL/*lpr* mice delayed the onset of proteinuria and preserved GFR and kidney weight in a time- and dose-dependent manner [53]. This study further suggests that SDG in flaxseed exerts renal protective effects similar to the whole flaxseed in this mouse model of lupus nephritis. The flaxseed meal used in the present study also contains very high amounts of SDG (e.g., 17.0 mg/g). Thus, it is possible that SDG may have contributed to the renal protective effects of flaxseed meal observed in the present study. Finally, it is also conceivable that the various cellular actions of lignans may represent a mechanism associated with the protective effects of flaxseed in a variety of chronic renal diseases, including diabetic nephropathy. Whether each one or all of these putative biochemical and cellular actions of lignans are involved in the renal protection by flaxseed in vivo remains to be determined.

CONCLUSION

We have shown for the first time that dietary protein substitution with flaxseed meal reduces proteinuria and glomerular and tubulointerstitial lesions in obese SHR/N-cp rats. These initial studies indicate that flaxseed meal is more effective than soy protein in reducing proteinuria and renal histologic injury in this model and that this beneficial effect is independent of the amount of protein intake and glycemic control. Which dietary component(s) present in flaxseed meal or soy protein is (are) responsible for the observed renal protective effect remains to be determined. The marked reduction in proteinuria and renal protective effects of flaxseed meal may have important therapeutic implications in patients with type II diabetes mellitus and diabetic nephropathy and, therefore deserve further study in humans with these disorders. Supplementation with flaxseed meal in the diet may provide a novel therapeutic strategy to reduce proteinuria and preserve renal function.

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