Omega-3 fatty acid rich diet prevents diabetic renal disease

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Garman JH, Mulroney S, Manigrasso M, Flynn E, Marie C. Omega-3 fatty acid rich diet prevents diabetic renal disease. Am J Physiol Renal Physiol 296:F306–F316, 2009.—Omega-3 polyunsaturated fatty acids (n-3 PUFA) show beneficial effects in cardiovascular disease, IgA, and diabetic nephropathy; however, the mechanisms underlying these benefits are unknown. The study was performed in male Sprague-Dawley rats randomly divided into four treatment groups: nondiabetic (ND), streptozotocin-induced diabetic (D), diabetic and fed a high n-3 PUFA diet (D+canola), and diabetic and fed a high n-6 (omega-6) PUFA diet (D+corn). Study treatments were carried out for 30 wk. D+canola significantly decreased diabetes-associated increases in urine albumin excretion (ND 17.8 ± 6.4; D 97.3 ± 9.4; D+canola 8.3 ± 2.2 mg/day); systolic blood pressure (ND 153 ± 9; D 198 ± 7; D+canola 162 ± 9 mmHg); glomerulosclerosis (ND 0.6 ± 0.2; D 1.8 ± 0.2; D+canola 0.8 ± 0.1 AU); and tubulointerstitial fibrosis in the renal cortex (ND 1.2 ± 0.2; D 2.0 ± 0.2; D+canola 1.1 ± 0.1) and the inner stripe of the outer medulla (ND 1.0 ± 0.2; D 2.1 ± 0.2; D+canola 1.1 ± 0.2 AU). D+corn also exerted renoprotection, but not to the same degree as D+canola (urine albumin excretion, 33.9 ± 6.1 mg/day; systolic blood pressure, D+corn 177 ± 6 mmHg; glomerulosclerosis, D+corn 1.2 ± 0.3 AU; cortical tubulointerstitial fibrosis, D+corn 1.6 ± 0.1 AU; medullary tubulointerstitial fibrosis, D+corn 1.5 ± 0.1 AU). In addition, D+canola attenuated D-associated increase in collagen type I and type IV, IL-6, MCP-1, transforming growth factor-β, and CD68 expression. These observations indicate a beneficial effect of high dietary intake of n-3 PUFA in reducing diabetic renal disease.

Diabetic nephropathy is the leading cause of chronic renal disease, end-stage renal disease, and the need for kidney dialysis and transplantation in the Western world (16). The currently available treatments for diabetic nephropathy (e.g., angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, β blockers, and diuretics) are antihypertensive drugs which slow the progression of renal disease, but do not totally stop or reverse existing disease (12, 16). Additionally, thiazolidinediones (e.g., pioglitazone and rosiglitazone) are used in the treatment of diabetic renal disease but have adverse cardiac effects (29). Research indicates that diet plays a significant role in the progression of diabetic renal complications; however, specific suggestions for improvements in diet are lacking (4, 27). The aim of this study is to examine the effect of diet modification on the prevention and treatment of diabetic renal disease.

Omega-3 polyunsaturated fatty acids (n-3 PUFA) have proven anti-inflammatory effects (14, 50) and are beneficial in the treatment of cardiovascular disease (49), IgA nephropathy (43), and cyclosporine nephrotoxicity (41) and in patients undergoing dialysis (18). Epidemiological studies suggest that n-3 PUFA slow the progression of renal dysfunction, e.g., prevent the decline in creatinine clearance in healthy older people (28), lessen the risk of albuminuria in young type 1 diabetic patients (35), and slow the progression of albuminuria in older patients with type 2 diabetes (44). Furthermore, dietary PUFA have been shown to have renoprotective effects in experimental models of diabetes (3, 19). However, the mechanisms by which n-3 PUFA exert this renoprotection are unclear. In addition, n-3 PUFA deficiency has been noted in the streptozotocin (STZ)-induced diabetic rat (23), suggesting that omega-3 PUFA supplementation may reduce complications associated with diabetes. Thus the aim of the present study was to examine some of the mechanisms by which a diet rich in canola, as a source of n-3 PUFA, reduces the development of diabetic renal disease in an experimental model.

MATERIALS AND METHODS

Animals and treatments. Male Sprague-Dawley rats (10 wk, Harlan, Madison, WI) were randomly divided into four treatment groups: nondiabetic (ND; n = 4), diabetic (D; n = 5), diabetic fed a corn oil-supplemented diet (D+corn; n = 5), and diabetic fed a corn oil-supplemented diet (D+corn; n = 5). All diets were based on low-salt rat chow (Harlan 7034, 0.10% Na) either without supplementation (ND and D) or supplemented con canola (D+canola, Mazola, ACH Food Companies, Memphis, TN) or corn oil (D+corn, Mazola, ACH Food Companies). Oil was added at a dose of 200 g/kg. The base diet contained 25, 60, and 15% of calories from protein, carbohydrates, and fat, respectively, while the canola- and corn oil-supplemented diet contained ~15, 35, and 50% of calories from protein, carbohydrates, and fat, respectively. The corn oil group served as a macronutrient control for the canola group. Food and water were given ad libitum. Diet supplementation started 2 wk before induction of diabetes and continued for the duration of the study (30 wk). Given the anticipated increase in food intake in the diabetic rats, low-sodium chow was chosen to prevent kidney damage due to sodium load, while still providing a diet with sufficient sodium (33).

During the treatment period (30 wk), body weight and blood glucose (FreeStyle, TheraSense, Alameda, CA) were measured weekly. The animals were placed in metabolic cages for 24 h every 4 wk for measurement of food and water consumption, urine output, and albumin concentration. At 29 wk of treatment, systolic blood pressure was measured by noninvasive tail-cuff sphygmomanometry (Narco Bio-Systems, Houston, TX). At 30 wk, the animals were weighed, anesthetized with pentobarbital sodium (40 mg/kg ip), and blood was collected (via cardiac puncture) for measurement of creatinine concentration. The kidneys were excised, weighed, and then either snap frozen in liquid nitrogen for protein analysis or immersion fixed with 10.220.33.1 on October 21, 2017 http://ajprenal.physiology.org/ Downloaded from http://ajprenal.org.org by 10.220.33.1 on October 21, 2017

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HistoChoice (Amresco, Solon, OH) for histological analysis. All experiments were performed according to the guidelines recommended by the National Institutes of Health and approved by the Georgetown University Animal Care and Use Committee.

Induction of diabetes. Diabetes was induced with a single intraperitoneal injection of STZ (Sigma, St. Louis, MO) at a dose of 55 mg/kg body wt in 0.1 M citrate buffer (pH 4.5) after an overnight fast. Nondiabetic rats were given a single intraperitoneal injection of citrate buffer. Blood glucose was measured 24 h following induction of diabetes using the FreeStyle glucometer, and only rats with blood glucose >200 mg/dl were included in the study. Throughout the study, the rats were injected twice weekly with 5 U of glargine insulin (Lantus, Aventis Pharmaceuticals, Kansas City, MO) to prevent excessive weight loss and mortality.

Urinary albumin excretion and creatinine clearance. Urine albumin concentration was measured using the Neprherit II albumin kit (Exocell, Philadelphia, PA), according to the manufacturer’s protocol. The rate of urine albumin excretion (UAE) was calculated based on urine albumin concentration and 24-h urine output. Urine and plasma creatinine concentrations were measured using a kit (BioAssay Systems, Hayward, CA), according to the manufacturer’s protocol. Creatinine clearance (CrCl) was calculated based on plasma and urine creatinine concentrations and 24-h urine output.

Glomerulosclerotic index. Periodic acid–Schiff (PAS)-stained paraffin sections (4 μm) were examined under a light microscope to assess the degree of glomerular damage, defined as mesangial matrix expansion, capillary dilation, and glomerular tuft occlusion. Approximately 60 glomeruli/sample were randomly selected and evaluated at ×400 using a semiquantitative scoring method (32) as follows: grade 0, no obvious sclerosis (normal); grade 1, sclerotic area <25% (minimal sclerosis); grade 2, sclerotic area 25–50% (moderate sclerosis); grade 3, sclerotic area 50–75% (moderate-severe sclerosis); and grade 4, sclerotic area 75–100% (severe sclerosis). The glomerulosclerotic index (GSI) was calculated using the weighted average of the evaluated glomeruli, as previously described (32). All evaluations were performed with the observer masked to the treatment group.

Tubulointerstitial fibrosis index. Masson’s trichrome-stained paraffin sections (4 μm) were examined under a light microscope to assess the degree of tubulointerstitial fibrosis defined as tubular atrophy or dilatation, presence of inflammatory cells, and deposition of extracellular matrix (ECM). Approximately 30 fields (at ×400)/sample were randomly selected and evaluated for degree of tubulointerstitial damage, in both the renal cortex and medulla using a semiquantitative scoring method (32) as follows: grade 0, no obvious damage; grade 1, affected area <10%; grade 2, affected area 10–25%; grade 3, affected area 25–75%; and grade 4, affected area 75–100%. The tubulointerstitial fibrosis index (TIFF) was calculated using the weighted average of the evaluated fields as previously described (32). All evaluations were performed with the observer masked to the treatment group.

Immunohistochemistry. Paraffin sections (4 μm) were dewaxed and hydrated. For transforming growth factor (TGF)-β, antigen retrieval was performed by heating the slides in citrate buffer (1.8 mM citric acid, 8.2 mM trisodium citrate) in a microwave for 10 min at ~90°C. For collagen type I and type IV, antigen retrieval was performed by incubating the slides in 1% trypsin with 0.1% albumin for 15 min. No antigen retrieval was performed for CD68 and nestin. The slides were serially incubated with 6% H2O2 in methanol for 10 min for blocking endogenous peroxidase, avidin, and biotin (Vector; Burlingame, CA) for 15 min (for blocking endogenous avidin/biotin), and 10% normal goat serum or 0.1% albumin (for collagen IV only) for 30 min at room temperature (for blocking nonspecific reactions). Sections were rinsed three times with PBS between each incubation and then incubated with antisera against TGF-β (1:400; Santa Cruz Biotechnology, Santa Cruz, CA), CD68 (1:100, Serotec, Oxford, UK), nestin (1:200, Millipore, Billerica, MA), collagen type I (1:400, Sigma) or collagen IV (1:200; Southern Biotech, Birmingham, AL) overnight at 4°C. Slides were rinsed with PBS, incubated with biotinylated IgG raised against rabbit (Sigma), mouse (Vector), or goat (Dako) at room temperature, rinsed with PBS, and incubated with avidin-biotin complex (Vector) for 1 h at room temperature. A positive immunoreaction was identified after incubation with 3’-3’-diaminobenzidine tetrahydrochloride dihydrate (DAB; chromogen, Dako) and counterstaining with Mayer’s hematoxylin.

Nestin immunoreactivity was assessed in 10 randomly selected glomeruli/section and quantitated using image-analysis software (NI-SElements, ver. 2.32, Nikon Instruments, Melville, NY). The data are expressed as the percentage of area stained for nestin per glomerulus. Macrophage number was assessed by counting the number of CD68-positive cells in 20 random fields per section and expressed per field of view.

Western blotting. For kidney cortex, tissue was homogenized in 80 mM Tris buffer (pH 7.3) containing protease inhibitor Cocktail Set III (Calbiochem, EMD Chemicals, San Jose, CA). For TGF-β, neprhin, IL-6, and MCP-1, homogenized protein samples were denatured at 95°C for 15 min. For collagen type I and type IV, homogenized protein samples were not heat denatured. Protein samples, 15 μg for TGF-β, 6 μg for neprhin, IL-6, and MCP-1, and 2 μg for collagen type I and type IV, were loaded onto 4–15% precast SDS-PAGE gels (Bio-Rad, Hercules, CA) and transferred to a nitrocellulose membrane. The membranes were incubated first with 5% nonfat milk for blocking nonspecific reactions and then with antisera against TGF-β (1:600, R&D Systems, Minneapolis, MN), neprhin (1:1,000, Abcam, Cambridge, MA), IL-6 (1:1,000, Abcam), MCP-1 (1:1,000, Abcam), collagen type I (1:1,000, Sigma), or collagen type IV (1:1,000; Chemicon, Temecula, CA) at 4°C overnight. The membranes were washed, incubated with anti-mouse IgG conjugated to horseradish peroxidase (1:10,000; KPL, Gaithersburg, MD), and proteins were visualized by enhanced chemiluminescence (KPL). For TGF-β, IL-6, and MCP-1, the membrane was stripped and incubated with anti-β-actin (1:3000, Cell Signaling Technology). Because the samples used for collagen type I and type IV were not heat denatured, β-actin was not accessible for probing. Therefore, for collagen type I and type IV, to verify equal levels of protein loading, another gel with identical loading was stained with Coomassie blue. The densities of specific bands were quantitated by densitometry using Scion Image, ver. 4.02 (Scion, www.scioncorp.com). The densities of specific bands were then normalized to the total amount of protein loaded in each well following densitometric analysis of β-actin or Coomassie blue staining.

Statistical analysis. Statistical analysis was performed using Prism ver. 4.00 (GraphPad, San Diego, CA). Data for ND, D, and D+canola were analyzed using a one-way ANOVA with a post hoc comparison using Tukey’s multiple comparison test. Because the corn oil group served as a control for D+canola, data for D+corn was compared with D+canola using an unpaired t-test. A value of P < 0.05 was considered statistically significant. Unless otherwise indicated, all values are presented as means ± SE.

RESULTS

Blood glucose, body weight, food intake, and kidney weight. Diabetic (D) animals showed a 5.6-fold increase in blood glucose, a 2.3-fold increase in kidney/body weight, and a 0.71-fold decrease in body weight despite greater food consumption compared with ND (Table 1). The D+canola animals showed a 4.2-fold increase in blood glucose, and 0.79-fold decrease in body weight compared with ND. The D+corn animals showed a 3.9-fold increase in blood glucose, and 0.80-fold decrease in body weight compared with ND. There was no difference in the ratio of kidney weight to body weight for D+canola or D+corn compared with ND, but they both showed a significant reduction in kidney/body weight com-
Table 1. Effects of diabetes and canola supplementation on metabolic parameters

<table>
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<tr>
<th>Parameter</th>
<th>ND</th>
<th>D</th>
<th>D+canola</th>
<th>D+corn</th>
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<tr>
<td>Blood glucose, mg/dl</td>
<td>70±2</td>
<td>395±23*</td>
<td>293±26‡</td>
<td>274±22‡</td>
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<td>Body weight, g</td>
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<td>382±30*</td>
<td>425±26†</td>
<td>430±6†</td>
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<td>Food intake, kcal/day</td>
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<td>128±6*</td>
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<tr>
<td>Kidney/body weight</td>
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<td>1.15±0.08*</td>
<td>0.77±0.10§</td>
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<tr>
<td>Kidney weight, g</td>
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<td>3.18±0.19*</td>
<td>3.36±0.20‡</td>
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Values are means ± SE ND, nondiabetic; D, diabetic; D+canola, diabetic animals fed a diet rich in omega-3 polyunsaturated fatty acids (n-3 PUFA); D+corn, diabetic animals fed a diet rich in omega-6 PUFA (n-6 PUFA). *P < 0.01 vs. ND. †P < 0.05 vs. ND. ‡P < 0.05 vs. D.

pared with D. There were no significant differences between D+canola and D+corn in blood glucose, body weight, food intake, or kidney/body weight.

**UAE, CrCl, and systolic blood pressure.** Diabetes (D) was associated with a 5.5-fold increase in UAE compared with ND (Fig. 1A). D+canola and D+corn reduced the diabetes-associated increase in UAE by 11.7- and 2.9-fold, respectively (Fig. 1A). CrCl was 2.2 ± 0.1, 1.8 ± 0.3, 2.1 ± 0.5, and 1.8 ± 0.3 (ml/min) for the ND, D, D+canola, and D+corn animals, respectively. No differences in CrCl were observed between any of the treatment groups. The D animals exhibited a 1.29-fold increase in systolic blood pressure compared with ND animals (Fig. 1B). This increase in systolic blood pressure associated with diabetes was completely prevented in the D+canola group. No differences in systolic blood pressure were observed between D+canola and D+corn groups.

**Glomerulosclerosis and tubulointerstitial fibrosis.** The D animals exhibited moderate glomerular and tubulointerstitial changes characterized by mild mesangial expansion, accumulation of ECM proteins, capillary dilatation, tubular dilatation, and accumulation of inflammatory cells (Figs. 2 and 3). These changes were completely absent in the D+canola group and partially attenuated in the D+corn group. The semiquantitative analysis of the degree of renal pathology showed a 3.0-fold increase in glomerulosclerosis (Fig. 2, B and C), a 1.7-fold increase in tubulointerstitial fibrosis in the renal cortex (Fig. 3A), and a 2.1-fold increase in tubulointerstitial fibrosis in the inner stripe of the outer medulla (Fig. 3B) in the D compared with ND group. All of these changes were completely absent in the D+canola group. The D+corn group showed partially attenuation of these changes compared with the ND group, although it exhibited significantly increased tubulointerstitial fibrosis in both the cortex and medulla compared with D+canola (Figs. 2 and 3).

**Nestin and nephrin.** Nestin is an intermediate filament, recently identified as one of the molecular markers of podocytes (47). Nestin-positive cells identifying podocytes were observed throughout the glomerular tuft in the ND animals (Fig. 4A), while D animals showed both nestin-positive and nestin-negative cells in the glomerular tuft (Fig. 4A). Quantitative analysis of the percentage of nestin-positive cells in the glomerulus showed a 3.6-fold decrease in the area of glomerular tuft occupied by nestin-positive cells (Fig. 4B), and this was completely attenuated in the D+canola group (Fig. 4B). Nephrin is an integral component of the glomerular slit diaphragm and is involved in podocyte foot process formation and maintenance of podocyte integrity (15). Nephrin protein expression, as measured by Western blotting, was reduced by 1.2-fold in the D compared with the ND group (Fig. 4C), and this was partially attenuated in the D+canola group.

**TGF-β.** In the D group, TGF-β was immunolocalized to proximal and distal tubules as well as mesangial cells (Fig. 5A). D+canola completely attenuated the increased intensity of immunostaining for TGF-β associated with diabetes. Protein expression supported the immunohistochemical findings in that D showed a 2.5-fold increase in TGF-β protein expression compared with ND (Fig. 5B) and this increase was attenuated with D+canola.

**CD68, IL-6, and MCP-1.** Diabetes was associated with a 9.5-fold increase in the abundance of CD68-positive cells, indicating the presence of macrophages (Fig. 6, A and B). In addition, IL-6 and MCP-1 protein expression was increased 1.7- and 1.5-fold, respectively, in the D compared with ND animals (Fig. 6, C and D). This increase in the abundance of CD68-positive cells was prevented in the D+canola group (Fig. 6, A and B). These increases in CD68, IL-6, and MCP-1 were prevented in the D+canola animals (Fig. 6, A–D).

![Fig. 1. Urine albumin excretion (UAE) and systolic blood pressure. A: UAE was increased in diabetic (D) and reduced in diabetic mice fed a diet high in omega-3 polyunsaturated fatty acids (n-3 PUFA; D+canola) compared with nondiabetic (ND) animals. Diabetic animals fed a diet high in omega-6 PUFA (n-6 PUFA; D+corn) only partially attenuated the diabetes-associated increase in UAE. B: systolic blood pressure was increased in D and reduced in D+canola animals compared with ND. D+corn only partially attenuated the diabetes-associated increase in blood pressure. Values are means ± SE.](http://ajprenal.physiology.org/)
Collagen type I and type IV protein expression. By immunohistochemistry, collagen type I (Fig. 7A) and collagen type IV (Fig. 7B) were localized to tubulointerstitial spaces and basement membranes, respectively. There was an apparent increase in the intensity of immunostaining in the D compared with ND animals, and this change was attenuated in the D/canola group (Fig. 7, A and B). These observations were confirmed with Western blot analysis showing a 2.4-fold increase in collagen type I protein (Fig. 7C) and a 1.6-fold increase in collagen type IV protein (Fig. 7D) expression in D compared with ND animals. Treatment with canola completely attenuated these changes (Fig. 7, C and D).

**DISCUSSION**

Our data demonstrate that a diet high in n-3 PUFA (as in canola oil), but not n-6 PUFA (as in corn oil), completely prevents the development of renal disease characteristic of longer-term (30 wk) diabetes. Specifically, animals fed a n-3 PUFA-rich diet did not exhibit increased urine albumin excretion, glomerulosclerosis, tubulointerstitial fibrosis, hypertension, and inflammation characteristic of diabetic renal disease. These observations suggest that an n-3 PUFA rich diet may be effective in the prevention and treatment of diabetic renal disease.

In humans, albuminuria is a strong predictor of progressive decline in renal function (38). Similarly, in the STZ animal model of diabetes, albuminuria correlates with renal injury. In the current study, diabetic animals showed a significant increase in urine albumin excretion, while treatment with canola reduced urine albumin excretion to levels of those observed in nondiabetic animals. Clinical studies have shown that treatment with n-3 PUFA lowers the risk of development of albuminuria in young type 1 diabetic patients (35) and slows the progression of albuminuria in older patients with type 2 diabetes (44). Eicosapentaenoic acid ethyl ester reduces albuminuria in STZ-induced Sprague-Dawley rats (3, 19).

*Fig. 2. Glomerulosclerosis. A: periodic acid-Schiff-stained sections of the renal cortex. g, Glomerulus; pt, proximal tubule; dt, distal tubule; arrowheads, mesangial expansion; +, dilated capillaries. Original magnification ×400. B: glomerulosclerotic index (GSI). C: percentage of glomeruli in each grade of glomerulosclerosis."

**Table:**

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<tr>
<th>GSI Grade</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ND</td>
<td>56.4±12.0</td>
</tr>
<tr>
<td>D</td>
<td>14.9±3.1*</td>
</tr>
<tr>
<td>D+canola</td>
<td>44.6±4.8*</td>
</tr>
<tr>
<td>D+corn</td>
<td>31.3±13.0</td>
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*P < 0.01 vs. ND; †P < 0.05 vs D in each grade of glomerulosclerosis.*
ever, this is not a universal finding, as one study reports no differences in the rate of urinary albumin excretion in patients with type 1 and type 2 diabetes (31). It should be noted that this study was performed only over a 12-wk period and included only a small number of patients (n = 6) per treatment group. Our study did not identify any changes in creatinine clearance among any of the treatment groups. However, it is well known that, unlike diabetic nephropathy in humans, a decline in creatinine clearance is not consistently observed in the STZ-induced model of diabetic renal disease. However, the absence of any changes in creatinine clearance does not imply that there are no changes in renal function or renal injury, and further
analyses were performed to determine the effects of n-3 PUFA on diabetic renal disease.

Hypertension is a common complication of diabetes in humans and often accompanies renal disease (20). However, in the STZ-induced Sprague-Dawley rats the appearance of hypertension is variable, with few studies reporting hypertension (2, 13), while most other studies report normal blood pressure (5, 37). The exact reasons for these contradictory findings are unclear, but most likely they are due to the duration of diabetes, degree of insulin treatment, and method of blood pressure measurement. The current study demonstrated an increase in systolic blood pressure in diabetic animals compared with nondiabetic animals after 30 wk of diabetes, as measured by tail-cuff sphygmomanometry. This increase in blood pressure was not observed in diabetic animals fed either a n-3 PUFA or a n-6 PUFA diet. Diets high in PUFA have been shown to have cardioprotective and antihypertensive effects. The Mediterranean diet, which is high in olive oil (both n-3 and n-6 PUFA rich), has been shown to lower blood pressure (48). Blood pressure also decreases in type 1 diabetic patients receiving

Fig. 4. Renal cortical nestin and nephrin immunolocalization and expression. A: nestin immunolocalization in the glomerulus shown as brown staining. Original magnification ×400. B: quantitative analysis of nestin expression. C: densitometric scans of nephrin protein expression in arbitrary units (AU) expressed as the ratio of nephrin to β-actin. Values are means ± SE.
supplemental cod liver oil (a rich source of n-3 PUFA) (25). Omega-3 PUFA supplementation reduces hypertension in TGR(mRen-2)27 (24) and Dahl salt-sensitive (36) rats, as well as in partially nephrectomized dogs (8). These data are consistent with the antihypertensive effect of omega-3 PUFA.

Glomerulosclerosis and tubulointerstitial fibrosis are hallmarks of diabetic renal disease (39). Studies have shown that while lowering blood pressure and albuminuria is paramount in the treatment of diabetic renal disease, but concomitant organ protection dramatically slows disease progression (1, 11). In the current study, diabetic animals showed greatly increased glomerulosclerosis and tubulointerstitial fibrosis after 32 wk of treatment. This pathology was completely prevented by treatment with n-3 PUFA (canola oil), demonstrating that in addition to being able to prevent albuminuria and hypertension, canola is also effective in attenuating renal structural damage associated with diabetes. In other models of renal disease, n-3 PUFA have also shown renoprotective effects. Treatment with fish oil ameliorates diabetes-associated glomerulosclerosis and tubulointerstitial fibrosis (3). In both a rat (9) and dog model (8) of renal ablation, n-3 PUFA significantly reduced glomerulosclerosis compared with control animals. Type 2 diabetic KKAy/Ta mice showed a renoprotective effect of n-3 PUFA via reduced glomerulosclerosis and tubulointerstitial fibrosis (22). Our studies show that n-6 PUFA also have some renoprotective effects with respect to partially attenuating glomerulosclerosis and tubulointerstitial fibrosis, but the effects were not as renoprotective as were the effects of n-3 PUFA. Additionally, in the dog model of renal insufficiency, n-6 PUFA had a significant deleterious effect. These data indicate that n-3 PUFA treatment is superior to that of n-6 PUFA in attenuating renal injury. Based on these observations, our attempt to elucidate the potential mechanisms underlying the renoprotective effects of PUFA concentrated on n-3 PUFA alone. One of the mechanisms that contributes to the development of glomerulosclerosis and tubulointerstitial fibrosis is accumulation of ECM proteins, including collagen type I and type IV (7, 39). Our study shows that treatment with canola oil reduced expression of both collagen type I and type IV protein to levels of those observed in nondiabetic animals. In a rat model of polycystic kidney disease, n-3 PUFA ameliorate, while n-6 PUFA exacerbate, fibrosis (30).

Studies over the past several years have shown that podocyte injury plays a key role in the development of diabetic renal disease. Specifically, apoptosis, foot process detachment, and ultimately podocyte loss are thought to contribute to albuminuria, glomerulosclerosis, and declining renal function. (40, 46).

Our study shows that the expression of nephrin and nestin, two podocyte markers (15, 47), are...
reduced in diabetes and that this reduction in completely attenuated in the animals fed a high-n-3 PUFA diet. These observations indicate that n-3 PUFA are renoprotective through preserving podocyte integrity.

Upregulation of the TGF-β pathway is the key event in the pathogenesis of end-organ complications associated with diabetes, including diabetic renal disease (51). The current study shows that TGF-β protein levels are increased in the diabetic renal cortex, while TGF-β protein levels are normalized to the level of nondiabetic controls in the canola-fed rats. While n-3 PUFA have been shown to reduce TGF-β protein expression in experimental cardiomyopathy (45), no studies to date have shown the effects of n-3 PUFA on TGF-β protein expression in the diabetic kidney. Inflammation is also an important pathogenic factor in the development of diabetic renal disease. The present study shows an increased density of activated macrophages and IL-6, and MCP-1 protein expression in the diabetic renal cortex, consistent with increased tissue inflammation. Treatment with n-3 PUFA attenuated these changes, demonstrating their anti-inflammatory effects in diabetes.

High blood glucose is believed to be the primary cause of diabetic end-organ complications (26), and improved blood glucose control is one of the most critical factors in managing diabetes and its related end-organ damage. Clinical trials have shown that low-carbohydrate diets improve blood glucose control (6, 21). Substituting fat for carbohydrates in the diet could lower blood glucose, but studies have shown that high-fat diets contribute to the development of atherosclerosis (34) and metabolic syndrome (17). However, when the fat composition of diets is dissected, trans and saturated fatty acids appear to be the most damaging (e.g., contributing to atherosclerosis and metabolic syndrome), while PUFA often prove to be beneficial in preventing atherosclerosis and metabolic syndrome (10, 42). This suggests that displacing dietary carbohydrates with PUFA could reduce blood glucose without causing fat-induced pathology. Our data show a decrease in blood glucose levels associated with high fat and low carbohydrate consumption, e.g., canola and corn diets. In numerous trials involving both type 1 and type 2 diabetic patients, treatment with n-3 PUFA, however, failed to change either fasting blood glucose or HbA1c (14). In these studies, the supplemental fat contributed ~1% of calories, hardly enough to displace a significant amount of dietary carbohydrates. In the current study, added fat contributed 40% of calories, significantly reducing dietary carbohydrate intake. Thus it is likely that some of the renoprotective effects of high-PUFA diets are mediated through lowering blood glucose via reducing dietary carbohydrate intake. In addition to controlling for dietary fat content, corn oil was used to control for PUFA content, as it is low in n-3 PUFA, but high in n-6 PUFA. While the n-3

Fig. 6. Inflammation. A: CD68-positive cells shown as brown staining. Original magnification ×400. B: quantitation of CD68-positive cell density. C: densitometric scans of IL-6 protein expression in AU expressed as the ratio of IL-6 to β-actin. D: densitometric scans of MCP-1 protein expression in AU expressed as the ratio of MCP-1 to β-actin. Values are means ± SE.
PUFA-rich diet (as in canola) was effective in completely attenuating various measures of diabetic kidney disease, including albuminuria, glomerulosclerosis, and tubulointerstitial fibrosis, the n-6 PUFA-rich diet was not as effective in reducing these parameters. Both canola and corn diets reduced blood glucose to the same degree, but canola was more effective in reducing renal injury. Our conclusion is that high-PUFA diets are renoprotective but that n-3 PUFA-rich diets provide greater protection than high-n-6 PUFA diets.

In summary, our study demonstrates that canola, a rich source of dietary n-3 PUFA, prevents albuminuria, glomerulosclerosis, tubulointerstitial fibrosis, inflammation, and increased systolic blood pressure associated with long-term diabetes. This study underscores the importance of further examination of the effects that diet can have on the prevention of diabetic organ complications.

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