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Effect of dietary fish oil, vitamin E, and probucol on renal injury in the rat

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Dietary fish oil, vitamin E, and probucol have been considered in a variety of human and experimental models of kidney disease. Using subtotal nephrectomized cholesterol-fed rats as a model for progressive kidney disease, we examined the effect of 5% dietary fish oil, or a combination of 5% dietary fish oil with 500 IU vitamin E/kg diet or 1% probucol on renal injury. Three-month-old Sprague Dawley rats were fed a control diet (C group) or a cholesterol supplemented (2%) diet (Ch group) containing either fish oil (FO group) or fish oil plus vitamin E (FO+E group) or fish oil plus probucol (FO+P group). After 4 weeks of dietary treatment, the right kidney was electrocoagulated and the left kidney nephrectomized. After 8 weeks, 24-hour urine was collected before sacrifice. No effect of the dietary treatments was noted on serum creatinine, blood urea nitrogen, or proteinuria, except that proteinuria was highest in FO+P group. Rats receiving the cholesterol diets had higher serum low density lipoprotein (LDL) + very low density lipoprotein (VLDL) cholesterol ($P < 0.05$). In contrast, rats in the FO+P group had the lowest serum total cholesterol and LDL+VLDL cholesterol among all groups. The FO group had 26% lower kidney α-tocopherol concentrations than the C group. However, inclusion of vitamin E in the diet (FO+E group) increased the kidney α-tocopherol status to a level comparable to that in the C group, whereas inclusion of probucol in fish oil diet (FO+P group) did not improve the kidney α-tocopherol status. Rats fed the cholesterol diet had a 2.5-fold higher glomerular segmental sclerosis (GSS) score and 1.5-fold higher glomerular macrophage (GM) subpopulation than the C group. These effects of the cholesterol diet were ameliorated by a fish oil diet (FO group: GSS by 30%, GM by 24%). The inclusion of vitamin E in the fish oil diet (FO+E group) did not further improve the GSS score or GM subpopulation. However, inclusion of probucol in fish oil diet (FO+P group) lowered the GSS score by 73% and reduced GM subpopulation by 83% compared with the Ch group. These remarkable changes can be attributed to the powerful hypocholesterolemic activity of probucol. Our findings indicate that progression of glomerular sclerosis in the rat remnant kidney model of progressive kidney disease can be significantly modulated with fish oil treatment. (J. Nutr. Biochem. 10: 539–546, 1999)

Keywords: vitamin E; probucol; fish oil; remnant kidney model; glomerulonephritis; macrophage

Introduction

It has been suggested that hypercholesterolemia contributes to progressive renal disease.[1–3] Increasing dietary cholesterol in normal rats has been shown to increase plasma cholesterol levels and induce glomerulosclerosis.[4] Additionally, the glomeruli of guinea pigs fed a high cholesterol diet demonstrated prominent glomerular monocyte/macrophage infiltration.[5] Administration of lipid lowering agents to animals with progressive renal disease and hyperlipidemia has been shown to reduce glomerulosclerosis and proteinuria.[6,7]

The mechanism by which an increased cholesterol level leads to renal injury is unclear. However, the morphologic similarity between glomerulosclerosis and atherosclerosis suggests that common mechanism(s) may be involved in tissue injury.[2,3] Several prospective human studies and animal experiments have suggested that fish oil and vitamin E have beneficial effects on atherosclerosis and coronary

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heart disease.[8,9] There is also some evidence indicating that these nutrients may prevent chronic renal failure.[10–14] Fish oil has been shown to be beneficial for treating human primary glomerulonephritis[10,15] and preventing the evolution of glomerulosclerosis in the rat nephritis model.[11] Fish oil has anti-inflammatory properties and has been shown to reduce proteinuria in some models of nephritis.[16–19] Vitamin E also has been suggested to limit the development of glomeruloscleroses in diabetic nephropathy, probably through inhibition of lipid peroxidation.[13,14]

The remnant kidney model of chronic renal failure, which is a condition with subtotal nephrectomy, is a commonly used experimental model for studying progressive kidney injury. Following subtotal nephrectomy the remnant nephron subsequently undergoes hyperfiltration with evolution of the proteinuria, hypertension, and glomerulosclerosis. The remnant kidney model is postulated to represent the paramount common pathway for all chronic progressive kidney diseases.[20] Using this model, we found that administration of 1% dietary probucol, a cholesterol lowering agent with strong antioxidant properties,[21] reduced renal injury and proteinuria in cholesterol-fed rats.[22] Thus, in addition to lowering cholesterol, probucol as an antioxidant may also contribute to the reduction of renal injury by preventing low density lipoprotein (LDL) oxidation and lipid peroxidation. Because the beneficial effect of vitamin E and probucol on atherosclerosis in experimental animals appears mediated in part through common pathways, vitamin E may have the same beneficial effect as probucol on the progression of glomerulosclerosis. In this study, we investigated the capability of dietary fish oil to slow the progression of renal injury in cholesterol-fed rats with subtotal nephrectomy.

Because fish oil is highly polyunsaturated and is prone to oxidation, we sought to examine whether the combination of fish oil with vitamin E or probucol further enhances the efficacy of fish oil in slowing the progression of renal injury in this model.

Materials and methods

Animals and diet

Male viral antibody-free Sprague Dawley rats, aged 3 months and weighing 342 ± 8 g, were purchased from Charles River Breeding Laboratories (Kingston, NY USA) and housed individually in suspended wire mesh stainless steel cages in a temperature and humidity controlled room with a 12-hour light/dark cycle. Rats were randomly assigned to five dietary treatment groups, with 12 rats per group. The control group (C) received a modified semisynthetic AIN-76A diet (Dyets Inc., Bethlehem, PA USA)[23] containing 6.2% corn oil. The cholesterol group (Ch) received the control diet containing 2% cholesterol (Dyets, Inc.). The fish oil group (FO) received the cholesterol supplemented diet containing 5% by weight menhaden fish oil (Sigma Chemical Co., St. Louis, MO USA) substituted for 5% of the corn oil. As previously reported,[24] corn oil contains 65 ± 0.6% linoleic acid.[25] Therefore, 1.2% corn oil in this diet provided approximately 1% by weight linoleic acid to prevent essential fatty acid deficiency. The fish oil and vitamin E (FO+E) group received the cholesterol supplemented diet containing 5% by weight fish oil and 500 IU vitamin E (dl-α-tocopherylacetate; Bioserv Frenchtown, NY USA)/kg diet. The fish oil and probucol (FO+P) group received the cholesterol supplemented diet containing 5% by weight fish oil and 1% by weight probucol (Sigma Chemical Co). Fresh diets were fed daily just before the dark cycle to minimize food exposure to air prior to consumption. Any leftover diet was discarded. The bulk of the diets, without oil and in the form of powder, was stored at 4°C. Oils were mixed in smaller batches. After addition of the oils, the diets were flushed with nitrogen and kept in −20°C. Food and water were provided ad libitum.

Procedures

After 4 weeks of dietary treatment, the rats were anesthetized with 5% isoflurane (Ohmeda, Liberty Corner, NJ USA) inhalation and 5 mg/kg body weight xylazine (Miles, Shawnee Mission, KA USA). Following the onset of anesthesia, a 2-cm incision was made on the right lumbar skin, and a right lateral laparotomy was performed. The capsule of the right kidney was removed, and electrocautery of the cortex was performed with an electrocoagulator (Surgitron, Ellman International Manufacturing Inc., Hewlett, NY USA) according to the procedure of Boudet et al.[26] Following a 7-day recovery and ligation of the hila, a left nephrectomy was performed using the same anesthetic procedure. Body weight was measured before and after each surgical procedure and monitored daily for 1 week or until body weight returned to within 5% of the original value; body weight was measured weekly thereafter. Urine and blood were collected before and after the fourth and eighth weeks of nephrectomy. At baseline and 4 weeks after the surgical procedures, blood samples were collected from the tail vein following heat lamp dilatation while the animals were restrained in a rigid rodent restrainer (Lab Products, Maywood, NJ USA). After 8 weeks of nephrectomy, food was withheld from the animals overnight, and after 24 hours, urine was collected using metabolic cages (Lab Products). The animals were then sacrificed by terminal exsanguination following anesthesia with isoflurane. Blood was collected from the vena cava at week 8, and serum was separated by centrifugation at 300 × g for 15 minutes at 4°C. The kidney remnant was removed, and a portion was fixed in 10% formalin solution, a portion in 70% ethanol, and the remainder frozen in liquid nitrogen and stored at −70°C for vitamin E and fatty acid analysis. This protocol was reviewed and approved by the institutional Animal Care and Use Committee.

Analytical methods

Serum total cholesterol (TCh),[27] triglyceride (TG),[28] high density lipoprotein (HDL) cholesterol,[29,30] and blood urea nitrogen (BUN)[31] were measured by an enzymatic method using automated COBAS FARA (Roche Diagnostic System, Nutley, NJ USA). LDL + very low density lipoprotein (VLDL) cholesterol were calculated from TG and TCh values. Urinary protein concentration was measured by the method of Biuret.[32,33] Serum creatinine was measured as described by Larsen.[34]

The fatty acid profile of the kidney tissue was determined by gas chromatography. Briefly, the kidney sample was homogenized in chloroform:methanol (1:1, v:v) and lipids were extracted as described by Caruso et al.[35] The extract was dried under nitrogen, and residue was resuspended in 1.0 mL benzene. The fatty acids were methylated with 5% methanolic HCl at 70°C for 2 hours.[36] One microliter of fatty acid methylester was injected into a Hewlett Packard model 5890 gas chromatograph (Wilmington, DE USA) equipped with a 30 m AT-WAX (Alltech, Deerfield, IL USA) capillary column with an inner diameter of 0.25 mm and a film thickness of 0.25 μm. The oven temperature was programmed from 160°C to 250°C at 2°C/min without initial temperature hold and 10 minutes final temperature hold. The split ratio was 20:1 with helium as the carrier gas.

Kidney vitamin E was measured by reverse-phase high perfor-

Table 1 Final body weight and body weight gain

Treatment group	Final body weight (g)	Body weight gain (g)
C	465 ± 28[a]	153 ± 22[a]
Ch	451 ± 23[a]	117 ± 25[b]
FO	467 ± 25[a]	127 ± 25[b]
FO+E	471 ± 28[a]	133 ± 23[b]
FO+P	513 ± 37[b]	184 ± 41[a]

Final body weight and body weight gain from initial body weight in control (C) rats fed a modified AIN-76A diet and in groups treated with a diet containing 2% cholesterol (Ch), 2% cholesterol + 5% fish oil (FO), 2% cholesterol + 5% fish oil + 500 IU vitamin E/kg (FO+E), and 2% cholesterol + 5% fish oil + 1% probucol (FO+P). Values are mean ± SD. Means in a column not sharing a common superscript are significantly different at $P < 0.05$.

mance liquid chromatography (HPLC).[37] Briefly, after homogenization in the buffer, the kidney sample was saponified with 30% KOH in the presence of 2% pyrogallol (Sigma Chemical Co.) at 60°C for 30 minutes. Tocol (a gift from Hoffmann-La Roche, Nutley, NJ USA) was added to the mixture as an internal standard. α-Tocopherol was extracted into 2.5 mL hexane containing 0.002% butylated hydroxytoluene (BHT), dried under a stream of nitrogen, and reconstituted in 60 μL of methanol. α-Tocopherol was separated by HPLC using a 3 μm C_{18} reverse phase column (Perkin-Elmer, Norwalk, CT USA) with 100% methanol as the mobile phase. The eluted α-tocopherol peak was detected with a Perkin-Elmer 650-15 spectrofluorometer set at 292 nm excitation and 330 nm emission and integrated with a Waters 860 system (Cambridge, MA USA).

Histology

Histologic examination of the remnant kidney was conducted in a blinded fashion with light microscopy using periodic acid-Schiff staining[38] of formaldehyde-fixed tissue sections to assess glomerulosclerosis. Fifty glomeruli were examined at 140× magnification and the number of glomeruli showing segmental sclerosis was expressed as a percentage (GSS score) of the total number of glomeruli counted for each rat.

Glomerular macrophage subpopulations were examined by immunoenzymatic staining of ethanol-fixed tissue sections using Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA USA) and mouse monoclonal antibody against rat macrophages (ED-1: Chemicon International Inc., Temecula, CA USA). The immunoenzymatic staining specimens were evaluated in a blinded and coded fashion by two independent observers. The number of glomeruli having ED-1 positive cells was expressed as a percentage of the total number of glomeruli counted for each rat.

Statistical analysis

All data are expressed as means ± SD. Comparisons between groups were assessed by analysis of variance followed by Student's *t*-test with Bonferroni corrections for multiple comparisons. Differences were considered significant at a *P*-value of less than 0.05.

Results

The final body weights of rats in the FO+P group were significantly higher than those of the other groups (*Table 1*). Although we did not measure food intake, weekly food consumption in the FO+P group was noticeably higher than in the other groups. Body weight gain at the end of 8 weeks was also higher in this group compared with those of rats in the Ch, FO, and FO+E groups (*Table 1*). Feeding 2% cholesterol without other dietary treatments resulted in the lowest weight gain. Inclusion of FO or FO+E increased weight gain, but not significantly. Weight gain was significantly higher in the FO+P group than in the Ch group.

There were no significant differences in serum creatinine and BUN concentration between dietary treatment groups after 4 or 8 weeks of nephrectomy (*Table 2*). Because body weight gain was affected by dietary treatments, serum creatinine and BUN were adjusted per gram of body weight. This adjustment did not result in a significant difference between treatment groups for these indices of protein metabolism.

Serum lipid profile data are shown in *Table 3*. TCh was not markedly affected by the cholesterol feeding (Ch group versus C group). However, serum TG and TCh concentrations were the lowest in FO+P rats ($P < 0.05$). The concentration of HDL cholesterol in all groups of rats fed diets supplemented with cholesterol was lower than that of the C group. Conversely, the concentration of LDL+VLDL cholesterol in the Ch and FO groups was higher than in the C group and probucol but not vitamin E significantly reduced this concentration in the FO+P group.

At 4 weeks, 24-hour urinary protein per gram of body weight in the FO+P group was not different from that of the other groups; however, at 8 weeks postnephrectomy, rats in the FO+P group who had a higher weight gain (*Table 1*) had significantly higher levels of urinary protein than did

Table 2 Serum creatinine and blood urea nitrogen concentrations

Treatment group	Creatinine*		BUN*	
	4 wk	8 wk	4 wk	8 wk
C	207.7 ± 29.1	166.2 ± 17.7	21.77 ± 3.57	21.78 ± 2.86
Ch	193.6 ± 20.3	149.4 ± 19.4	25.35 ± 3.21	22.49 ± 2.86
FO	197.1 ± 21.2	149.4 ± 18.6	23.92 ± 4.28	22.49 ± 2.86
FO+E	197.1 ± 29.2	157.3 ± 29.1	24.99 ± 4.29	23.56 ± 4.28
FO+P	185.6 ± 20.3	152.9 ± 17.7	20.35 ± 4.27	19.99 ± 5.35

Effect of dietary treatment (see footnote of *Table 1* and experimental design) on serum creatinine and blood urea nitrogen (BUN) concentrations (adjusted per gram of body weight) after 4 and 8 weeks of nephrectomy. Values are mean ± SD; $n = 12$.
*$\mu mol/L/g \times 10^{-3}$.

Table 3 Serum lipid profile following 8 weeks of dietary treatment

Treatment group	TG	Cholesterol*		
		Total	HDL	(VLDL+LDL)
C	0.70 ± 0.19[a]	3.19 ± 0.52[a]	1.86 ± 0.21[a]	1.24 ± 0.34[a]
Ch	0.55 ± 0.27[ab]	3.21 ± 1.01[a]	1.11 ± 0.34[b]	2.15 ± 0.80[b]
FO	0.61 ± 0.12[ab]	2.77 ± 0.54[ab]	0.83 ± 0.13[b]	1.92 ± 0.60[b]
FO+E	0.64 ± 0.18[ab]	2.59 ± 0.49[ab]	0.93 ± 0.23[b]	1.71 ± 0.41[ab]
FO+P	0.41 ± 0.11[b]	1.73 ± 0.44[b]	0.83 ± 0.15[b]	1.04 ± 0.34[a]

Effect of dietary treatment (see footnote of *Table 1* and experimental design) on serum total cholesterol, triglyceride (TG), and high density lipoprotein (HDL) cholesterol and very low density lipoprotein (VLDL) + low density lipoprotein (LDL) cholesterol concentrations 8 weeks after nephrectomy. Values are mean ± SD, $n = 12$. Values in a column not sharing a common superscript are significantly different at $P < 0.05$.
*mmol/L.

rats in the other groups (*Table 4*). Therefore, relative to the other dietary groups, a higher food intake and thus a higher protein intake may have contributed to the high urinary excretion of protein in this group.

The kidneys of rats in the FO group had α-tocopherol levels that were 28% lower than those of rats in the Ch group and 26% lower than those of rats in the C group, but significantly lower (38%) than those of rats in the FO+E group (*Figure 1*). Thus, inclusion of vitamin E in the FO diet improved the kidney α-tocopherol status to the level of rats in the Ch and C groups (*Figure 1*). The addition of probucol to the fish oil diet did not prevent the fish oil-induced decrease of α-tocopherol concentration in the kidney. Interestingly, kidney α-tocopherol concentration in the FO+P group was the lowest among all the dietary groups receiving cholesterol (*Figure 1*). This effect is most likely associated with this group's low level of lipoprotein, which results in a low deposition of α-tocopherol in kidney (*Table 3*).

Fish oil treatment significantly altered the fatty acid composition of kidney tissue (*Table 5*). The concentration of eicosapentaenoic acid [EPA, 20:5 (n-3)] on average was 34-fold higher and docosahexaenoic acid [DHA, 22:6 (n-3)] was approximately 3.8-fold higher in kidneys from the FO, FO+E, and FO+P groups compared with those of rats who did not receive fish oil. In contrast, the concentration of arachidonic acid [AA, 20:4 (n-6)] was 50% lower in the fish oil groups than in the other dietary treatments. The presence of vitamin E or probucol in the diet of the fish oil groups did not have any effect on EPA, DHA, or AA concentrations in kidney. The concentration of linoleic acid [18:2 (n-6)] in the kidneys of FO and FO+E groups was lower than that in the Ch group, whereas in the kidneys of rats fed FO+P, the level of this fatty acid was comparable to those of rats in the Ch group. Fish oil also increased the concentration of 14:0, 16:0, and 16:1 fatty acids in FO and FO+E groups and 16:0 fatty acid in the FO+P group.

Cholesterol treatment (Ch group) increased the GSS score in the kidneys 2.5-fold ($P < 0.05$) compared with the C group (*Figure 2*). This effect of cholesterol was reduced by 30% in FO rats, 31% in FO+E rats, and 73% in FO+P rats, so there was no statistical difference between the last group and C group. A representative photomicrograph (*Figure 3A*) shows the presence of GSS with mesangial cell proliferation in the glomerulus of a kidney from the Ch group. Supplementing the diet of rats with 2% cholesterol alone also increased GM subpopulation by 1.5-fold ($P < 0.05$) compared with the C group (*Figure 4*). This effect of the cholesterol in the diet was reduced ($P < 0.05$) by 24% in the FO group, 28% in the FO+E group, and remarkably 83% in the FO+P group (*Figure 4*). There were no

Table 4 Urinary protein excretion*

Treatment group	4 wk	8 wk
C	1.95 ± 0.52[a]	2.99 ± 0.76[a]
C	2.16 ± 1.15[a]	2.56 ± 0.80[a]
FO	2.45 ± 0.90[a]	2.46 ± 0.74[a]
FO+E	3.18 ± 0.86[a]	2.78 ± 0.67[a]
FO+P	2.65 ± 0.94[a]	4.01 ± 0.89[b]

Effect of dietary treatments (see footnote of *Table 1* and experimental design) on 24-hour urinary protein excretion (adjusted per gram of body weight) after 4 and 8 weeks of nephrectomy. Values are mean ± SD, $n = 12$. Values in a column not sharing a common superscript are significantly different at $P < 0.05$.
*mg/24 hr/g body weight.

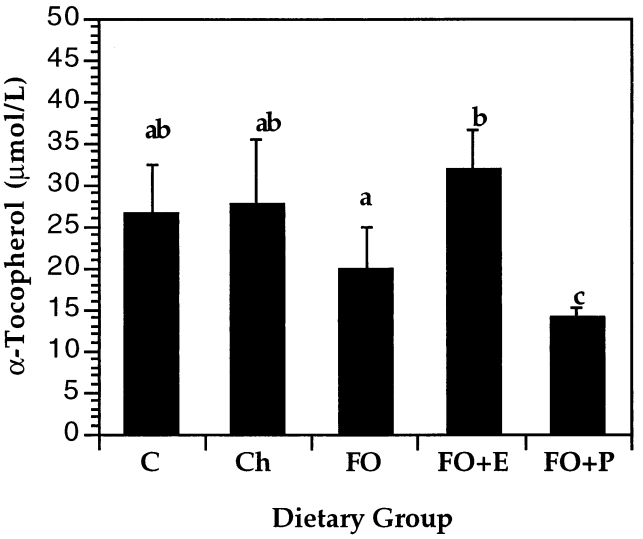

Figure 1 Effect of dietary fish oil, vitamin E, and probucol on kidney α-tocopherol concentration. Each bar represents mean ± SD, $n = 12$. Dietary groups are described in the experimental design. Bars not sharing a common superscript are different ($P < 0.05$).

Table 5 Fatty acid profile in kidney

Fatty acid	C	Ch	FO	FO+E	FO+P
14:0	0.31 ± 0.05^{a}	0.35 ± 0.06^{a}	0.70 ± 0.4^{b}	1.00 ± 0.60^{b}	0.52 ± 0.07^{a}
16:0	22.0 ± 0.3^{a}	21.7 ± 0.7^{a}	24.2 ± 1.3^{b}	25.1 ± 1.8^{b}	24.4 ± 0.4^{b}
16:1	0.77 ± 0.09^{a}	0.88 ± 0.34^{a}	2.70 ± 0.96^{bc}	3.92 ± 1.90^{bc}	2.01 ± 0.19^{ac}
18:0	18.6 ± 0.8^{a}	18.1 ± 0.7^{ab}	17.5 ± 1.6^{ab}	15.9 ± 2.4^{b}	17.9 ± 0.5^{ab}
18:1 (n-9)	7.6 ± 0.9^{a}	8.1 ± 0.7^{a}	9.0 ± 2.1^{a}	11.1 ± 4.4^{a}	7.9 ± 0.4^{a}
18:1 (n-7)	2.8 ± 0.1^{a}	3.4 ± 0.1^{b}	3.1 ± 0.2^{ab}	3.0 ± 0.2^{ab}	3.0 ± 0.2^{ab}
18:2 (n-6)	13.2 ± 1.7^{b}	15.7 ± 0.5^{a}	13.2 ± 0.3^{b}	11.8 ± 1.6^{c}	14.8 ± 0.4^{ab}
20:3 (n-6)	0.41 ± 0.03^{a}	0.48 ± 0.11^{a}	0.13 ± 0.02^{b}	0.14 ± 0.2^{b}	0.15 ± 0.01^{b}
20:4 (n-6)	31.6 ± 1.5^{a}	29.7 ± 1.1^{a}	14.7 ± 2.2^{b}	13.0 ± 2.8^{b}	15.0 ± 0.8^{b}
20:5 (n-3)	0.30 ± 0.18^{a}	0.14 ± 0.19^{a}	9.6 ± 1.2^{b}	9.7 ± 1.6^{b}	9.1 ± 0.3^{b}
22:5 (n-3)	0.67 ± 0.12^{a}	0.58 ± 0.25^{a}	0.12 ± 0.03^{b}	0.11 ± 0.06^{b}	0.17 ± 0.03^{b}
22:6 (n-3)	1.7 ± 0.2^{a}	0.83 ± 0.13^{b}	5.1 ± 0.2^{c}	5.1 ± 0.6^{c}	5.2 ± 0.7^{c}

Weight percent of kidney fatty acids from each dietary treatment (see footnote of *Table 1* and experimental design). Values are mean ± SD, $n = 5$. Values in a row not sharing a common superscript are significantly different at $P < 0.05$.

significant differences among the dietary groups in the histopathologic changes within the interstitium. A representative photomicrograph (*Figure 3B*) shows infiltration of macrophages into the glomerulus as detected by specific ED-1 monoclonal antibody in a kidney section from the Ch group.

Discussion

This study demonstrates that dietary fish oil can slow the progression of glomerulosclerosis in rats with remnant kidney nephrons. Supplementing a fish oil diet with 1% probucol remarkably reduced glomerulosclerosis, whereas addition of 500 IU vitamin E/kg body weight in the fish oil diet did not further increase the fish oil effect. This was apparent from the histologic examination of kidney tissues of rats in FO, FO+P, and FO+E groups. Both the GSS score and GM subpopulation were lower in animals treated with these diets. Probucol, which has both cholesterol-lowering and antioxidant properties, significantly reversed the cholesterol-induced increase in GSS score by 73% so that the rats in the FO+P group were almost comparable histologically to those fed the control diet (*Figure 2*). In our

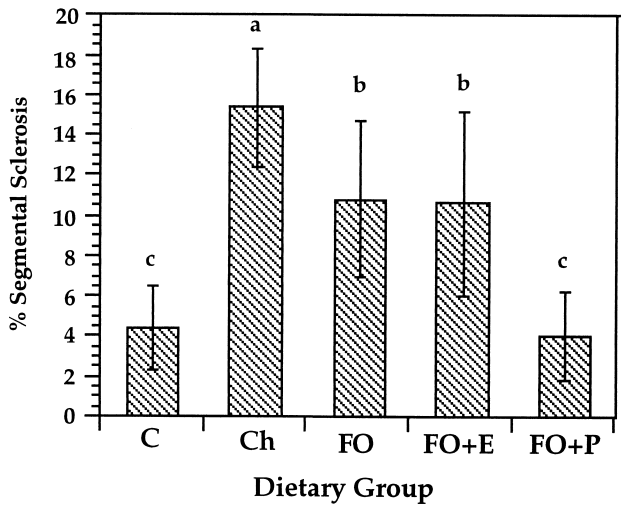

Figure 2 Effect of dietary fish oil, vitamin E, and probucol on glomerular segmental sclerosis score. Each bar represents mean ± SD, $n = 12$. Dietary groups are described in the experimental design. Bars not sharing a common superscript are different ($P < 0.05$).

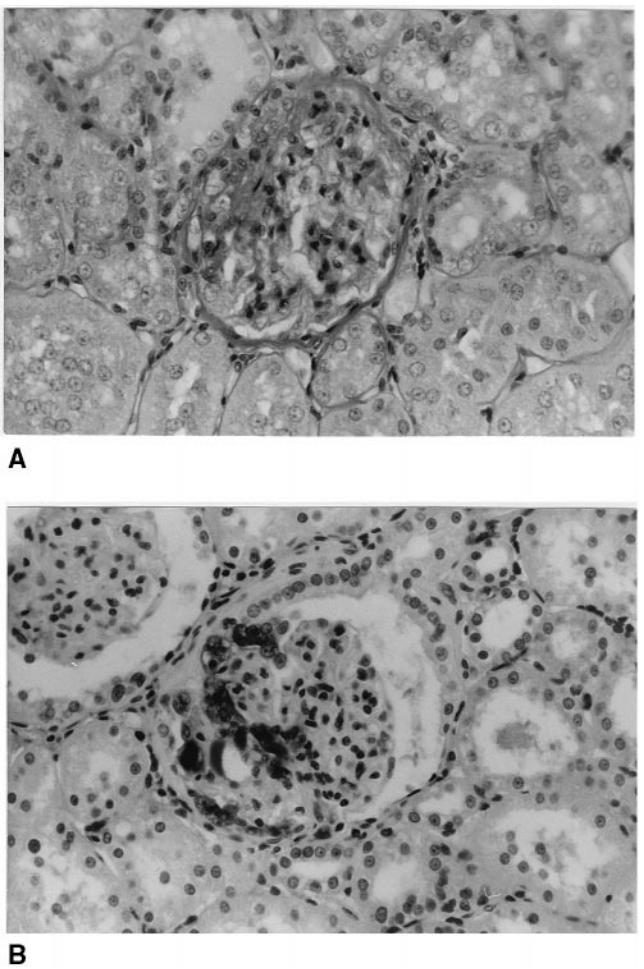

Figure 3 Representative photomicrographs from glomerulus of rats with remnant kidney treated with 2% dietary cholesterol for 8 weeks. (*A*) Segmental sclerosis is shown in upper left portion of glomerulus. (*B*) Macrophage infiltrated into the underlying glomerulus are stained with ED-1 monoclonal antibody and shown in upper left portion of glomerulus.

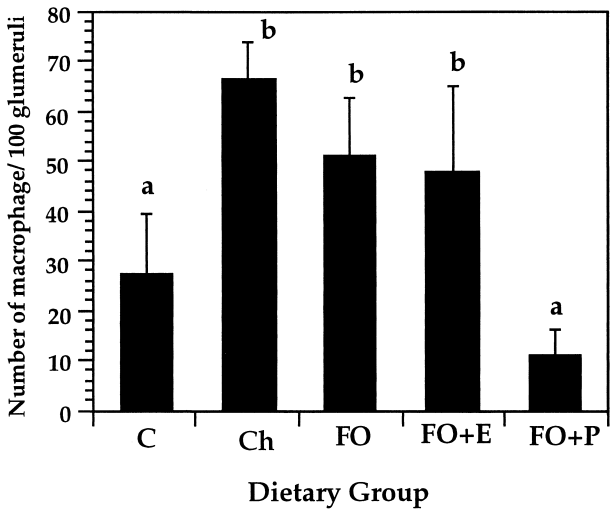

Figure 4 Effect of dietary fish oil, vitamin E, and probucol on glomerular macrophage subpopulation. Each bar represents mean ± SD, $n = 12$. Dietary groups are described in the experimental design. Bars not sharing a common superscript are different ($P < 0.05$).

earlier study[22] we found that treating cholesterol-fed rats with 1% probucol for 8 weeks also reduced GSS by 72% in this model of kidney injury. Therefore, the dramatic effect we observed in the FO+P group can be attributed to probucol treatment.

Tissue requirements and utilization of α-tocopherol are higher when levels of highly oxidizable (n-3) polyunsaturated fatty acids (PUFA) are high.[24] Thus, rats in the FO group had lower kidney α-tocopherol than those in the C and Ch groups (*Figure 1*). Including 500 IU vitamin E/kg in the diet of the FO+E group increased the kidney α-tocopherol concentrations to the control levels. Therefore, the decrease in GM subpopulation in the FO+E group was slightly better (but not statistically different) than that of the rats treated with fish oil alone (28% versus 24%). The striking effect in this index of kidney inflammatory reaction was also observed with fish oil when given together with probucol (FO+P group), which reduced the cholesterol-induced increase in the GM subpopulation by 83%. Again, such remarkable reduction in the GM subpopulation can be attributed to probucol, because in our earlier study,[22] probucol treatment alone reduced the GM subpopulation by 95%.

Although we did not observe a significantly greater level of serum TCh in the Ch group, increases in serum LDL+VLDL cholesterol in this group appeared to be the major contributing factor to the increase in GSS score and GM subpopulation, as established in this model. Probucol has been reported to decrease the serum cholesterol level in the model of subtotal nephrectomy as well as in the model of obstructive nephropathy.[39,40] It appears that the decrease in serum TCh and LDL+VLDL cholesterol level by probucol was the major contributing factor for the decreased GSS score in the FO+P group. Hyperlipidemia may contribute to glomerular injury, probably in the same manner that cholesterol alters major vessel walls.[41] Data suggest a potential role for lipids in the progression of renal disease, although the evidence is circumstantial. A causal relationship has not been clearly established.

The effect of fish oil in the reduction of the GSS score in this study may be related to a lesser extent to its effect on LDL+VLDL cholesterol in which only a slight reduction was detected. Rather, the anti-inflammatory effect of fish oil may have substantially contributed to the reduction of GSS score via modulation of monocyte and macrophage activation. Fish oil and its principal (n-3) PUFA, EPA, and DHA possess both anti-inflammatory and anti-atherosclerotic properties.[42] Rats fed diets containing fish oil, (i.e., the FO, FO+E, and FO+P groups) had approximately 50% lower kidney AA, approximately 34-fold higher EPA, and approximately 3.8-fold higher DHA levels. The (n-3) PUFA inhibit production of inflammatory prostaglandins and leukotrienes by competing with AA for cyclooxygenase and lipoxygenase enzymes. Prostaglandins of the 3-series and leukotrienes of the 5-series produced from (n-3) PUFA are less potent than prostaglandins of the 2-series and leukotrienes of the 4-series in inflammatory reactions. Thus, the effect of fish oil as reflected in the GSS score is perhaps mediated through a variety of mechanisms.

Reactive oxygen species (ROS) have been suggested to be involved in the pathogenesis of several forms of renal injury, including acute ischemic renal failure,[43] glomerulonephritis,[44] toxic nephropathies caused by aminoglycosides,[45] glycerol-induced renal failure, and obstructive nephropathy in the rat[39] and other experimental animals. However, the role of ROS in the pathogenesis of chronic progressive renal disease remains to be defined. The rate of oxygen consumption in nephrons of sub-totally nephrectomized rats is approximately three times that observed before subtotal renal ablation.[7] It has been postulated that increased oxygen consumption by the remnant kidney may lead to enhanced formation of oxygen radicals, which in turn may cause further cell injury when the level of antioxidants is inadequate.[46] In addition, it has been shown that renal cortical malondialdehyde, a product of lipid peroxidation, progressively increases in the remnant kidney model.[11,15] This observation further supports the concept that ROS are involved in the pathology associated with the remnant kidney model.[47,48]

Recent studies support a role for oxidized LDL in the pathogenesis of atherosclerosis.[49] By using specific monoclonal antibodies, the presence of oxidized LDL has been demonstrated in atherosclerotic lesions in humans and experimental animals[50] as well as in the glomeruli of the cholesterol-fed nephrotic rats.[51] The lesions of glomerulosclerosis in rats with subtotal nephrectomy resemble atherosclerotic lesions.[2,3] Thus, oxidized LDL may also play a pivotal role in the pathogenesis of chronic renal failure associated with increased cholesterol levels.[52] Oxidized LDL is a powerful macrophage chemoattractant.[53] Therefore, the increase in serum LDL+VLDL cholesterol found in the Ch group might contribute to LDL oxidation in the kidney, activation of macrophages, and production of chemoattractants for glomerular infiltration of monocytes and macrophages. Production of ROS by activated macrophages could further increase lipid peroxidation of PUFA in nephrotic cell membranes and the glomerular injury observed here as a higher GSS score. Therefore, the reduced infiltration of GM subpopulation into the renal parenchyme of rats in the FO+P group is most likely related to the combined

antioxidant function and hypocholesterolemic effect of probucol.

Reports on the effect of fish oil in proteinuria in this model are controversial. Barcelli et al.[19] and Clark et al.[10] reported that fish oil prevented proteinuria in the rat remnant nephron model. In this study, although all the nephrectomized rats had relatively higher creatinine and BUN levels, proteinuria was not significantly affected by nephrectomy. Scharschmidt et al.[54] found no effect of fish oil on urinary excretion of protein after 14 weeks of treatment. In contrast to our previous study, in which a decrease in GM subpopulation was accompanied by a decrease in urinary protein and serum creatinine levels,[22] we found that fish oil or fish oil supplemented with vitamin E or probucol had no effect on creatinine and BUN concentrations, despite the significant decrease in GSS score and GM subpopulation infiltration in the kidney. Urinary protein, however, was higher in the FO+P group at the end of the 8 weeks of this study. Because body weight gain in the FO+P group was significantly higher than in other groups, the increase of a high excretion of protein in urine in this group could have been related to high intake of protein due to high food consumption. Inclusion of pair-fed group(s) using a control diet would have been useful to delineate the mechanism underlying the higher proteinuria observed with the consumption of the FO+P diet, despite its effect on the improvement of kidney injury, which was observed histologically.

In summary, the hypolipidemic and anti-inflammatory actions of fish oil, the strong hypocholesterolemic and antioxidant properties of probucol, and to a lesser extent, the antioxidant effects of vitamin E combined with fish oil significantly alter histologic indices associated with the reduced progression of glomerulosclerosis induced by high levels of dietary cholesterol in the remnant kidney model of progressive kidney disease in the rat.

References

1 Moorhead, J.F., Chan, M.K., El Nahas, M., and Varghese, Z. (1982). Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. *Lancet* **2,** 1309–1311

2 Keane, W., Kasiske, B.L., and O'Donnell, M.P. (1988). Lipids and progressive glomerulosclerosis, a model analogous to atherosclerosis. *Am. J. Nephrol.* **8,** 261–271

3 Diamond, J.R. and Karnovsky, M.J. (1988). Focal and segmental glomerulosclerosis: Analogies to atherosclerosis. *Kidney Int.* **33,** 917–924

4 Peric-Golia, L. and Peric-Golia, M. (1983). Aortic and renal lesions in hypercholesterolemic adult male virgin Sprague-Dawley rats. *Atherosclerosis* **46,** 57–65

5 French, S.W., Yamanaka, W., and Ostwald, R. (1967). Dietary induced glomerulosclerosis in the guinea pig. *Arch. Pathol.* **83,** 204–210

6 Kasiske, B.L., O'Donnell, M.P., and Garvis, W.J. (1988). Pharmacologic treatment of hyperlipidemia reduces glomerular injury in rat 5/6 nephrectomy model of chronic renal failure. *Circ. Res.* **62,** 367–374

7 Harris, D.C., Tay, C., Egan, M.A., and Stewart, A. (1993). Altered metabolism in the ex vivo remnant kidney. I. Effects of time, substrate and perfusion pressure. *Nephron* **64,** 410–416

8 Kromhout, D., Bosschieter, E.B., and Coulander, C. (1985). The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *New Engl. J. Med.* **312,** 1205–1209

9 Meydani, M. (1995). Vitamin E. *Lancet* **345,** 170–175

10 Clark, W.F., Parbtani, A., and Jevnikar, A.M. (1989). Fish oil therapy in kidney disease. In *Health Effect of Fish and Fish Oils* (R.K. Chandra, ed.), pp. 303–320, St. Johns, Newfoundland:ARTS Biomedical Publishers

11 Clark, W.F., Parbtani, A., Philbrick, D.J., Spanner, E., Huff, M.W., and Holub, B.J. (1993). Dietary protein restriction versus fish oil supplementation in the chronic remnant nephron model. *Clin. Nephrol.* **39,** 295–304

12 Shohat, J. and Boner, G. (1993). Role of lipids in the progression of renal disease in chronic renal failure: Evidence from animal studies and pathogenesis. *Israel J. Med. Sci.* **29,** 228–239

13 Trachtman, H. (1994). Vitamin E prevents glucose-induced lipid peroxidation and increased collagen production in cultured rat mesangial cells. *Microvasc. Res.* **47,** 232–239

14 Trachtman, H., Futterweit, S., Prenner, J., and Hanon, S. (1994). Antioxidants reverse the antiproliferative effect of high glucose and advanced glycosylation end products in cultured rat mesangial cells. *Biochem. Bioph. Res. Co.* **199,** 346–352

15 Hamazaki, T., Tateno, S., and Shishido, H. (1984). Eicosapentaenoic acid and IgA nephropathy. *Lancet* **I,** 1017–1018

16 Prickett, J.D., Robinson, D.R., and Steinberg, A.D. (1981). Dietary enrichment with the polyunsaturated fatty acid eicosapentenoic acid prevents proteinuria and prolongs survival in NZBxNZW/F1 mice. *J. Clin. Invest.* **68,** 556–559

17 Robinson, D.R., Prickett, J.D., Makoul, G.T., Steinberg, A.D., and Calvin, R.B. (1986). Dietary fish oil reduces progression of established renal disease in (NZBxNZW) F1 mice and delays renal disease in BXSB, and MRL/1 strains. *Arthritis Rheum.* **29,** 539–546

18 Wheeler, D.C., Nair, D.R., Persaud, J.W., Jeremy, J.Y., Chappell, M.E., Varghese, Z., and Moorhead, J.F. (1991). Effects of dietary fatty acids in an animal model of focal glomerulosclerosis. *Kidney Int.* **39,** 930–937

19 Barcelli, U.O., Miyata, J., Ito, Y., Gallon, L., Laskarzewski, P., Weill, N., Hitzmann, R., and Pollak, V.E. (1986). Beneficial effects of polyunsaturated fatty acids in partially nephrectomized rats. *Prostaglandins* **32,** 211–219

20 Brenner, B.M., Mayer, T.W., and Hostetter, T.H. (1982). Dietary protein intake and the progressive nature of kidney disease: The role of homodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *New Engl. J. Med.* **307,** 652–659

21 Parthasarathy, S., Young, S.G., and Witzum, J.L. (1986). Probucol inhibits oxidative modification of low density lipoprotein. *J. Clin. Invest.* **77,** 641–644

22 Mune, M., Iteya, I., Matoba, K., Ohtani, H., Tujimoto, H., and Yukawa, S. (1996). Effect of probucol on the progression of remnant kidney of rats. *Lipid Peroxide Research* **20,** 163–165

23 American Institute of Nutrition (1977). Report of American Institute of Nutrition Ad Hoc Committee for Nutritional Studies. *J. Nutr.* **107,** 1340–1349

24 Meydani, S.N., Shapiro, A.C., Meydani, M., Macauley, J.B., and Blumberg, J.B. (1987). Effect of age and dietary fat (fish, corn and coconut oils) on tocopherol status of C57BL/6Nia mice. *Lipids* **22,** 345–350

25 Hellerstein, M.K., Meydani, S.N., Meydani, M., Wu, D., and Dinarello, C.A. (1989). Interleukin-1-induced anorexia in the rat. Influence of prostaglandins. *J. Clin. Invest.* **84,** 228–235

26 Boudet, J., Man, N.K., Pils, P., Sausse, A., and Brentano, J.L.F. (1978). Experimental chronic renal failure in the rat by electrocoagulation of the renal cortex. *Kidney Int.* **14,** 82–86

27 Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.* **28,** 470–475

28 Esders, T.W. and Mickrina, C.A. (1979). Purification and properties of L-alpha-glycerophosphate oxidase from streptococcus faecium ATCC 12755. *J. Biol. Chem.* **254,** 2710–2715

29 Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., and Fu, P.C. (1974). Dextran sulfate-Mg precipitation procedure for quantitation of high-density lipoprotein cholesterol. *Clin. Chem.* **28,** 1379–1388

30 Warnick, G., Benderson, J., and Albers, J. (1982). Dextran sulfate-Mg precipitation procedure for quantitation of high-density lipoprotein cholesterol. *Clin. Chem.* **28,** 1379–1388

31 Tiffany, T.O., Jansen, J.M., Burtis, C.A., Overton, J.B., and Scott, C.D. (1972). Enzymatic kinetic rates and end-point analyses of

substrate by use of a GCMS-AEC fast analyzer. *Clin. Chem.* **18,** 829–840
32 Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, T.J., and Schaeffer, R. (1981). A candidate reference method for determination of total protein in serum I: Development and validation. *Clin. Chem.* **27,** 1642–1650
33 NCCLS approved Standard (1979). *ACS-1, Specification for Standardized Protein Solution (Bovine Serum Albumin).* Villanova, PA: National Committee for Clinical Laboratory Standards
34 Larsen, K. (1972). Creatinine assay by a reaction kinetic principle. *Clin. Chim. Acta.* **41,** 209–217
35 Caruso, U., Fowler, M., Erceg, M., and Romano, C. (1991). Determination of very-long-chain fatty acids in plasma by a simplified gas chromatographic-mass spectrometric procedure. *J. Chromatogr*. **562,** 147–152
36 Sukhija, P.S. and Palmquist, D.L. (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. *J. Agr. Food Chem.* **36,** 1202
37 Meydani, M., Evans, W., Handelman, G., Biddle, L., Fielding, R.A., Meydani, S.N., Burrill, J., Fiatarone, M.A., Blumberg, J.B., and Cannon, J.G. (1993). Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. *Am. J. Physiol.* **33,** R992–R998
38 McManus, J.F.A. (1993). The periodic acid applied to the kidney. *Am. J. Path.* **21,** 613–663
39 Modi, K.S., Morrissey, J., Shah, S.V., Schreiner, G.F., and Klahr, S. (1990). Effects of probucol on renal function in rats with bilateral ureteral obstruction. *Kidney Int.* **38,** 843–850
40 Modi, K.S., Schreiner, G.F., Purkerson, M.L., and Klahr, S. (1992). Effects of probucol in renal function and structure in rats with subtotal kidney ablation. *J. Lab. Clin. Med.* **120,** 310–317
41 Moorhead, J.F., Chan, M.K., and Vaghese, Z. (1986). Role of abnormalities of lipid metabolism in the progression of renal disease. In *Progressive Nature of Renal Disease. Contemporary Issues in Nephrology* (W.E. Mich, B.M. Brenner, and S.J. Stein, eds.), pp. 133–148. Churchill Livingstone, New York, NY USA
42 Simopoulos, A.P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* **54,** 438–463
43 Paller, M.S., Hoidal, J.R., and Ferris, T.F. (1984). Oxygen free radicals in ischemic acute renal failure in the rat. *J. Clin. Invest.* **75,** 1156–1164
44 Rehan, A., Johnson, K.J., and Wiggins, R.C. (1984). Evidence for the role of oxygen radicals in acute nephrotoxic nephritis. *Lab. Invest.* **51,** 396–402
45 Walker, P.D. and Shah, S.V. (1988). Evidence suggesting a role for hydroxyl radical in gentamicin induced acute renal failure in rats. *J. Clin. Invest.* **81,** 334–341
46 Schrier, R.W., Harris, D.C.H., and Chan, L. (1986). Tubular hypermetabolism as a factor in the progression of chronic renal failure. *Am. J. Kidney Dis.* **12,** 243–249
47 Nath, K.A., Croat, A.J., and Hostetter, T.H. (1990). Oxygen consumption and oxidant stress in surviving nephrons. *Am. J. Physiol.* **258,** F1354–1362
48 Kobayashi, S. and Venkatachalam, M.A. (1992). Differential effects of calorie restriction on glomeruli and tubules of the remnant kidney. *Kidney Int.* **42,** 710–717
49 Steinberg, D., Parthasarathy, S., and Carew, T.E. (1989). Beyond cholesterol: Modification of low density lipoprotein that increase its atherogenicity. *New Engl. J. Med.* **320,** 915–924
50 Rosenfeld, M.E., Palinski, W., and Yla-Herttuala, S. (1990). Distribution of oxidation specific lipid-protein adducts and apolipoprotein B in atherosclerotic lesions of varying severity from WHHL rabbits. *Arteriosclerosis* **10,** 336–349
51 Magil, A.B., Frochlich, J.J., Innis, S.M., and Steinbrecher, U.P. (1993). Oxidized low-density lipoprotein in experimental focal glomerulosclerosis. *Kidney Int.* **43,** 1243–1250
52 Wheeler, D.C., Chana, R.S., Topley, N., Petersen, M.M., Davies, M., and Williams, J.D. (1984). Oxidation of low density lipoprotein by mesangial cells may promote glomerular injury. *Kidney Int.* **45,** 1628–1636
53 Quinn, M.T., Parthasarathy, S., Fong, L.G., and Steinberg, D. (1987). Oxidatively modified low density lipoproteins: A potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc. Natl. Acad. Sci. USA* **84,** 2995–2998
54 Scharschmidt, L.A., Gibbons, N.B., McGarry, L., Berger, P., Axelrod, M., Janis, R., and Ko, Y.H. (1987). Effects of dietary fish oil on renal insufficiency in rats with subtotal nephrectomy. *Kidney Int.* **32,** 700–709