Effects of fish oil on glomerular function in rats with diabetes mellitus

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Abstract The mechanisms responsible for hyperfiltration in diabetes mellitus (DM) as well as for the initiation and progression of diabetic nephropathy are not fully elucidated. Enhanced prostaglandin E_2 (PGE_2) production has been invoked in the former and thromboxane (TXB_2) and hyperlipidemia in the latter. Fish oil (FO)-enriched diets can favorably alter eicosanoid synthesis and serum lipid profiles. We therefore examined the effects of a FO-enriched diet on glomerular filtration (GFR), proteinuria, glomerular eicosanoid production, and serum lipids in rats with streptozotocin-induced DM (STZ-DM). Groups of 5-8 rats with STZ-DM were maintained on low insulin and then pair-fed with isocaloric diets enriched with either FO (20% w/w) or beef tallow (BT; 20% w/w). GFR was determined in the same animals at onset of diet and after 8 and 20 weeks on the respective diets by [^{14}C]inulin clearance using implanted osmotic minipumps each time. Significant hyperfiltration was present initially and GFR did not change on either diet for 20 weeks, in spite of a significant and greater than 50% decrease in all prostaglandins (PGE_2, TXB_2, $PGF_{2\alpha}$, 6-keto, $PGF_{1\alpha}$) produced by glomeruli isolated from DM/FO as compared to DM/BT or control rats. FO diet completely corrected the hypertriglyceridemia of diabetes and significantly reduced the mild and early proteinuria of DM. ▮▮ The decrease in proteinuria and the correction of hyperlipidemia of DM by a FO-enriched diet may be beneficial in the long term not only for the development of diabetic glomerulopathy, but also for the accelerated atherosclerosis of DM.—Sinha, A. K., L. A. Scharschmidt, R. Neuwirth, H. Holthofer, N. Gibbons, C. M. Arbeeny, and D. Schlondorff. Effects of fish oil on glomerular function in rats with diabetes mellitus. *J. Lipid Res.* 1990. **31:** 1219–1228.

Supplementary key words eicosanoids • lipids • kidney • glomerulus

Diabetic nephropathy is the most common cause of end stage renal disease. The mechanisms responsible for the initiation and progression of diabetic nephropathy are not completely elucidated. Among the many contributing factors proposed are alterations in glomerular hemodynamics, prostaglandin and thromboxane production, and lipid metabolism (1–8). A state of glomerular hyperfiltration has been consistently demonstrated in patients with newly diagnosed insulin-dependent diabetes mellitus (DM) (2, 4), as well as in the early stages of experimental diabetes in animals (3, 8). These early glomerular hemodynamic disturbances have been implicated in the pathogenesis of the diabetic glomerulopathy, a hypothesis supported by a considerable body of evidence both in animal models of DM and in humans (9, 10). Among the factors contributing to the hyperfiltration are atrial natriuretic peptide (11), altered response to vasoactive hormones (12), and changes in locally generated vasoactive eicosanoids (13). Increased prostaglandin synthesis has been shown in glomeruli isolated from rats with streptozotocin-induced diabetes (14–16). Furthermore, enhanced thromboxane synthesis by platelets in DM has been proposed to contribute to the microangiopathy of DM (17, 18). Thromboxane has also been implicated as an etiological factor in other forms of renal disease (19). In addition to these reports, there are several others describing alterations in eicosanoid production in systemic vasculature (20). There is, therefore, evidence from a number of investigators documenting abnormalities in renal and extrarenal arachidonate metabolism in diabetes. The significance of these findings with regard to hyperfiltration, however, remains unclear. Clearance studies in rats with experimental DM and in patients with DM have provided conflicting results. Some studies show correction of hyperfiltration by prostaglandin inhibition (16, 21), but others do not (12, 22, 23). The reasons for these discrepant results are not fully apparent but may relate to the underlying volume status of the diabetic animals, to duration of prostaglandin inhibition, and effects of anesthesia, etc.

Little is known about the role of hyperlipidemia in glomerulopathy of DM. There are, however, increased lipid deposits in diabetic glomeruli (7) and marked

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Abbreviations: DM, diabetes mellitus; PGE_2, prostaglandin E_2; TXB_2, thromboxane; FO, fish oil; GFR, glomerular filtration rate; STZ, streptozotocin; BT, beef tallow; EPA, eicosapentaenoic acid.

hyperlipidemia is characteristic of DM both in humans and animals (7, 24). Hyperlipidemia in general has been invoked as a factor contributing to the development of glomerular sclerosis (7). In addition, the increased nonenzymatic glycosylation of the elevated low density lipoproteins (LDL) in DM, may contribute to the increased incidence of microvascular disease and glomerulopathy of DM (25).

Fish oil, containing n-3 (ω3) polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) slows the course of atherosclerosis (26, 27). This is though to be related to the lowering of plasma lipids by these polyunsaturated fats (27, 28) and to a change in prostaglandin-thromboxane production (29, 30). In contrast to the natural substrate arachidonic acid, eicosapentaenoic acid is poorly converted by cyclooxygenase and generates a biologically inactive thromboxane (31). The latter may contribute to the beneficial effects of diets enriched in fish oil.

In the present study, we examined the effects of a FO-enriched diet on GFR, proteinuria, glomerular eicosanoid production, and serum lipids in rats with streptozotocin-induced DM. We used FO both as an experimental tool, to address the potential contribution of glomerular eicosanoids and hyperlipidemia to glomerular hyperfiltration and to diabetic renal disease, and also as a potential therapy, to determine whether there might be a role for a FO-enriched diet in the prevention or attenuation of diabetic nephropathy.

Our results indicate that decreasing glomerular PG synthesis by FO diet does not influence the hyperfiltration of rats with DM, which persists unaltered over 20 weeks. The FO diet does, however, correct the hyperlipidemia and decrease the modest proteinuria of early DM and may thus be of potential benefit.

METHODS

Induction of disease

Diabetes mellitus was induced in female Sprague-Dawley rats (Charles River, Wilmington, MA) by a single intravenous injection of freshly prepared streptozotocin (STZ; Sigma Chemical Corp., St. Louis, MO) (60 mg/kg) in citrate buffer (0.05 M citric acid, pH 4.5) under light anesthesia. Within 48 h of the STZ injection, animals became diabetic. Only those with blood glucose values above 250 mg/dl were included in the study. Morning glucose concentration was determined biweekly, initially for the first 3–4 weeks (to titrate the dose of insulin required) and bimonthly thereafter with a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). All rats were housed in individual cages, had free access to water, and were given weighed amounts of powdered rat chow

(Purina) daily. (For details of diets see below.) The diabetic rats received daily subcutaneous injections of insulin (Ultralente Iletin I, Lilly, Indianapolis, IN) to maintain blood glucose concentration between 350 and 500 mg/dl. Daily insulin dose averaged 0.5 units. Normal rats matched for age and weight at the beginning of the study were carried as nondiabetic controls. These control rats received vehicle injections instead of streptozotocin. Body weight was recorded on a weekly basis throughout the 20-week period of the study.

Diets (Table 1)

After diabetes was established and the insulin dose per animal was determined (approximately 4 weeks after streptozotocin), the rats were given measured amounts of standard laboratory rodent chow (Laboratory Rodent Chow 50011 Ralston, Purina Co.) daily in order to establish the feeding pattern and to determine daily food consumption. They were then divided into two dietary subgroups commencing in the middle of the first clearance period. From this time on the DM rats were pair-fed isocaloric diets that differed only in the composition of fat, but not in the total amount of fat.

The basic diet consisted of a fat-free powder TD84010 (Teklad Test Diets, Madison, WI). To this was added dietary fat, either in the form of melted beef tallow (ICN Nutritional Biochemicals, Cleveland OH) for the DM/BT and control rats or in the form of whole menhaden oil, for the DM/FO rats (a generous gift from Mr. Anthony Bimbo, Zapata Haynie, Reedville, VA). Menhaden oil, a rich source of EPA and other n-3 fatty acids, is highly refined and processed with more than 95% of the fatty acids in the triglyceride from. Fatty acid analysis of the dietary lipids as percent of the total dietary fatty acids is depicted in Table 1. All nutrients, minerals, and vitamins were equal in each diet and in compliance with recommended nutritional requirements. The composition of both control and experimental diets after the addition of fat was: carbohydrate 50% w/w; protein 19% w/w; fat 21% w/w; cellulose 5% w/w; minerals 4% w/w (Teklad AIN 76,170915); and vitamins (including vitamin E to inhibit oxidation of fatty acids in the chow), 1% w/w (Teklad 40060). Sufficient safflower oil was added to provide 2% essential fatty acids. Prior to use, the menhaden oil was stored in a refrigerated room under nitrogen. Freshly prepared and measured amounts of food were provided 3 times a week with the rats taking the food ad libitum. The animals were maintained on these two different diets over a period of 20 weeks. The weight of the food ingested by the DM/FO rats was equal to that consumed by the DM/BT rats (range 20–25 g/day). Nondiabetic control rats were fed the beef tallow-supplemented diet or regular Purina rat chow (average food consumption 10–15 g/day).

TABLE 1. Fatty acid analysis of dietary lipids

Fatty Acid	Menhaden Oil (EPA-Enriched Diet)	Beef Tallow (Control Diet)	Safflower Oil (To Provide Essential Fatty Acids)
14:0	8.2	2.7	0.11
16:0	13.5	23.9	6.38
16:1	13.5	5.3	0.57
18:0	5.5	17.7	2.45
18:1	12.5	41.2	11.9
18:2	5.2	6.1	73.03
20:3 (DHG)	1.4	<0.05	
20:4 (AA)	1.5	<0.05	
20:5 (EPA)	14.4	<0.05	<0.05
18:3, 22:5, 22:6	14.0		

Diabetic rats received either 20% (w/w) menhaden oil or 20% (w/w) beef tallow. Sufficient safflower oil was added to each diet to provide equal amounts of dietary linoleate and arachidonate. Values represent percent of total fatty acid content; DHG, dihomo-γ-linolenic acid; 22:6, docosahexanoic acid.

Measurement of glomerular filtration rate by inulin clearance

Three weeks after the induction of diabetes and stabilization of blood glucose values at 400 mg/dl, the animals were prepared for determination of glomerular filtration rate (GFR) by the method of Jobin and Bonjour (32). Rats were placed in metabolic cages at least 1 week prior to clearance studies so that they could adjust to their new environment. Osmotic minipumps (Model No. 2001, Alza Corporation, Palo Alto, CA) were inserted into the peritoneal cavity or subcutaneously in the neck under light anesthesia and sterile conditions. These pumps deliver fluid at a constant rate of 1 μl/h. In the present study pumps were filled with 200 μl of an aqueous solution containing 30 μCi of [^{14}C]inulin (Amersham Corporation, Arlington Heights, IL).

After insertion of the pump, a 12-h stabilization and equilibration period was allowed prior to starting 24-h urine collections. Urine was collected and measured over five to seven consecutive 24-h periods, each collection starting and ending at 11:00 AM. At the end of each 24-h interval, 200 μl of tail blood was collected from each animal into heparinized capillary tubes. Before blood samples were collected, the tails were thoroughly washed under running water in order to prevent urinary contamination. Radioactivity in urine and plasma was measured by scintillation spectrometry (LKB Wallac, 1215 Rackbeta, II, liquid scintillation counter, Finland), and the clearance of [^{14}C]inulin was determined. With this method we obtained three to six serial values of 24-h GFR in unrestrained, awake animals. In preliminary experiments in six control rats, we established that inulin clearance determined by this method remained stable over a 6-day period (variation of less than 10% per rat). The pumps were taken out at the end of the clearance period, i.e., 8 to 10 days after implantation. Rats were returned to their individual cages at the end of each clearance period. In addition to the initial clearances, clearances were repeated in some rats after 9 weeks into

the study and in all animals at 20 weeks, i.e., at the end of the study. For each animal the mean of three to five consecutive 24-h clearance periods was used as a single value.

Urinary protein estimation

Twenty-four hour urinary protein excretion rates were determined at initiation of the diet changes (i.e., 4 weeks after induction of DM) and after 20 weeks using the Bradford method (Bio-Rad Protein Assay, Bio-Rad, Richmond, CA) (33). Protein excretion was measured on two consecutive 24-h urine samples and expressed as mean of the 2-day collections (mg/24 h).

Measurement of blood pressure

Systolic blood pressure was measured by the tail cuff method at the initiation of the study, at bi-monthly intervals thereafter, and at the end of the study. During this procedure the animals were placed under light ether anesthesia and pressures were recorded by a single channel recorder-amplifier (Grass Instruments, MA).

Termination of study

At the end of the 20-week study period, rats were anesthetized with pentobarbital sodium injection (50 mg/kg) i.p., and the abdominal aorta was exposed through a midline incision. Blood was drawn from the aorta into EDTA-containing syringes for lipid studies. The kidneys were rapidly removed, decapsulated, and weighed. The left kidney was immediately placed in iced phosphate-buffered saline solution, pH 7.23, and processed for isolation of glomeruli. From the right kidney, cortical slices were either immediately fixed in 4% buffered formalin solution for histological examination or rapidly frozen in liquid nitrogen and stored at − 70°C for immunofluorescent microscopy. Kidney samples were also examined for lectin binding as published previously (34).

Preparation and isolation of glomeruli

Glomeruli were prepared by a sequential sieving technique, as described previously (35, 36). The renal capsule was removed, cortical tissue was minced with a razor blade and passed sequentially through sieves of 150 μm, 100 μm, and 75 μm mesh size in phosphate-buffered saline, pH 7.23. The glomerular suspension was then centrifuged at 500 g for 5 min; the resulting preparation contained 95% glomeruli as assessed by light microscopy.

Kreb's Ringer bicarbonate buffer containing 1 mg/ml glucose (KRBG) equilibrated with 95% O_2 and 5% CO_2 was used for the experimental incubations. After centrifugation the glomerular preparation was gently resuspended in 4 ml of the KRBG at 37°C. Aliquots (1.5 ml) of the glomerular suspensions (700–1200 glomeruli per tube) were incubated in duplicate for 10 min at 37°C in a shaking water bath. Incubations were terminated by sedimenting the glomeruli in an Eppendorf microfuge for 10 sec and removing 1 ml supernate for subsequent assay of prostaglandins. The glomeruli in the pellet were dissolved in 1 N NaOH and protein content was determined by the Bradford method (33).

Assay for prostaglandins

$PGF_{2\alpha}$, PGE_2, 6-keto $PGF_{1\alpha}$, and TXB_2 were determined in duplicate by enzyme immunoassay (EIA) method as reported (37). The PGE_2 antibody was obtained from the Pasteur Institut (Paris, France). Antibodies for $PGF_{2\alpha}$, 6-keto $PGF_{1\alpha}$, and TXB_2 were obtained from Laboratories Des Stallergenes (France). Cross-reactivity of PGE_3 with the PGE_2 antibody in our assay was determined to be 10–12%.

Serum lipid analysis

Cholesterol was measured by the Fermco enzymatic method for free cholesterol and cholesteryl esters (Fermco Biochemical, Elk Grove Village, IL). Triglycerides were measured enzymatically using the Worthington Diagnostic Reagent Set (Cooper Biochemical, Diagnostic Division, Malvern, PA). For analysis of the individual lipoproteins, plasma from two to four rats in each group was pooled and separated by sequential ultracentrifugation following the method of Havel, Eder, and Bragdon (38). Glycosylated hemoglobin was determined by an affinity chromatographic method using the Glycaffin-Hb kit (GlycAffin System, Isolab, Akron, OH).

Statistics

Statistical analysis was done by Student's t test for unpaired data. All results presented represent means ± SEM.

RESULTS

General parameters (Fig. 1)

Blood glucose and body weights of the two groups of diabetic rats and of the normal controls on beef tallow diet are depicted in **Fig. 1**. Weight gains of the diabetic rats were comparable in the two dietary groups (3 g/week), but less than that of nondiabetic control rats fed the beef tallow diet (5 g/week). In the DM rats, blood sugars, regardless of dietary groups, were maintained around 400 mg/dl throughout the study, with no difference in insulin requirement between the two dietary groups (DM/FO: 0.5 ± 0.1 U/day; DM/BT: 0.5 ± 0.1 U/day). This was also confirmed by determinations of glycosylated hemoglobin which were 13.3% in DM/FO and 12.5% in DM/BT rats versus 7% in nondiabetic controls.

Blood pressures obtained by tail method were comparable between normal control rats (114 ± 5 mm Hg) and diabetic rats in either dietary group (DM/BT at initiation of diet 112 ± 6, at time of killing 113 ± 7; DM/FO at initiation of diet 118 ± 6, at time of killing 118 ± 6).

Renal function

The effects of diabetes and diets on GFR and kidney weights are shown in **Fig. 2** and **Table 2**. GFR expressed per kg body weight is shown in Fig. 2 which also includes data on additional control groups fed BT-supplemented chow or FO-supplemented chow. GFR in control rats fed the different diets was comparable in spite of marked lowering of eicosanoid synthesis by FO (**Fig. 3**). Further-
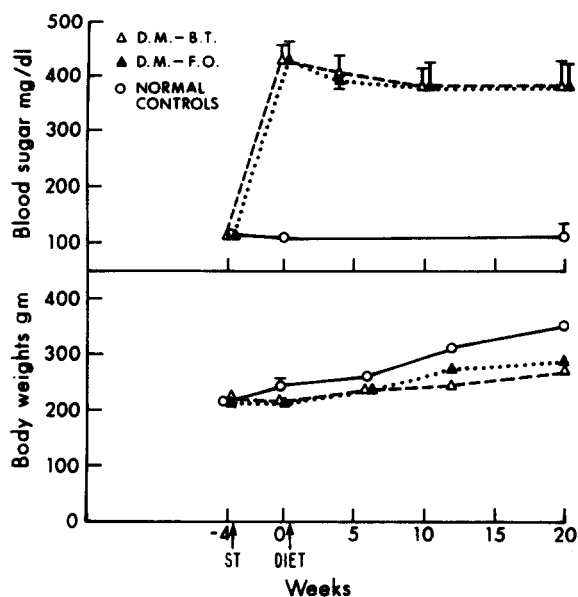

Fig. 1. Body weight (in grams) and blood sugar values of diabetic rats on beef tallow (DM/BT) or fish oil (DM/FO) diets and of nondiabetic controls. DM was induced by streptozotocin (ST) and different diets were initiated at 0 week, as indicated. Values are means ± SEM of six normal control rats, eight rats for DM/FO, and five rats for DM/BT. Standard errors for body weights were small and fall within the respective symbols.
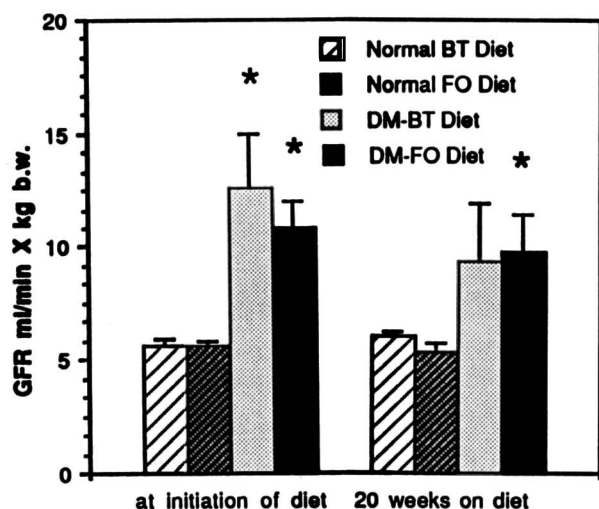
Fig. 2. Glomerular filtration rates (GFR) in ml/min per kg body weight) of diabetic rats on fish oil (DM/FO) or beef tallow (DM/BT) diets and of normal controls on the same diets at the initiation of the diet and after 20 weeks on the respective diet. Glomerular filtration was repeatedly determined in the same awake rats as described in Methods. Values are means ± SEM from six rats each for the normal control groups, from five rats with DM/BT, and from eight rats with DM/FO. Asterisks indicate $P<0.05$ or better as compared to normal controls on the respective diet.

more, BT diet had no effect on GFR in normal rats as compared to regular rat chow diet (BT diet: GFR 1.4 ± 0.2 ml/min, regular rat chow: 1.4 ± 0.1 ml/min; n = 6 for each group). After 4 weeks of streptozotocin-induced DM and at the initiation of the different diets, DM rats on either BT or FO diet had enhanced GFRs as compared to control rats on either BT or FO diet. This was true irrespective of expression of GFR per rat (Table 2) or per kg body weight (Fig. 2). GFR remained stable throughout the 20-week study period in the DM rats and was not significantly affected by the fish oil-enriched diet (Fig. 2 and Table 2). GFR of normal nondiabetic rats was also not affected by the diets. After 8 weeks, GFR was 10.3 ± 1.8 ml/min · kg body weight^{-1} in DM/BT and 9.1 ± 1.5 in DM/FO rats versus 5.4 ± 0.3 in control rats. As DM rats gained less weight than control rats, the GFR/kg body

weight remained elevated in DM rats on FO diet (Fig. 2). Kidneys from DM rats weighed 30% more than those from control rats (Table 2). This increase in renal weight in DM was also not affected by the FO diet. Thus the fish oil-enriched diet did not influence glomerular or renal hypertrophy in DM rats.

Urine volumes and 24-h protein excretion are shown in **Table 3**. At the initiation of the diets, all diabetic rats had the same degree of polyuria. Daily urine volumes decreased to a comparable degree over time in both dietary groups. Before initiation of the experimental diets, diabetic rats in both groups exhibited moderate proteinuria (approximately 10 mg of protein per day) as compared to nondiabetic control rats (1.8 mg/day). After 16 weeks on a fish oil diet, the protein excretion was reduced by more than 50% to 4.6 ± 0.9 mg/day ($P<0.01$) in the DM/FO rats, whereas the DM/BT rats maintained the elevated rate of protein excretion (10 mg protein/day) throughout the study. Thus the fish oil-enriched diet significantly reduced the increased urinary protein excretion of rats with early DM in spite of no change in glomerular hyperfiltration.

Prostaglandin synthesis by isolated glomeruli

At the end of the 20-week study period, prostaglandin synthesis was evaluated in glomeruli isolated from the different groups of rats. As shown in Fig. 3, production of PGE_2 and TXB_2 after 20 weeks was comparable among normal controls on BT and DM/BT rats on the diets. Furthermore, in normal control rats fed the BT diet for 20 weeks, there was no effect on eicosanoid synthesis by isolated glomeruli as compared to normal rat chow (PGE_2: 3.9 ± 0.3 ng/mg protein for regular rat chow and 3.8 ± 0.6 for BT diet; TXB_2: 2.1 ± 0.2 ng/mg protein for regular diet and 2.0 ± 0.5 for BT diet). Synthesis of $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$ remained slightly higher in glomeruli from DM/BT rats ($PGF_{2\alpha}$:10.4 ± 0.8 ng/mg protein × 10 min ($P<0.02$), 6-keto $PGF_{1\alpha}$: 4.1 ± 0.6) after 20 weeks of diabetes compared to the normal controls on BT ($PGF_{2\alpha}$: 7.8 ± 0.3; 6-keto $PGF_{1\alpha}$: 2.7 ± 0.3). The fish oil diet significantly reduced production of all eicosanoids measured by 50–60% in glomeruli from

TABLE 2. Glomerular filtration rates and kidney weights in different groups of rats

Measurement	Normal Controls		DM Rats	
	BT Diet (6)	FO Diet (6)	BT Diet (5)	FO Diet (8)
Filtration rate (ml/min × rat)				
At initiation of diet	1.4 ± 0.1	1.4 ± 0.1	2.6 ± 0.2*	2.5 ± 0.1*
After 20 wk of diet	2.0 ± 0.3	1.8 ± 0.4	2.6 ± 0.3	2.8 ± 0.2
Kidney weight (g/kidney)				
After 20 wk of diet	1.0 ± 0.1	1.0 ± 0.1	1.3 ± 0.1*	1.3 ± 0.1*

Numbers in parentheses indicate number of rats per group.
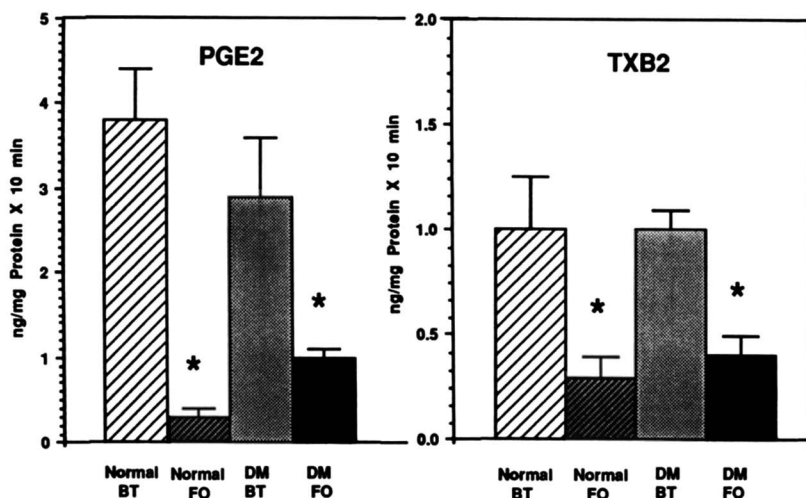*$P < 0.05$ or better by group comparison of DM rats with normal controls on the respective diets.

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Fig. 3. Prostaglandin synthesis by glomeruli isolated from normal control rats or diabetic rats on either beef tallow (BT) or fish oil (FO) diets at the end of the 20-week study period. Glomeruli were isolated and then incubated, and prostaglandins were determined as described in Methods. Results are means ± SEM from six controls on BT and five controls on FO and of five DM/BT and eight DM/FO rats. Asterisks indicate $P<0.01$ or better for normal/BT versus normal/FO or for DM/FO versus DM/BT.

normal FO rats or from DM/FO rats as compared to normal BT or DM/BT rats.

Plasma lipids

Within 4 weeks after induction of DM and before assignment to the dietary groups, all DM rats had elevated triglycerides compared to normal controls (diabetic rats 263 ± 42 mg/dl vs normal control rats 146 ± 6 mg/dl). At this time there were no differences in plasma cholesterol (diabetic rats 65 ± 6 mg/dl vs normal control rats 77 ± 1 mg/dl). Four weeks after initiation of the experimental diets, diabetic rats on the beef tallow diet (DM/BT) showed further rise in triglyceride (1086 ± 288 mg/dl) whereas diabetic rats fed fish oil (DM/FO) had stabilized their triglyceride levels at 341 ± 87 mg/dl. Cholesterol levels in both dietary groups remained unchanged (DM/BT 81 ± 12; DM/FO 96 ± 20 mg/dl). Throughout the rest of the experimental dietary period, diabetic rats fed beef tallow diets maintained their hyper-

triglyceridemia such that, at the time of killing, levels were 1162 ± 325 mg/dl (**Fig. 4**). In contrast, diabetic rats on fish oil diet (DM/FO) had significantly lower triglyceride levels (DM/FO: 125 ± 25 mg/dl) which were not different from those of normal control rats, (72 ± 2 mg/dl) (see Fig. 4). By the time of killing, cholesterol levels of the DM/BT rats had risen to 150 ± 41 mg/dl, a value numerically but not statistically higher than that in DM/FO rats (77 ± 10 mg/dl) or in normal controls on BT (108 ± 9) (Fig. 4).

Lipoprotein analysis

At the end of the 20-week study period, lipoprotein analysis was carried out in two series of experiments on blood samples pooled from two to four rats in each experimental group. **Fig. 5** gives the mean values for the lipoproteins from the two series of experiments. While no statistical evaluation can be performed on the lipoprotein fractions, it appears evident that the fish oil diet reduced the elevated levels of VLDL and also LDL in the diabetic rats, consistent with the marked reduction in triglycerides and the modest decrease in cholesterol. The fish oil diet had no effect on HDL in the DM rats.

Thus, fish oil diets prevented the rise in triglycerides in the streptozotocin rats and nonsignificantly attenuated the rise in cholesterol.

Histology

By light microscopy, none of the kidneys from either the DM/BT or DM/FO rats after the 20-week study period manifested overt diabetic changes such as mesangial expansion or basement membrane thickening.

By immunofluorescence microscopy, a faint glomerular staining for IgG was observed in one rat each from the DM/BT and DM/FO groups. No immunofluorescence for complement C_3 was noted in either group. We also examined binding for the lectins of peanut agglutinin

TABLE 3. Urine volume and 24-h protein excretion

Measurement	Normal Control (6)	DM/BT (5)	DM/FO (8)
Urine volume (ml/day)			
Initial		100 ± 12	87 ± 15
Final	16 ± 1	44 ± 2	50 ± 12
Urinary protein (mg/day)			
Initial		10.3 ± 2.1	10.6 ± 1.8
Final	1.8 ± 0.3	10.5 ± 3.8	4.6 ± 0.9*

Urine volumes and 24-h urinary protein excretion of diabetic rats on either beef tallow-enriched (DM/BT) or fish oil-enriched (DM/FO) diets compared to nondiabetic controls fed beef tallow chow. Initial values were obtained 4 weeks after the induction of diabetes, immediately before the start of experimental diets. Final values represent measurements made after 20 weeks on experimental diets. Values represent mean ± SEM; numbers in parentheses indicate number of rats in each group.

*$P < 0.01$ DM/FO final versus DM/FO initial.
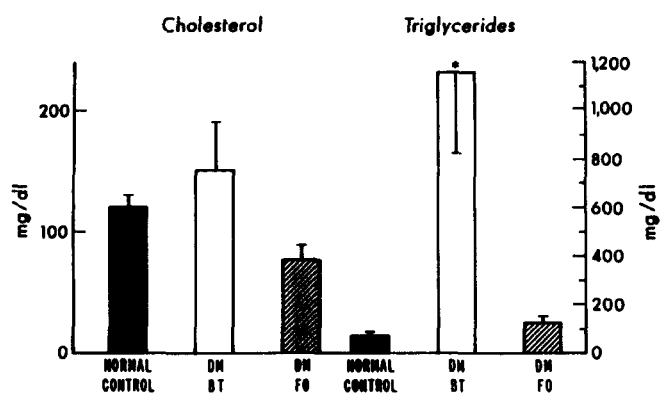
Cholesterol   Triglycerides

Fig. 4. Plasma cholesterol and triglyceride levels in diabetic rats after 20 weeks on beef tallow (DM/BT) or fish oil (DM/FO) diets. Values for normal control rats on beef tallow diet are shown for comparison. Values are means ± SEM from six normal controls, five DM/BT, and eight DM/FO rats. *$P < 0.01$ DM/FO vs DM/BT or normal controls.

(*Arachis hypogaea*) and of horse germ (*Dolichos biflorus*), as these have been noted to become positive in glomeruli of human DM. (34). After the 20-week study period, fluorescence for these lectins was uniformly absent in both groups of diabetic rats and in the normal controls.

DISCUSSION

The purpose of the present studies was to examine whether a fish oil-supplemented diet, rich in n–3 polyunsaturated fatty acids, could influence renal function in normal rats and in rats with streptozotocin-induced DM. The fish oil would be expected to alter eicosanoid synthesis and plasma lipids, factors that have been implicated in contributing to the development of diabetic nephropathy. Our results show that the fish oil diet significantly lowered eicosanoid production by glomeruli isolated from the normal FO rats and the DM/FO rats as compared to normal BT rats or the DM/BT rats. In spite of this 50% reduction in glomerular prostaglandin synthesis, glomerular filtration rates remained stable in the control group and remained elevated in the DM/FO and DM/BT rats over the 20-week study period. Fish oil diet did, however, totally correct the marked hypertriglyceridemia of DM and did decrease the modest proteinuria observed in all rats with DM.

Our present studies support the conclusion that chronically enhanced glomerular prostaglandin synthesis does not contribute in a major fashion to hyperfiltration of DM rats. This conclusion is based on several observations. In contrast to the uniformly enhanced glomerular prostaglandin production early in streptozotocin-induced DM (12, 14, 15, 39), glomeruli isolated from the rats after 20 weeks of DM showed only a moderate increase in synthesis of $PGF_{2\alpha}$ but not in PGE_2 or thromboxane produc-

tion, as compared to control rats. This finding would be consistent with the observation by Craven, Caines, and DeRubertis (16) who reported enhanced glomerular prostaglandin synthesis 3 days but not 4 weeks after administration of streptozotocin to rats. Glomeruli isolated from normal control/FO or DM/FO rats showed 50% or greater decrease in the production of all eicosanoids measured, as compared to those from either nondiabetic rats fed BT diet or DM/BT rats. This finding is consistent with previous observations from our laboratory on the effect of fish oil on prostaglandin production in both isolated glomeruli and mesangial cells in culture (40). In these previous studies (40) we had demonstrated that eicosapentaenoic acid, the major replacement in the fish oil diet, is a poor substrate for cyclooxygenase as compared to arachidonic acid, as also observed in other systems (30, 41, 42). The other major n–3 fatty acid in menhaden oil, docosahexanoic acid, is also a poor cyclooxygenase substrate (43). Based on these previous observations we also believe it to be unlikely that glomeruli produced large amounts of prostaglandins of the three series, for which either eicosapentaenoic or docosahexanoic acid would be the precursor.

The use of the osmotic minipumps allowed us to determine GFR in the same rats repeatedly over the entire study period. Furthermore, this method does not require restraining or instrumentation, so that the rats are fully awake, active, and have their usual free access to food and water. Our results show that, in spite of a normal (DM/BT rats) or even a 50% reduction (DM/FO rats) of glomerular prostaglandin synthesis, awake, unrestrained DM rats maintain their glomerular filtration rates for at least 20 weeks. When GFR was expressed as ml/min for each rat, the diabetic rats had significant hyperfiltration initially, which, however, after 20 weeks was no longer significantly elevated over that of weight-matched normal controls. Diabetic rats on either FO or BT diet gained less weight than control rats. When GFR
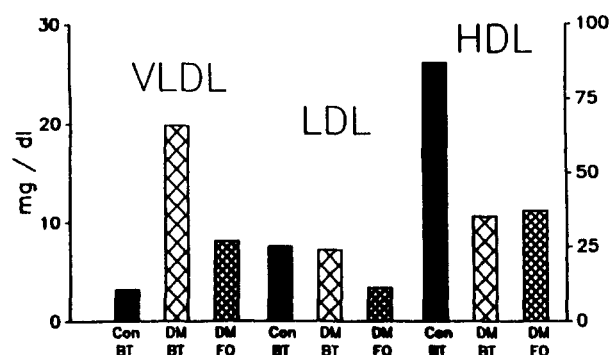

Fig. 5. Plasma lipoprotein values for the diabetic rats on beef tallow (DM/BT) or fish oil (DM/FO) diets. Normal rats on the beef tallow diet (Con BT) are shown for comparison. Values are means from two series of experiments, each performed on pooled blood samples from two to four rats per group; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

was expressed per kg body weight, the DM rats maintained significantly elevated filtration rates. Based on our clearance studies we cannot, however, exclude the possibility that the altered prostaglandin synthesis in the DM/FO rats may have influenced intraglomerular hemodynamic forces. Overall, the failure to change the GFR in DM rats by reducing PG synthesis is similar to results obtained with cyclooxygenase inhibitors in patients with DM (22, 23) and in our previous acute studies in rats (12).

The second finding of interest in our studies concerns the effect of FO diet on the hyperlipidemia of DM. It has been suggested that the abnormal lipid metabolism of DM may play a role in the development of diabetic glomerulopathy (7). Enrichment of diet with FO can improve plasma lipid profiles and delay the development of vascular disease (27). In general, FO diet predominantly decreases LDL and VLDL, with no consistent effect on HDL (44–47). Triglycerides are also markedly reduced by FO (28, 44). Our present results are entirely consistent with these previous reports, in that we observed a marked decline in the DM/FO rats of the severe hypertriglyceridemia that developed in the DM/BT rats. As is to be expected, this decrease in the hypertriglyceridemia was reflected by a fall in the VLDL levels. A less pronounced decline occurred in the cholesterol levels of the DM/FO rats. As HDL levels were not appreciably influenced by the diet, FO feeding improved the VLDL to HDL ratio in the DM rats, a finding that is generally considered to have a beneficial influence on the development of diabetic vascular complications. The mechanism for the beneficial effect of FO on serum lipids is not fully understood but has been related to inhibition of hepatic triglyceride and apolipoprotein B synthesis (27, 45, 47).

Mild proteinuria (about 10 mg/day) was observed in all DM rats at the initiation of the diet (i.e., 4 weeks after induction of DM) as compared to nondiabetic control rats (2 mg/day). This mild and early proteinuria has also been observed in other studies on experimental DM (48, 49). The proteinuria persisted throughout the 20-week study period in the DM/BT rats, but was significantly reduced in the DM/FO fats. At present we can only speculate on the reasons for the reduced proteinuria in the DM rats on FO diet. It does not appear to relate to urinary flow rates, as these were comparable in both DM/FO and DM/BT rats. Differences in glycosylation of proteins has been implicated in the pathogenesis of proteinuria in DM (50) but this is also unlikely, as both groups had comparable blood sugars and glycosylated hemoglobin levels. Potentially, the decrease in proteinuria may relate to the reduced prostaglandin synthesis in glomeruli of DM/FO rats. Inhibition of prostaglandin synthesis has been shown to decrease proteinuria in a variety of glomerular diseases (51), and can occur without a concomitant fall in GFR (52, 53). For example, intraglomerular prostaglandins and thromboxane can influence glomerular capillary permeability (51–53) or alter intraglomerular hydraulic pressure, factors that can contribute to proteinuria in DM (54). Correction of the hyperlipidemia in the DM/FO rats could represent an additional factor, as control of hyperlipidemia may protect against development of proteinuria and progression of renal disease in some nondiabetic models of glomerular disease (7). Unfortunately, our results do not allow us to discern between these various possibilities nor do they indicate whether this decrease in proteinuria in DM/FO rats may be beneficial in the long run. Our studies were carried out for 20 weeks of DM, a period after which no significant pathological changes were found by light or immunofluorescence microscopy in either group of DM rats, as was also observed by others (55–58). We believe, however, that our initial findings with the fish oil-enriched diets in rats with experimental DM should encourage further long term studies. The decrease in proteinuria and the correction of the hyperlipidemia in the DM/FO rats may eventually prove to be beneficial not only for the development of diabetic glomerulopathy but also for the accelerated atherosclerosis of DM.

REFERENCES

1. Hostetter, T. H. 1985. Diabetic nephropathy. *N. Engl. J. Med.* **312:** 642–644.
2. Mogensen, C. E. 1971. Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes mellitus. *Scand. J. Clin. Lab. Invest.* **28:** 91–100.
3. Hostetter, T. H., J. L. Troy, and B. M. Brenner. 1981. Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int.* **19:** 410–415.
4. Christiansen, J., J. Gammelgaard, M. Frandsen, and H. H. Parving. 1981. Increased kidney size, glomerular filtration role and renal plasma flow in short term insulin-dependent diabetes mellitus. *Diabetologia.* **20:** 451–456.
5. Zatz, R., T. W. Meyer, H. G. Renneke, and B. M. Brenner. 1985. Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc. Natl. Acad. Sci. USA.* **82:** 5963–5967.
6. Esmatjes, E., M. R. Fernandez, I. Halperin, J. Camps, J. Gaya, V. Arroyo, F. Rivera, and D. Figuerola. 1985. Renal hemodynamic abnormalities in patients with short term

insulin-dependent diabetes mellitus: role of renal prostaglandins. *J. Clin. Endocrinol. Metab.* **60:** 1231–1236.

7. Moorhead, J. F., M. K. Chan, and Z. Varghese. 1986. The role of abnormalities of lipid metabolism in the progression of renal disease. *Contemp. Issues Nephrol.* **14:** 133–148.

8. Jensen, P. K., J. S. Christiansen, K. Steven, and H. H. Parving. 1981. Renal function in streptozotocin-diabetic rats. *Diabetologia.* **21:** 409–414.

9. Hostetter, T. H., H. G. Rennke, and B. M. Brenner. 1982. The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am. J. Med.* **72:** 375–380.

10. Zatz, R., and B. M. Brenner. 1986. Diabetic microangiopathy: the hemodynamic view. *Am. J. Med.* **80:** 443–453.

11. Ortola, F. V., B. J. Ballermann, S. Anderson, R. E. Mendez, and B. M. Brenner. 1987. Elevated plasma atrial natriuretic peptide levels in diabetic rats: potential mediator of hyperfiltration. *J. Clin. Invest.* **80:** 670–674.

12. Bank, N., M. A. G. Lahorra, H. S. Aynedjian, and D. Schlondorff. 1988. Vasoregulatory hormones and the hyperfiltration of diabetes. *Am. J. Physiol.* **254:** F202–F209.

13. Gerrard, J. M., M. J. Stuart, G. H. Rao, M. W. Steffes, S. M. Mauer, D. M. Brown, and J. G. White. 1980. Alteration in the balance of prostaglandin and thromboxane synthesis in diabetic rats. *J. Lab. Clin. Med.* **95:** 950–958.

14. Kreisberg, J. I., and P. Y. Patel. 1983. The effects of insulin, glucose and diabetes on prostaglandin production by rat kidney glomeruli and cultured glomerular mesangial cells. *Prostaglandins Leukotrienes Med.* **11:** 431–442.

15. Schambelan, M., S. Blake, J. Sraer, M. Bens, M. P. Nivez, and F. Wahbe. 1985. Increased prostaglandin production by glomeruli isolated from rats with streptozotocin-induced diabetes mellitus. *J. Clin. Invest.* **75:** 404–412.

16. Craven, P. A., M. A. Caines, and F. R. DeRubertis. 1987. Sequential alterations in glomerular prostaglandin and thromboxane synthesis in diabetic rats: relationship to the hyperfiltration of early diabetes. *Metabolism.* **36:** 95–103.

17. Halushka, P. V., R. C. Rogers, C. B. Loadholt, H. Wohltman, R. Mayfield, S. McCoy, and J. A. Colwell. 1981. Increased platelet prostaglandin and thromboxane synthesis in diabetes mellitus. *Horm. Metab. Res.* **11 (Supp. 1):** 7–11.

18. Colwell, J. A., P. D. Wincour, and P. V. Halushka. 1983. Do platelets have anything to do with diabetic microvascular disease? *Diabetes.* **32 (Suppl. 2):** 14–19.

19. Purkerson, M. L., J. H. Joist, J. Yates, A. Valdes, A. Morrison, and S. Klahr. 1985. Inhibition of thromboxane synthesis ameliorates and the progressive kidney disease of rats with subtotal renal ablation. *Proc. Natl. Acad. Sci. USA.* **82:** 193–197.

20. Harrison, H. E., A. H. Reece, and M. Johnson. 1978. Decreased vascular prostacyclin in experimental diabetes. *Life Sci.* **23:** 351–355.

21. Jensen, P. K., K. Steven, H. Blaehr, J. S. Christiansen, and H-H. Parving. 1986. Effects of indomethacin on glomerular hemodynamics in experimental diabetes. *Kidney Int.* **29:** 490–495.

22. Christiansen, J. S., B. Feldt-Rasmussen, and H. H. Parving. 1985. Short-term inhibition of prostaglandin synthesis has no effect on the elevated glomerular filtration rate of early insulin-dependent diabetes. *Diab. Med.* **2:** 17–20.

23. Linne, T., A. Konner, S. Rudberg, and B. Persson. 1988. Prostaglandin synthesis inhibition does not normalize GFR or decrease albumin excretion in moderately hyperfiltering diabetes mellitus. *Kidney Int.* **33:** 199 (Abstract).

24. Bar-on, H., P. S. Roheim, and H. A. Eder. 1976. Serum lipoproteins and apolipoproteins in rats with streptozotocin-induced diabetes. *J. Clin. Invest.* **57:** 714–721.

25. Brownlee, M., H. Vlassara, and A. Cerami. 1984. Nonenzymatic glycosylation in the pathogenesis of diabetic complications. *Ann. Intern. Med.* **101:** 527–537.

26. Dyerberg, J., and H. O. Bang. 1979. Atherogenesis and hemostasis in Eskimos: the role of prostaglandin-3 family. *Hemostasis.* **8:** 227–233.

27. von Schacky, C. 1987. Prophylaxis of atherosclerosis with marine omega-3 fatty acids: a comprehensive strategy. *Ann. Intern. Med.* **107:** 890–899.

28. von Schacky, C., S. Fisher, and P. C. Weber. 1985. Long term effects of dietary omega-3 fatty acids upon plasma and cellular lipids, platelet formation and eicosanoid formation in man. *J. Clin. Invest.* **76:** 1626–1631.

29. Needleman, P., A. Raz, M. S. Minkes, J. A. Ferrendeli, and H. Sprecher. 1979. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. Natl. Acad. Sci.* **76:** 944–948.

30. Spector, A. A., T. L. Kaduce, P. H. Figard, K. C. Norton, J. C. Hoak, and R. L. Czervionke. 1983. Eicosapentaenoic acid and prostacyclin production by cultured human endothelial cells. *J. Lipid Res.* **24:** 1595–1604.

31. Barcelli, U., and V. E. Pollak. 1985. Is there a role for polyunsaturated fatty acids in the prevention of renal disease and renal failure? *Nephron.* **41:** 209–212.

32. Jobin, J., and J. P. Bonjour. 1985. Measurement of glomerular filtration rate in conscious unrestrained rats with inulin infused by implanted osmotic pumps. *Am. J. Physiol.* **248:** F734–F738.

33. Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein using the principle of protein dye-binding. *Anal. Biochem.* **72:** 248–254.

34. Holthofer, H., E. Pettersson, and T. Tornroth. 1987. Diabetes mellitus-associated changes in glomerular glycocompounds. *Histochem. J.* **19:** 351–356.

35. Scharschmidt, L. A., J. G. Douglas, and M. J. Dunn. 1986. Angiotensin II and eicosanoids in the control of glomerular size in the rat and human. *Am. J. Physiol.* **250:** F348–F356.

36. Schlondorff, D., S. Roczniak, J. A. Satriano, and V. W. Folkert. 1980. Prostaglandin synthesis by isolated rat glomeruli: effect of angiotensin II. *Am. J. Physiol.* **239:** F486–F495.

37. Pradelles, P., J. Grassi, and J. Maclouf. 1985. Enzyme immunoassays of eicosanoids using acetylcholine esterase as label: an alternative to radioimmunoassay. *Anal. Chem.* **57:** 1170–1173.

38. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.

39. Barnett, R., L. A. Scharschmidt, and D. O. Schlondorff. 1987. Comparison of glomerular contractile response and mesangial arachidonate metabolism in BB and streptozotocin diabetes. *Diabetes.* **36:** 1468–1475.

40. Scharschmidt, L. A., R. Neuwirth, and N. B. Gibbons. 1989. Eicosapentaenoic and dihomogammalinolenic acid metabolism by cultured mesangial cells. *Am. J. Physiol.* **256:** C101–C108.

41. von Schacky, C., W. Siess, S. Fischer, and P. C. Weber. 1985. A comparative study of eicosapentaenoic acid metabolism by human platelets in vivo and in vitro. *J. Lipid Res.* **26:** 457–464.

42. Fisher, S., and P. C. Weber. 1984. Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. *Nature.* **307:** 165–167.

43. Corey, E. J., S. Chuan, and J. R. Cashman. 1983. Docosa-hexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. *Proc. Natl. Acad. Sci.* **80:** 3581–3584.

44. Philipson, B. E., D. W. Rothrock, W. E. Connor, W. S. Harris, and D. R. Illingworth. 1985. Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oil in patients with hypertriglyceridemia. *N. Engl. J. Med.* **312:** 1210–1216.

45. Simons, L. A., J. B. Hickie, and S. Balasubramaniam. 1985. On the effects of n-3 fatty acids (Max Epa) on plas-ma lipids and lipoproteins in patients with hyperlipidemia. *Atherosclerosis.* **54:** 75–88.

46. Nestel, P. J., W. E. Connor, M. F. Reardon, S. Connor, S. Wong, and R. Boston. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J. Clin. Invest.* **74:** 82–89.

47. Illingworth, D. R., W. S. Harris, and W. E. Connor. 1984. Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. *Arteriosclerosis.* **4:** 270–275.

48. Pennel, J. P., M. M. Millard, and M. H. Ashby. 1981. Pro-teinuria in the acutely diabetic rat and its response to in-sulin treatment. *Diabetologia.* **21:** 54–57.

49. Rasch, R., and C. E. Mogensen. 1980. Urinary excretion of albumin and total protein in normal and streptozotocin diabetic rats. *Acta Endocrinol.* **95:** 376–381.

50. Copeland, K. R., R. W. Yatscoff, J. A. Thlivens, A. Mehta, and B. Penhur. 1987. Non-enzymatic glycation and altered renal structure and function in the diabetic rat. *Kidney Int.* **32:** 664–670.

51. Vriesendorp, R., A. J. Donker, D. de Zeeuw, P. E. de Jong, G. K. ver Hem, and J. R. Brentjens. 1986. Effects of nonsteroidal anti-inflammatory drugs on proteinuria. *Am. J. Med.* **81:** 84–94.

52. Schlondorff, D. 1986. Renal prostaglandin synthesis: sites of production and specific actions of prostaglandins. *Am. J. Med.* **81:** 1–11.

53. Scharschmidt, L. A., M. S. Simonson, and M. J. Dunn. 1986. Gomerular prostaglandins, angiotensin II, and nonsteroidal anti-inflammatory drugs. *Am. J. Med.* **81:** 30–42.

54. Zatz, R., B. R. Dunn, T. W. Meyer, S. Anderson, H. G. Rennke, and B. M. Brenner. 1986. Prevention of diabetic glomerulopathy by pharmacological amelioration of glo-merular capillary hypertension. *J. Clin. Invest.* **77:** 1925–1930.

55. Mauer, S. M., A. F. Michael, A. J. Fish, and D. M. Brown. 1972. Spontaneous immunoglobulin and complement deposition in glomeruli of diabetic rats. *Lab. Invest.* **27:** 488–494.

56. Mauer, S. M., M. W. Steffes, and D. M. Brown. 1981. The kidney in diabetes. *Am. J. Med.* **70:** 603–612.

57. Steffes, M. W., D. M. Brown, and S. M. Mauer. 1978. Dia-betic glomerulopathy following unilateral nephrectomy in the rat. *Diabetes.* **27:** 35–41.

58. Mauer, S. M., M. W. Steffes, E. N. Ellis, D. E. Sutherland, D. M. Brown, and F. C. Goetz. 1984. Structural–functional relationships in diabetic nephropathy. *J. Clin. Invest.* **74:** 1143–1155.