Menhaden Oil Feeding Increases Potential for Renal Free Radical Production in BHE/cdb Rats (43914)

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Abstract. **The effects of feeding 9% beef tallow (BT) or menhaden oil (MO) in a 10% fat-60% sucrose-20% protein diet on renal cortex fatty acid profile, renal lipid peroxide formation potential, and the blood pressure response to a norepinephrine challenge was studied. Male weanling BHE/cdb prediabetic rats were studied after 8 weeks of diet treatment. Half the rats were subjected to a norepinephrine challenge, and their mean arterial blood pressure was determined. Plasma renin and angiotensin II levels were determined in the presence or absence of the challenge. The source of dietary fat had no effect on these measurements. MO fed rats had a greater potential to form lipid free radicals in the kidney than BT fed rats despite the fact that the renal tissue from both groups had an equivalent number of unsaturations on a mole % basis. From these results we conclude that the accelerated renal disease in menhaden oil fed rats is not due to a diet fat effect on blood pressure regulation but might be due to a diet fat effect on free radical production. These free radicals can be cytotoxic and if produced in large amounts could result in a loss of glomerular cells. Whether this occurs and can be reversed by a change in diet was not determined.** [P.S.E.B.M. 1995, Vol 209]

Recent evidence has suggested that the time course for the development of diabetic nephropathy can be influenced by dietary ingredients (1–8). Both dietary fat and protein have been implicated. The effects of dietary unsaturated fatty acids (8) and/or cholesterol (1, 2, 6, 7) and that of arginine (3–5) have been studied. Cholesterol and beef tallow (BT), which contains cholesterol, have been shown to ameliorate the progression of renal disease in diabetic rats (2, 6, 7). A recent study on longevity in BHE/cdb rats fed a 1% corn oil-9% BT or menhaden oil (MO) diet showed that those rats fed BT lived longer and had less renal disease than those fed MO (7). The rats used for this study were from a strain that typically develops non-insulin-dependent diabetes mellitus (NIDDM) at mid-life (9, 10). Rats of this strain fed purified diets develop many of the symptoms of NIDDM (hyperinsulinemia, reduced pancreatic insulin stores, reduced responsiveness to a glucose challenge) at an earlier age than rats fed a stock diet. They also develop renal disease at an earlier age depending on the source of the dietary fat (6, 7).

Highly unsaturated fatty acids as in menhaden oil (MO) accelerate renal disease development (6, 7) possibly through a mechanism mediated by free radical attack of renal cells. Thus, we hypothesized that the diet effect on renal disease progress could be due to a diet effect on renal tissue fatty acid profile and its potential for free radical formation. We found that the fat source did have effects on the renal fatty acid profile and the potential to form free radicals. As the rats in our longevity study (7) developed renal disease, they also developed mild hypertension. Mild hypertension was observed at 400 days of age, whereas renal lesions were apparent as early as 200 days of age. In a follow-up study (6), we examined rats at 250 days of age and found that those who had already developed impaired glucose tolerance also had more severe renal disease.

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Diabetic rats fed MO had hypertrophied kidneys and more severe renal lesions than those fed BT. Diet had no effect on glomerular filtration rate but creatinine clearance rate was greater in the BT rats than in the MO rats. Urea clearance rates were greater in the MO rats than in the BT rats. The MO rats evidenced mild albuminurea and had levels of plasma 6-keto prostaglandin $F_{1\alpha}$ and renal leukotriene B_4 that were greater than the BT rats. These eicosanoids are measurable end products of the prostaglandin I_2 and cyclooxygenase pathways respectively. These pathways are also involved in the regulation of blood pressure through effects on vasoconstriction and relaxation (11). In rats fed a MO diet, the number of glomerular atrial natriuretic hormone receptors were decreased together with an increase in atrial natriuretic hormone receptor affinity (8). Atrial natriuretic hormone plays an important role in blood pressure regulation. So too does nitric oxide (from arginine), endothelin, angiotensin, renin, and several of the eicosanoids (12). Blood pressure can also be influenced by concurrent disease. Renal disease, hypertension, cardiovascular disease, and diabetes mellitus are frequent concurrent pathologies that share a common linkage to dietary fat (12).

Because renal disease can affect blood pressure and vice versa, we hypothesized that the difference due to diet fat in renal disease development in BHE/cdb rats might be due to a diet effect on blood pressure regulation. To test this hypothesis, BHE/cdb rats were fed either a BT or MO diet and their responses to a norepinephrine challenge were assessed. Mean arterial pressure was determined with or without a norepinephrine challenge.

Materials and Methods

Two groups of 24 weanling male BHE/cdb rats were used. One group was fed a 1% corn oil-9% BT diet, while the other was fed a 1% corn oil-9% MO diet. The cholesterol content was 0.018 and 0.945 mg/g in the MO and BT diet, respectively. The composition of the diets is shown in Table I, while the fatty acid profiles of these diets are shown in Table II. The fatty acid profiles were determined by gas chromatography (Perkin-Elmer Model 8500). Separation was accomplished with a Supelco GP 10% SP2330 on a 100/120 chromosorb WAW column; temperature was 200°C, while the injection temperature and detection temperature was 230°C. The flow rate was 20 ml/min. The standards were obtained from NuCheck (Elysian, MN) and covered the range of fatty acid chain length and saturation. As well, a standardized sample of rapeseed oil and a combination of fatty acids normally found in animal fat were used as controls. The diets used were identical to those used previously (6, 7). Extra vitamin E (0.4% all rac dl α tocopherol acetate (Amersham, Arlington Heights, IL), in addition to that provided by the AIN vitamin mix, was provided to assure adequacy of intake by those rats fed the MO diet (13). Because MO quickly oxidizes and can form free radicals, great care was taken to minimize autooxidation. The MO was added to the dry ingredients just before the animals were fed. Feeding commenced just before the onset of the dark period of the lighting cycle, fresh food was provided daily and the MO was stored at −20°C under a layer of nitrogen. The experiment was of 8 weeks duration. The rats were housed individually in hanging wire mesh cages in a room controlled for temperature (21° ± 1°C), humidity (40%–50%) and light (lights on 06:00–18:00). Animal care followed the recommendations of the American Association for Laboratory Animal Care as outlined in the National Institutes of Health (NIH) Publication 88-23, 1985, NIH Guide for the Care and Use of Laboratory Animals. The rats were weighed weekly and the average food intake/100 g body wt determined. Food and water were always available. The fatty acid profiles of the plasma and renal tissue were determined using gas chromatography using the methods outlined above for the diet fatty acids (courtesy of Dr. Ron Etheridge, Joint Nutrition Laboratories, University of Georgia). The plasma was harvested from blood drawn by heart puncture from anesthetized (0.15 mg sodium pentobarbital/100 g body wt) rats using heparin as an anticoagulant. This blood sample was also used for the determination of renin (14, 15) and angiotensin (Radioimmuno assay kit #NEA-026, DuPont NEN Wilmington, DE). Six rats per group were used for this assay. The kidneys were excised, chilled and weighed. A 300-mg sample of renal tissue was used for the determination of free radical production (16) and the remaining tissue was used for the preparation of the renal cortex plasma membranes using the ultracentrifugation techniques of Touster (17).

Six animals from each group were used for the norepinephrine challenge test. The rats were anesthe-

Table I. Composition of the Diets

Ingredient	Diet (%)	
	BT	MO
Casein	10	10
Lactalbumin	10	10
Sucrose	60	60
AIN vitamin mix	1	1
AIN mineral mix	4	4
Fiber (alphacell)	4.6	4.6
Corn oil[a]	1	1
Beef tallow	9	0
Menhaden oil[b]	0	9
α tocopherol	0.4	0.4

[a] Gift of Best Foods, Pointe-Claire, Quebec, Canada.
[b] Gift of Zapata Haynie, Reedsville, VA.

tized with 0.15:1 mg xylazine:ketamine at a dose of 0.1 cc/100 g body wt. Rats were fitted with indwelling femoral arterial catheters (PE-50 tubing, tapered 1 cm at the arterial end) to monitor direct femoral pressure. Analog signals for arterial pressure were derived from pulsatile signals using an Electromedics small volume displacement pressure transducer and a pressure processor amplifier. The MAP was recorded using a Gould Model RS 3200 recorder (Gould Electronics, Cleveland, OH). The instrument was calibrated using a mercury manometer. The mean arterial pressure (MAP) was determined before and immediately after the infusion of 0.003 ng/100 g body wt of norepinephrine. As soon as the MAP peaked the animal was sacrificed by pneumothorax and blood drawn by heart puncture. The kidneys from rats fed the two diets were also excised for renal cortex membrane preparation. The kidneys of two animals were pooled for this preparation to provide an n of six per diet group.

Results

Food intake was not affected by diet until Week 5. From Week 5 to 8, food intake was significantly less ($P < 0.05$) in the MO rats than in the BT rats (Fig. 1), but this difference in intake had no effect on body weight gain over the 8 week period (Fig. 2). Renal weight likewise was not affected by the dietary treatment (data not shown). The fatty acid profiles of the diet, plasma, and renal cortex are shown in Table II. Less than 100% of the total fatty acids extracted from these sources are shown. Omitted are those fatty acids that individually comprised less than 0.3 mole % of the total.

Significantly greater amounts of 16:0 (palmitic acid) 18:0 (stearic acid) and lesser amounts of 18:1 (oleic acid) and 20:4 (arachidonic acid) were observed in the renal cortex membranes of the MO rats compared with the BT rats. In the blood plasma, MO rats had more 12:0 (lauric acid) and 16:0 with less 20:4 than

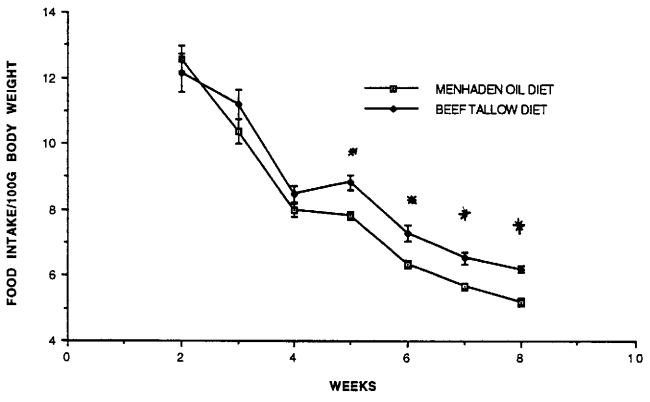

Figure 1. Weekly food intake (g/100 g body wt) for male BHE/cdb rats fed either a 1% corn oil-9% BT or MO diet. Each value is the mean ± SEM, $n = 24$.* Significantly different ($P < 0.05$) values.

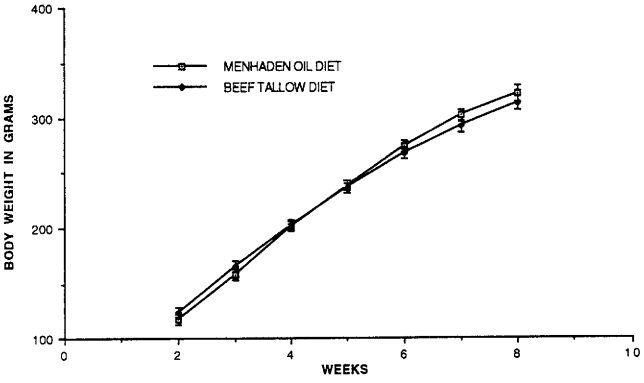

Figure 2. Body weights of male BHE/cdb rats fed either a 1% corn oil-9% BT or MO diet. Each value is the mean ± SEM, $n = 24$.

the BT rats. The MO diet had more 14:0, 16:0, and 16:1 fatty acids and less 18:0, 18:1, and 18:2 fatty acids than the BT diet. In addition, the MO diet had measurable amounts of 18:3, 20:4, and 22:4, while the BT diet had none of these fatty acids.

While the renal fatty acid profiles as shown in Table II indicates that the two groups of rats had a similar number of double bonds in their fatty acids on a mole % basis, the tissues from the MO rats had a greater potential for free radical formation (Table III). The basal amount of free radicals in this tissue was quite low but diet differences were observed. Tissue from MO rats had more free radicals than tissue from BT rats. The amounts of auto-oxidized fatty acids was higher than the basal amounts but not significantly different due to diet. The potential for free radical formation shown as the *in vitro* iron catalyzed free radicals was markedly greater in the MO rats than in the BT rats.

Lastly, Table IV shows the responses of these rats to a norepinephrine challenge test. As expected, the infusion of norepinephrine elicited a rise in blood pressure, a decrease in renin activity level, and an increase in angiotensin I level. There were no effects of diet on these measurements.

Discussion

Renal disease is a common feature of BHE/cdb rats and a common complication of diabetes mellitus in humans. The pathophysiology of this disease process is not fully understood. Raij and Keane (18) have proposed several mechanisms for renal disease development, one of which suggests that hemodynamic forces are important determinants of the disease process. BHE/cdb rats are not spontaneously hypertensive as are the SHR rats, but, because we had previously observed a modest rise at 400 days of age in MAP in the BHE/cdb rats of our longevity study (7), we hypothesized that the difference in renal disease severity due to the diet fat variable might be explained by a differ-

Table II. Fatty Acid Composition of the Diet, Plasma, and Kidney As Affected by Dietary Fat Source

Fatty acid[a] (mole %)	Diet[b]		Plasma		Kidney cortex plasma membrane	
	BT	MO	BT	MO	BT	MO
12:0	—	—	0.4 ± 0.1[c]	2.0 ± 0.8[d]	0.7 ± 0.3	1.3 ± 0.5
14:0	2.3	8.5	1.6 ± 5	2.8 ± 0.5	1.5 ± 0.2	1.7 ± 0.3
16:0	20.7	23.9	23.2 ± 0.4	26.2 ± 1.2[d]	24.3 ± 0.6	30.5 ± 0.4[d]
16:1	2.8	14.9	4.0 ± 1.1	3.7 ± 1.0	1.2 ± 0.3	1.74 ± 0.3
18:0	15.5	6.1	15.2 ± 1.2	16.7 ± 1.5	18.1 ± 1.2	21.7 ± 0.8[d]
18:1	46.5	21.5	38.7 ± 1.8	34.1 ± 2.9	24.4 ± 4	16.9 ± 1.4[d]
18:2	9.1	7.4	6.5 ± 0.6	7.7 ± 0.7	6.8 ± 0.5	7.1 ± 0.4
18:3	—	1.3	—	—	1.5 ± 0.3	1.8 ± 0.2
20:4	—	0.3	10.6 ± 1.0	3.7 ± 0.6[d]	18.4 ± 0.2	10.2 ± 0.6[d]
22:4	—	7.3	—	3.7 ± 0.6	—	5.6 ± 1.0

[a] Fatty acids having less than 0.3 mole% are not shown.
[b] BT = beef tallow; MO = menhaden oil.
[c] Mean ± SEM, $n = 6$.
[d] Diet effect is significant ($P < 0.05$).

ential effect of these fats on factors that affect blood pressure. Hence, we designed our experiment to include measurements of MAP and vasoactive peptides. The source of the diet fat had no effect on these measurements. Hence, we concluded that the diet effects on renal disease development could not be explained by a primary effect of diet fat on factors that regulate blood pressure. This conclusion supports the hypothesis of Anderson and Brenner that, while hypertension may accelerate glomerular pathology, it is not a prerequisite for diabetic renal disease (19). In the BHE/cdb rats of the longevity study changes in blood pressure were observed at a later age than the age at which renal disease was evident. Renal lesions were observed as early as 200 days of age. Thus, it would appear that elevated MAP was a consequence rather than a cause of renal disease in these rats.

In the present work, we tested the hypothesis that the differential effects of MO and BT on renal disease development was due to differences in the potential of these fats to form free radicals that could damage the renal tissue. Our data (Table III) partially support this hypothesis. The MO rats had renal tissue that had a greater potential for free radical formation (Table III) than did the BT rats, despite the fact that diet differences in the number of double bonds on a mole % basis did not exist (Table II). However, the fatty acid profile of a tissue is a static measurement and not revealing with respect to the metabolic movement of the fatty acids. The long chain polyunsaturated fatty acids, while comprising a small percentage of the total fatty acid pool, are more reactive and have greater potential for free radical formation than the monounsaturated acids. These fatty acid free radicals form and leave the fatty acid pool before the membrane fatty acids are deposited. These radicals could cause cellular and tissue damage without affecting cell number in the young rat. This then could set the stage for subsequent disease.

Thomas *et al.* (20) have shown that multiple lipid oxidation products in low-density lipoproteins induce interleukin-1β release from human blood mononuclear cells. The oxidized lipids of the low-density lipoproteins play a role in vascular disease by providing reactive products whose conformation is recognized by the macrophage scavenger receptor (21, 22). Cholesterol serves to downregulate the scavenger receptor. In the absence of this downregulation, lipids accumulate and lead to the formation of macrophage-derived

Table III. Effect of Diet Fat Source on Levels of Free Radicals in Renal Tissue from BHE/cdb Rats

	Diet[a]	
	BT	MO
Basal	0.22 ± 0.03[b]	0.29 ± 0.02[c]
Autooxidized fatty acids	0.92 ± 0.05	0.76 ± 0.15
Iron catalyzed free radicals	0.59 ± 0.04	8.41 ± 1.11[c]

[a] BT = beef tallow; MO = menhaden oil.
[b] Mean ± SEM; $n = 5$.
[c] Diet effect is significant ($P < 0.05$).

Table IV. Effects of Dietary Fat Source on Mean Arterial Pressure and Vasoactive Hormones in Norepinephrine-Stimulated and Control BHE/cdb Rats

	Diet[a]	
	BT	MO
Basal mean arterial pressure (mm Hg)	96 ± 4.6[b]	91 ± 3.7
NE-stimulated mean arterial pressure (mm Hg)	165 ± 14.4[c]	168 ± 7.4[c]
Renin activity (mg/ml/hr)	12.5 ± 0.3	12.2 ± 1.1
Angiotensin I (ng/ml plasma)	0.27 ± 0.01	0.32 ± 0.07

[a] BT = beef tallow; MO = menhaden oil; NE = norepinephrine.
[b] Mean ± SEM; $n = 6$.
[c] Effect of norepinephrine was significant ($P < 0.05$).

foam cells (23). This hypothesized pathway for atherosclerosis may also pertain to the glomerulosclerosis observed in our NIDDM-prone BHE/cdb rats. In another series of experiments (24), we showed that BT rats had greater rates of cholesterol uptake by the kidney than did MO rats at 77 days of age. BT rats had twice as much HDL cholesterol than MO rats and equivalent amounts of LDL. At this age, BT rats had more renal cholesterol and less hepatic cholesterol than did MO rats. As shown in Table II and III the MO rats had a greater number of double bonds in the long chain fatty acids and hence had a greater potential for the formation of lipid derived oxygen centered radicals in the kidney. They also had less cholesterol to protect the membrane lipid. The free radicals could react with renal proteins and provide reactive products recognized by the macrophage scavenger receptor. Because the cholesterol content of the renal tissue was lower in the MO fed rat, this receptor would not be down-regulated and macrophage-derived foam cells could develop to a greater extent. This then could account for the accelerated renal disease development in rats fed MO.

Alternatively, the glomerulosclerosis could result from direct damage of the membrane proteins of the glomerulus by the lipid oxidation products. There is considerable evidence that the vascular endothelium plays a critical role in mediating the pathophysiology of renal disease (24). Hyperfiltration, a characteristic of incipient renal disease could occur if damage to the vessels of the glomerulus has occurred. This damage, possibly by lipid free radicals, would explain our earlier report of deranged electrolyte flux and decreased creatinine clearance in MO rats compared with BT rats (6). Damage to the renal microvasculature is difficult to document, but we have documented in aging BHE/cdb rats a progressive thickening of the mesangial membrane, progressive glomerulosclerosis, and progressive changes in renal function (7). In addition, Stewart *et al.* have reported that free radical damage can occur before measurable amounts of oxidized lipids can be detected (25). In contrast however, Parimandi et al. (26) have reported that diabetic animals have greater free radical suppression activity than do nondiabetic animals. This suggests that, although free radical damage can occur and can explain the progressive nature of the renal disease in NIDDM-prone BHE/cdb rats, these rats also can suppress free radical formation and thus limit the extent of the damage. We have reported that the need for vitamin E by BHE/cdb rats is 10 times greater than that by normal rats (13). Vitamin E is an important dietary antioxidant, and an increased need for this nutrient suggests that the non-vitamin E free radical suppression system is not as active in these rats as in normal rats. Although we have measured the activities of glutathione peroxidase, superoxide dismutase, and catalase in BHE/cdb and control rats and found no strain differences (10), we have not asked whether there could be a genetic difference in these enzymes in rats fed MO or BT. Parimandi *et al.* (26) used streptozotocin-diabetic rats (a model for insulin dependent diabetes) not NIDDM rats, so whether this hypothesis is tenable in our rats will require further study to determine whether age and diet effects on free radical suppression can account for the progressive renal disease in BHE/cdb rats. Indeed, such study should include a cross-over design to determine whether free radical damage due to MO feeding could be reversed by feeding BT.

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