Early Renal Disease in BHE/cdb Rats Is Less in Rats Fed Beef Tallow Than in Rats Fed Menhaden Oil (43587)

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Abstract. **The effects of feeding a 1% corn oil-9% menhaden oil or beef tallow diet on the early phase of diabetic nephropathy in BHE/cdb rats was studied. The diet groups were subdivided into rats with or without impaired glucose tolerance. Those fed menhaden oil had renal hypertrophy, mild albuminuria, decreased creatinine clearance, increased urea clearance, and more severe lesion scores than rats fed beef tallow. No differences in glomerular filtration rate, Na^+,K^+-ATPase activity, sorbitol dehydrogenase, or inositol 1,4,5-phosphate were observed. Beef tallow-fed rats had higher serum triglyceride levels and renal cholesterol levels. Renal and hepatic fatty acid profiles reflected the fatty acid profile of the dietary fat. These results suggest that beef tallow conferred a protective effect on the renal tissues of these diabetes-prone rats.**
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In an earlier communication (1), we reported that BHE/cdb rats fed a 1% corn oil-9% beef tallow diet lived longer and had less renal disease than rats fed an equivalent menhaden oil diet. BHE/cdb rats develop noninsulin-dependent diabetes mellitus (NIDDM) as they age (2). Because of the frequency with which renal disease accompanies NIDDM in humans (3–10) and rats (11), it was not surprising to observe renal disease in the rats used for the longevity study. What was surprising was the differential effects, regarding the time course and severity of lesions, that the different dietary fats had on the renal disease process.

Reyes *et al.* (12) reported that feeding a cholesterol-enriched diet to streptozotocin-diabetic rats protected these rats from developing renal tubule lesions (the Armani-Epstein lesion) typical of severe diabetes. Since beef tallow contains cholesterol, we hypothesized that the above-described diet difference was due to its cholesterol content. However, since the Armani-Epstein lesion is somewhat different from the sclerotic glomerular change we observed earlier, we proposed some additional hypotheses about the nature of the dietary fat effect on the development of renal disease in these NIDDM rats. We hypothesized that the type of dietary fat might differentially affect the Na^+,K^+-ATPase or inositol phosphate cycle or the synthesis of key prostaglandins thought to be involved in the renal disease process. To test these hypotheses, we fed male weanling BHE rats a diet containing 1% corn oil and 9% menhaden oil (MO) or beef tallow (BT) until they were 250 days of age. At this age we assessed renal function in several ways and determined glucose tolerance as well as several other indicators of metabolic activity.

Materials and Methods

Two groups of 30 male weanling BHE/cdb (UGA colony) rats were used. They were individually housed in hanging wire mesh cages in a room controlled for temperature (21 ± 1), humidity (40–50%), and light (lights on from 0600 to 1800 hr). The animals were cared for according to the standards of care put forth by the American Association for Laboratory Animal Care and the appropriate federal agencies. The rats were weighed weekly, and food intakes were calculated so as to maintain consistency of intake between the groups. Diet composition in percentage by weight was as follows: corn oil, 1; beef tallow or menhaden oil (gift of Z. Haynie, Reidsville, VA), 9; casein, 10; lactalbu-

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min, 10; AIN vitamin mix, 1; AIN mineral mix, 4; fiber, 4.96; sucrose, 60; and vitamin E, 0.04. The diet ingredients were purchased from U.S. Biochemicals (Cleveland, OH). The fatty acid composition of the dietary fat was determined using gas chromatographic (Table I) techniques (courtesy of the Department of Food Science). Each day the dry ingredients were mixed with the fat in the amount estimated for the next 24-hr period. The menhaden oil was kept frozen and stored under a layer of nitrogen to prevent auto-oxidation, and mixing took place under a layer of nitrogen. The rats were maintained on these diets until they were 250 days of age.

At 240 days of age, the rats were tested for their glucose tolerance. The rats were starved for 16 hr before the test. Blood samples were collected from the cut tip of the tail before and 30, 60, and 120 min after a glucose challenge (1 g/kg, orally). Blood glucose was determined using glucose oxidase (Sigma kit 510, Sigma Chemical Co., St. Louis, MO).

At 248 days of age, the rats were placed in metabolism cages to collect urine over 24 hr. The rats were allowed food and water *ad libitum*. Total urine volume was determined and frozen in 2-ml aliquots for future analysis. The rats were then subdivided into two groups of 15.

At 250 days of age, two of these subgroups were used to test the glomerular filtration rate (GFR) by a modification of the methods of Michels *et al.* (13). The rats were anesthetized with an ip injection of 0.15 mg/100 g body wt of sodium pentobarbital. The femoral artery, contralateral vein, and ureter were catheterized.

Table I. Fatty Acid Composition of the Diets

Fatty acids[a]	Diet (mole % of total)[b]	
	Menhaden oil	Beef tallow
6,8,10,12:0	0.55	0.53
14:0	7.09	5.68
16:0	12.66	22.93
16:1	9.19	7.12
18:0	3.32	—
18:1	13.01	45.97
18:2	11.93	13.05
18:4	3.59	0.74
20:0	2.57	3.16
20:1	1.68	—
20:5	15.18	—
22:0	1.74	0.31
22:1	2.26	0.14
22:5	3.56	—
22:6	11.25	—
24:0	0.46	—

[a] Fatty acids are indicated by chain length, followed by number of double bonds. This analysis was performed by P. Koehler, Department of Food Science, University of Georgia (Athens, GA).
[b] The diet contained 1% corn oil and either beef tallow or menhaden oil.

The artery was infused with a 1.5 ml of a solution containing 4.0 g of inulin, 900 mg of NaCl, and 100 ml of H_2O. After the priming dose, an infusion of inulin was continued at a rate of 60 μl of solution/min. A 30-min equilibration period was allowed after the priming dose and beginning of the infusion before samples of blood and urine were collected.

After the equilibration period, urine was collected for 20 min into preweighed tubes. At the midpoint of the collection period, a blood sample was obtained via the femoral artery. After centrifugation, a 150-μl aliquot of the plasma was deproteinized with 3.2% trichloroacetic acid and centrifuged, and the supernatant was used to determine the inulin concentration (14).

After 20 min, the preweighed tube containing the urine was collected and weighed a second time to determine the weight of urine produced. Distilled water was then added to the urine in a test tube so that the total volume was 10 ml. The tubes were then vortexed to ensure complete mixing, and a 200-μl aliquot was removed, placed in a 16 × 100-mm test tube that contained 5.8 ml of H_2O, mixed, parafilmed, and used for inulin analysis (14).

The 20-min collection period was repeated three times. At the end of the third period, the rat was opened by a dorsal transverse incision. Blood was collected via heart puncture. The liver and kidneys were excised and weighed. The liver and one kidney were divided into 0.6-g samples and frozen at −20°C. A second kidney was placed into buffered formalin for histological evaluation. The blood was immediately spun for 20 min at 3500 rpm.

The handling of the serum and urine samples immediately after the 20-min intervals resulted in a ratio of 1.0 of the expected urinary inulin concentration to that in plasma. Renal clearance was determined as the amount of material appearing in the urine, considering the amount that the kidney had available in the plasma. The equation used for the calculation was as follows: GFR (ml/min) = $UV \times [Iu]/t \times [Ip]$, where UV is the urine volume (in milliliters), $[Iu]$ is the urinary inulin concentration, t is the time (in minutes), and $[IP]$ is the plasma inulin concentration.

The second group of 30 rats was decapitated at 250 days of age, blood was collected in heparized Vacutainers, and the liver and kidneys were excised. Once weighed, one kidney and a portion of the liver were placed in liquid nitrogen and then stored at −80°C pending analysis of inositol 1,4,5-phosphate (IP_3). The blood was centrifuged for 20 min at 3500 rpm, and the serum was withdrawn and stored in 1-ml aliquots at −20°C for further analysis. The second kidney was divided into two parts. Half of the kidney was placed in 5 ml of ice-cold sucrose solution (0.25 *M* at pH 6–7) and homogenized. The homogenizer was then rinsed with a second tube containing 5 ml of ice-cold sucrose,

and the washes were combined. The second half of the kidney was placed in 5 ml of ice-cold Trizma Base (pH 9) and homogenized. The homogenizer was rinsed with a second tube containing 5 ml Trizma Base (pH 9), and the two washes were combined.

A 0.05-ml aliquot of the sucrose homogenate was used to determine protein concentration by the biuret method. Another aliquot of the sucrose homogenate was diluted with 0.25 *M* ice-cold sucrose to a final concentration of 1 mg/ml of protein and used to determine the activity of the Na^+,K^+-ATPase by the modified methods of Myers and Slater (15). Na^+,K^+-ATPase was determined by subtraction of activity in an incubation medium containing $Ca2^+$ and Mg^{2+} from the activity in an incubation medium containing Na^+, K^+, Ca^{2+}, and Mg^{2+}. The inclusion of ouabain in the same incubation medium allowed for determination of the Na^+,K^+-ATPase activities of the plasma membrane in relation to other ATPase activities contained in the crude homogenate of the whole kidney. The ATPase activity was determined as the inorganic phosphorus released during the incubation, and the activities were expressed as micrograms of phosphorus liberated/mg protein/hr of incubation.

The Trizma base homogenate was centrifuged for 20 min at 3500 rpm. The supernatant was then diluted 10/1 and used for the determination of sorbitol dehydrogenase activity by method of Gerlach and Hiby (16).

Kidney samples, frozen at −80°C, were used for analysis of IP_3 (Amersham RIA kit TRK.790, Arlington Heights, IL) (17, 18). Leukotriene B_4 and 6-keto-prostaglandin F_1 concentrations were determined in matching serum and kidney samples by RIA kits (kits TRK.940 and TRK.790) purchased from Amersham.

The determinations of cholesterol content in the liver and kidney were analyzed by the methods of DeHoff (19), as improved by Cho (20). The lipids were first extracted from the tissue by the methods of Bligh and Dyer (21). The total cholesterol in serum was quantified enzymatically (Sigma kit 352). Sodium and potassium levels in the serum and urine were determined using a flame photometer. The stored urine and serum samples were analyzed for concentrations of albumin by a Biochemica test kit for albumin (Boehringer Mannheim, Indianapolis, IN) and for glucose and creatinine using kits purchased from Sigma (no. 510 and 555). Urea was determined using the methods of Brown (22). The values for creatinine and urea were used to determine clearance rates of both compounds as indicators of kidney dysfunction. The equation used for clearance rate was as follows: Cl (ml/min) = (μmol/liter [urine creatinine])/(μmol/liter [serum creatinine] $\times$ ml/$S \times 173/A$, where S is time in seconds, and A is body surface (12.54 wt $[g]^{0.60}$).

Triglyceride was quantified in the serum based on the method of Eggstein (Sigma kit 405). The fatty acid profiles of kidneys and livers were determined using gas chromatography. This set of determinations was provided by the Joint Nutrition Laboratory of the University of Georgia.

The mean values of each of the above measurements were compared using a two-way analysis of variance (ANOVA; SAS Institute, Cary, NC) for groups of unequal number.

Results

Neither diet nor glucose tolerance influenced food intake or body weight gain (Table II). The diet groups were segregated into two subgroups with respect to their glucose tolerance at 240 days of age. The results of the glucose tolerance test were used to segregate the diet groups into two subgroups: those that had a normal glucose tolerance and those that did not. The group of rats fed the MO diet had a greater number of rats with normal glucose tolerance (12 of 30) than those fed the BT diet (8 of 30). Although MO-fed rats exhibited more tolerance, their blood glucose values 60 min postchallenge were greater than those of rats fed the BT diet in both the normal and diabetic subgroups.

Feeding the MO diet resulted in significantly heavier livers and kidneys compared to those in rats fed the BT diet (Table II). This diet effect was also apparent when organ weight was expressed as a fraction of the total body weight. Although diabetes did not exert a significant effect on organ weights, the diet-diabetes interaction effect was significant. Relative kidney size differences were close to significant ($P < 0.06$) when diabetes was considered the determining factor. These effects on the kidney correlated with renal pathology. The severity of each lesion was subjectively scored from 0 (no significant microscopic lesion) to 5 (severe lesions). Kidneys from the MO-fed rats had an average lesion score of 2.00 for normal rats and 2.18 for diabetic rats compared to averages of 0.43 and 0.74, respectively, for normal and diabetic rats fed BT. The lesions evident in the kidneys were characterized by glomerulosclerosis with an increase in the mesangial matrix, tubular degeneration (foci of degenerative tubular epithelial cells that have large nuclei and appear immature), tubular casts (foci of hyaline casts within tubules), and mineralization (foci of calcium deposition in epithelial or interstitial tissues). Other changes included tubular atrophy and the presence of inflammatory cells. The lesions were similar to those reported previously (1), and the severity of the lesions was the only difference among the different treatment groups. This difference may be important because the age at which the lesions appeared was determined by the type of dietary fat consumed.

Striking differences between the groups were observed in the creatinine clearance rates (Table IV). Plasma creatinine concentrations were significantly af-

Table II. The Effects of Dietary Fat and Diabetes on Weight and Glucose Tolerance of Rats at 250 days of Age

Group	*n*	Initial weight (g)	Food intake (g/100 g body wt)[a]	Final weight (g)	Glucose tolerance (mmol/liter)			
					−1.5 m	30 m	60 m	120 m
MO, normal	12	49 ± 2[b]	4.99 ± .09	518 ± 17	6.40 ± 0.34	10.3 ± 0.51	11.40 ± 0.92	8.90 ± 0.38
MO, diabetic	18	49 ± 2	4.79 ± .07	546 ± 12	6.46 ± 0.20	12.0 ± 1.00	12.60 ± 0.75	11.14 ± 0.73
BT, normal	8	49 ± 3	5.34 ± .12	505 ± 18	6.07 ± 0.24	11.6 ± 0.93	9.38 ± 0.62	7.99 ± 0.39
BT, diabetic	22	48 ± 2	5.32 ± .06	527 ± 8	6.50 ± 0.20	11.5 ± 0.44	11.23 ± 0.44	10.41 ± 0.31
Least squares ANOVA table (*P*-values)								
Main effects								
Diet		NS	NS	NS	NS	NS	<0.05	NS
Diabetes		NS	NS	NS (<0.09)	NS	NS	<0.05	<0.0002
Interaction diet × diabetes		NS	NS	NS	NS	NS	<0.05	NS

NS, No significant difference.
[a] Grams food per 100 g body wt.
[b] Mean ± SE.

fected by the presence of the diabetic state. Diabetes increased plasma and urinary concentrations of creatinine in both diet groups. MO-fed rats excreted twice as much creatinine in urine/ml as those fed beef tallow. Collectively, the clearance rates became compromised in all groups except the BT-fed rats with normal glucose tolerance. The MO-fed rats notwithstanding their diabetic status had severely compromised creatinine clearance rates. These rates correlated well with the lesion scores reported in Table III for each group. Urea clearance was also affected by diet (Table IV). Both the plasma and urinary urea concentrations were higher in the rats fed the MO diet than in rats fed the BT diet. The diabetic state caused a slight reduction in urinary and plasma urea in both diet groups, although the effect of the abnormal glucose tolerance was not significant, nor was there a significant diet-diabetes interaction.

These changes in organ weight and clearance values were observed before any change in the glomerular filtration rate was observed (Table IV). The hypothesis that glomerular filtration rates would be increased was based on previous research which showed that at 250 days of age, BHE rats are just beginning to show evidence of renal disease. MO appears to have delayed the glucose intolerance, as seen in the 18 to 12 ratio of rats with diabetes to those without, but the nephrotic state was still present. At this age, before the complete manifestation of diabetes and before renal disease had progressed to failure, GFR was not significantly affected. Another characteristic of renal disease that has been cited frequently is the appearance of albumin in the urine. Table V gives the values for urinary albumin and glucose concentrations. Diet had a significant effect on the urinary albumin concentration, while it was without effect on the urinary glucose concentration. Rats fed the MO diet excreted more albumin than rats fed the BT diet. Diabetes was without effect on these values. The values for urinary albumin corresponded to the

Table III. The Effects of Dietary Fat and Diabetes on Organ Weight and Pathology

Group	*n*	Liver weight (g)	RLS (g/body wt)	Kidney weight (g)	RKS (g/body wt)	Kidney lesion scores[a]
MO, normal	12	20.58 ± 0.66[b]	3.98 ± 0.08	4.06 ± 0.28	0.83 ± 0.05	2.00 ± 0.45
MO, diabetic	18	21.78 ± 0.77	3.99 ± 0.10	4.28 ± 0.18	0.75 ± 0.30	2.18 ± 0.33
BT, normal	8	17.78 ± 1.08	3.50 ± 0.11	3.49 ± 0.22	0.70 ± 0.04	0.43 ± 0.43
BT, diabetic	22	18.40 ± 0.44	3.49 ± 0.06	3.49 ± 0.22	0.66 ± 0.02	0.74 ± 0.33
Least squares ANOVA table (*P*-values)						
Main effects						
Diet		<0.0001	<0.0001	<0.001	<0.004	<0.004
Diabetes		NS	NS	NS	NS (<0.06)	NS
Interaction diet × diabetes		NS (<0.06)	<0.05	<0.05	<0.05	<0.05

RLS = (Liver weight × 100)/body weight; RKS = (kidney weight × 100)/body weight. NS, No significant difference.
[a] Lesions were graded from 0 (no lesions) to 5 (severe lesions).
[b] Mean ± SE.

Table IV. The Effects of Dietary Fat and Diabetes on Creatinine, Urea, and Glomerular Filtration Rate

Group	*n*	Creatinine			Urea			GFR (ml/min)
		Plasma (mmol/liter)	Urine (mmol/liter)	Clearance (ml/sec)	Plasma (mmol/liter)	Urine (ml/sec)	Clearance (ml/sec)	
MO, normal	12	77.4 ± 5.3[a]	176.8 ± 8.84	0.50 ± 0.01	5.36 ± 0.71	27.4 ± 82.8	30.67 ± 8.4	1.39 ± 0.10 (6)
MO, diabetic	18	110.11 ± 13.26	176.8 ± 8.84	0.50 ± 0.01	5.00 ± 0.36	196.0 ± 57.5	27.38 ± 11.1	1.50 ± 0.12 (8)
BT, normal	8	69.45 ± 7.07	88.4 ± 7.4	1.67 ± 0.06	7.85 ± 1.43	127 ± 42.8	11.48 ± 2.1	1.48 ± .35 (6)
BT, diabetic	22	95.08 ± 8.84	88.4 ± 10.6	0.67 ± 0.01	6.78 ± 1.07	81.04 ± 15.35	12.18 ± 1.4	1.45 ± .02 (8)
Least squares ANOVA table (*P*-values)								
Main effects								
Diet		NS	<0.02	<0.07	<0.04	<0.03	<0.02	NS
Diabetes		<0.02	NS	NS	NS	NS	NS	NS
Interaction diet × diabetes		NS	<0.05	<0.0001	NS	<0.05	NS	NS

NS, No significant difference.
[a] Mean ± SE; *n* in parentheses if altered.

Table V. The Effects of Dietary Fat and Diabetes on Albumin and Glucose Levels in Urine

Group	*n*	Albumin			Glucose	
		Urine (mg/day)	Urine (mmol/day)	Urine (mmol/liter)	Urine (mmol/day)	Urine (mmol/liter)
MO, normal	12	1.0 ± 0.30[a]	0.1 ± 0.0	46 ± 7.0	107 ± 21	13 ± 2
MO, diabetic	18	1.5 ± 0.02	0.1 ± 0.0	59 ± 8.0	107 ± 14	12 ± 1
BT, normal	8	0.1 ± 0.00	0.0 ± 0.0	36 ± 3.2	103 ± 18	14 ± 1
BT, diabetic	22	0.2 ± 0.00	0.0 ± 0.0	40 ± .41	128 ± 12	11 ± 1
Least squares ANOVA table (*P*-values)						
Main effects						
Diet		<0.005	<0.02	<0.02	NS	NS
Diabetes		NS	NS	NS	NS	NS
Interaction diet × diabetes		NS	NS	NS	NS	NS

NS, No significant difference.
[a] Mean ± SE.

lesion scores and creatinine clearance values shown in Tables III and IV.

The amounts of sodium and potassium excreted in the urine were significantly affected by diet, but not by diabetes (Table VI). The rats fed the BT diet excreted almost twice as much sodium as the rats fed the MO diet. Diabetes and the diabetes-diet interaction effects were not significant at the $P < 0.05$ level. Potassium concentrations in plasma and urine were unaffected by diet and/or diabetic state; however, the total potassium excretion (mmol/day) was greater in the rats fed the BT diet than in rats fed the MO diet.

The prostaglandin values for plasma and kidney are presented in Table VII. Levels of leukotriene B4, a product of the cyclooxygenase pathway, were significantly higher in the kidneys of the MO-fed rats than in the BT-fed rats, but were unaffected by diet in the plasma. Conversely, 6-keto-prostaglandin $F_{1\alpha}$, a metabolite of prostaglandin I_2, was greater in the plasma, but not the kidney, of the MO rats than in those of the BT-fed rats. There was no effect of interaction of diet and diabetes on these values. Neither diet nor diabetes had an effect on the concentration of IP_3 values in the kidney. The inositol values were similar in all groups.

Rats fed the MO diet had lower plasma triglyceride levels than those fed beef tallow (Table VIII). Both diet and diabetes significantly affected serum triglyceride levels. The plasma cholesterol values were not different, but the liver and kidney cholesterol concentrations were significantly greater in the BT-fed rats than in the MO-fed rats. The effect of diabetes on these values was not significant; however, there was a significant diet-diabetes interaction effect on liver and plasma cholesterol concentrations.

Kidney Na^+,K^+-ATPase and sorbitol dehydrogenase activities were not affected by either diet or diabetes (data not shown). The activities of these enzymes were within the normal ranges reported by others for these tissues.

Table VI. The Effects of Dietary Fat and Diabetes on Sodium and Potassium

Group	n	Sodium: Plasma (mmol/liter)	Sodium: Urine (mmol/day)	Sodium: Urine (mmol/ml)[a]	Potassium: Plasma (mmol/liter)	Potassium: Urine (mmol/day)	Potassium: Urine (mmol/liter)
MO, normal	12	130 ± 2.2[b]	410 ± 6	40.02 ± 12.61	6.1 ± .26	0.98 ± 0.12	67.00 ± 10.49
MO, diabetic	18	146 ± 4.3	420 ± 5	26.53 ± 4.35	6.1 ± .26	1.10 ± 0.10	71.86 ± 11.76
BT, normal	8	137 ± 4.8	820 ± 20	46.98 ± 8.26	6.4 ± .26	1.69 ± 0.27	114.32 ± 16.37
BT, diabetic	22	139 ± 2.6	710 ± 14	32.62 ± 5.65	5.9 ± .26	1.46 ± 0.21	72.89 ± 11.00
Least squares ANOVA table (*P*-values)							
Main effects							
Diet		NS	<0.01	NS (<0.06)	NS	<0.02	NS
Diabetes		NS	NS	NS	NS	NS	NS
Interaction diet × diabetes		NS	NS (<0.06)	NS (<0.07)	NS	NS	NS (<0.07)

NS, No significant difference.
[a] Volume of urine excreted/24 hr, reported in Table IV.
[b] Mean ± SE.

Table VII. The Effects of Dietary Fat and Diabetes on Prostaglandins and Inositol

Group	6-Keto prostaglandin $F_{1\alpha}$[a]: Plasma (pmol/ml)	6-Keto prostaglandin $F_{1\alpha}$[a]: Kidney (nmol/g)	Leukotriene B4[b]: Plasma (pmol/ml)	Leukotriene B4[b]: Kidney (nmol/g)	Kidney IP_3 (nmol/g)
MO, normal	3.30 ± 0.6 (6)[c]	0.15 ± 0.018 (6)	24.4 ± 2.8 (6)	0.211 ± 0.03 (6)	13.97 ± 3.76 (6)
MO, diabetic	3.65 ± 0.04 (4)	0.143 ± 0.01 (4)	20.1 ± 5.3 (4)	0.178 ± 0.04 (4)	11.26 ± 3.05 (9)
BT, normal	0.54 ± 0.05 (3)	0.055 ± 0.01 (3)	14.9 ± 4.2 (3)	0.075 ± 0.05 (3)	13.44 ± 2.32 (6)
BT, diabetic	1.70 ± 0.5 (8)	0.123 ± 0.07 (8)	21.7 ± 4.6 (8)	0.090 ± 0.01 (8)	13.61 ± 3.70 (9)
Least squares ANOVA table (*P*-values)					
Main effects					
Diet	<0.003	NS	NS	<0.001	NS
Diabetic	NS	NS	NS	NS (<0.06)	NS
Interaction diet × diabetes	NS (<0.01)	NS	NS	NS (<0.07)	NS

NS, No significant difference.
[a] 6-Keto prostaglandin $F_{1\alpha}$ was run on unextracted plasma and homogenate.
[b] Leukotriene B4 was run on extracted plasma and homogenate.
[c] Mean ± SE; *n* in parentheses.

Table VIII. The Effects of Dietary Fat and Diabetes on Triglycerides and Cholesterol

Group	Plasma triglycerides (mmol/liter)	Cholesterol: Plasma (mmol/liter)	Cholesterol: Liver (mmol/mg)	Cholesterol: Kidney (mmol/mg)
MO, normal	1.21 ± 0.10 (12)[a]	5.51 ± 1.71 (12)	20.23 ± 3.41 (11)	3.63 ± 0.72 (6)
MO, diabetic	1.11 ± 0.06 (18)	2.63 ± 0.23 (18)	24.57 ± 3.29 (18)	3.00 ± 0.52 (9)
BT, normal	2.06 ± 0.27 (8)	3.89 ± 0.28 (8)	12.48 ± 2.66 (8)	6.56 ± 0.52 (6)
BT, diabetic	2.16 ± 0.14 (22)	4.44 ± 0.48 (22)	10.86 ± 1.63 (19)	6.98 ± 3.98 (12)
Least squares ANOVA table (*P*-values)				
Main effects				
Diet	<0.0001	NS (<0.07)	<0.0002	<0.05
Diabetes	<0.03	NS	NS	NS
Interaction diet × diabetes	<0.05	<0.04	0.03	NS

NS, No significant difference.
[a] Mean ± SE; *n* in parentheses.

Effects of Diet on Renal and Hepatic Fatty Acid Composition. As with the tissue cholesterol levels, the type of dietary fat significantly altered the fatty acid profiles of both tissues (Tables IX and X). Rats fed the two diets had tissue fatty acids that reflected the dietary fatty acids shown in Table I. The long chain polyunsaturated fatty acids and the ω-3 fatty acids were found in the tissues from rats fed the MO diet, whereas more of the 16:1 and 18:1 fatty acids were found in the tissues of the rats fed the BT diets. Diabetes had only a marginal effect on the tissue fatty acid profiles.

Discussion

The results of the present work confirm and support our earlier report (1) that feeding animals a diet containing 9% MO hastens the development of renal disease in BHE/cdb rats compared to feeding a diet containing 9% BT. Whereas our earlier study reported on the presence of moderate to severe renal lesions in 300-day-old rats, the present work reports on the presence of lesions in 250-day-old rats. This age was selected because our earlier study on longevity showed that at this age, individual rats fed this MO diet died as early as 275 days of age. When killed at 300 days of age, rats fed this diet had disease that already was far advanced. In the present work we wanted to study some of the early tissue changes in the kidney so that we would have a better picture of the disease process and the role of diet therein.

At 250 days of age, glomerular filtration rates were normal, and diet had no effect on this functional measurement of the kidneys. This assured us that we were studying the early phase of the disease process, as described by Osterby and others (4–10, 13). Creatinine clearance was slightly less, and urea clearance was greater in the rats fed the MO diet than in those fed the BT diet. Again, this indicated that we were studying the early phase of the disease. This was supported by the lesion scores and the observation of renal hypertrophy and mild albuminuria. All of these findings are typical of early renal disease and are similar to the signs of early disease reported by Zamlauski-Tucker *et al.* (23) These investigators studied the progressive changes in glomerular function of diabetic BB rats.

The question that arises is how could diet affect the severity of the renal disease in BHE/cdb rats? The report of Reyes *et al.* (12) on the effects of dietary cholesterol on renal tubule lesions and the fact that tallow contains more cholesterol than does MO suggest that it may have been this component of the fat that affected the time course of the renal disease. Indeed, the kidneys of the BT-fed rats contained nearly twice as much cholesterol as the kidneys from the rats fed MO. In contrast, the livers from the BT-fed rats had less cholesterol than the MO-fed rats, and there were no striking differences in plasma cholesterol levels. Cholesterol is an important component of the membrane lipid bilayer. It together with the ratio of saturated

Table IX. The Effects of Dietary Fat and Diabetes on Fatty Acid Composition of Kidney

Fatty acid[a]	MO normal (mole %)	MO diabetic (mole %)	BT normal (mole %)	BT diabetic (mole %)	ANOVA (*P*-values)		
					Diet	Diabetes	Diet × diabetes
12:0 or fewer	0.44 ± 0.18[b]	0.73 ± 0.13	0.45 ± 0.10	0.92 ± 0.24	0.05	0.05	NS
13:0	0.14 ± 0.04	0.22 ± 0.08	0.06 ± 0.03	0.27 ± 0.00	NS	0.08	NS
14:0	3.23 ± 0.31	3.79 ± 0.32	1.42 ± 0.04	1.48 ± 0.11	0.0001	NS	NS
15:0	1.07 ± 0.13	1.08 ± 0.09	0.60 ± 0.05	0.82 ± 0.11	0.002	NS	NS
16:0	28.91 ± 0.46	27.87 ± 0.54	23.78 ± 1.05	23.21 ± 0.08	0.0001	NS	NS
16:1	8.7 ± 0.79	8.80 ± 0.59	5.42 ± 0.84	4.68 ± 0.5	0.0001	NS	NS
17:0	0.59 ± 0.06	0.75 ± 0.05	0.49 ± 0.04	0.45 ± 0.03	0.0003	NS	NS
18:0	13.82 ± 1.26	12.39 ± 1.11	11.20 ± 0.77	13.82 ± 1.02	NS	NS	NS
18:1	24.32 ± 1.30	24.85 ± 1.12	41.14 ± 1.49	34.68 ± 2.35	0.0001	0.09	0.05
18:2	7.59 ± 0.27	7.94 ± 0.25	5.35 ± 0.36	5.86 ± 0.13	0.0001	NS	NS
20:0	0.15 ± 0.11	0.06 ± 0.03	0.07 ± 0.02	0.12 ± 0.04	NS	NS	NS
18:3	0.07 ± 0.02	0.23 ± 0.11	0 ± 0	0 ± 0	—[c]		
20:1	0.32 ± 0.10	0.51 ± 0.06	0.35 ± 0.04	0.27 ± 0.04	NS	0.03	0.006
21:0	0.04 ± 0.02	0.05 ± 0.01	0.01 ± 0	0.01 ± 0	0.006	NS	0.05
20:2	0 ± 0	0 ± 0	0.12 ± 0.05	0.03 ± 0.01	—		
22:0	0.23 ± 0.03	0.22 ± 0.03	0.26 ± 0.08	0.13 ± 0.11	NS	NS	NS
20:5	5.95 ± 0.86	5.45 ± 0.75	7.70 ± 0.82	10.70 ± 1.28	0.05	NS	NS
22:1	0.26 ± 0.04	0.26 ± 0.07	0 ± 0	0 ± 0	—		
20:4	2.72 ± 0.27	2.55 ± 0.27	0.09 ± 0.03	0.09 ± 0.01	0.0001	NS	NS
24:0	1.83 ± 0.24	1.84 ± 0.34	1.23 ± 0.29	2.10 ± 0.19	0.05	NS	NS
22:6	0.34 ± 0.10	0.38 ± 0.12	0.42 ± 0.09	0.68 ± 0.08	0.08	NS	NS

NS, No significant difference. —, When values were 0, statistical comparisons were not made.
[a] Designations used: number of carbons in chain followed by number of double bonds.
[b] Mean ± SE; *n* = 5.

Table X. The Effects of Dietary Fat and Diabetes on Fatty Acid Composition of Liver

Fatty acid[a]	MO normal (mole %)	MO diabetic (mole %)	BT normal (mole %)	BT diabetic (mole %)	ANOVA (*P*-values)		
					Diet	Diabetes	Diet × diabetes
12:0 or fewer	0.05 ± 0.03	0.08 ± .02	0.12 ± 0.05	0.12 ± 0.03	NS	NS	NS
14:0	2.06 ± 0.47	1.54 ± 0.12	1.22 ± 0.11	1.32 ± 0.07	0.05	NS	NS
15:0	0.99 ± 0.29	0.57 ± 0.07	0.43 ± 0.03	0.49 ± 0.03	0.05	NS	NS
16:0	30.31 ± 0.97	29.54 ± 0.25	26.16 ± 0.91	25.74 ± 0.81	0.0001	NS	NS
16:1	11.66 ± 0.87	12.42 ± 0.54	7.46 ± 0.90	8.37 ± 0.68	0.0001	NS	0.05
17:0	0.86 ± 0.08	0.99 ± 0.05	0.65 ± 0.03	0.72 ± 0.03	0.0002	0.06	0.05
18:0	9.78 ± 1.06	8.62 ± 0.74	9.84 ± 1.12	8.60 ± 0.50	NS	NS	NS
18:1	27.82 ± 1.46	30.09 ± 1.44	42.59 ± 0.93	43.48 ± 0.74	0.0001	NS	NS
18:2	7.98 ± 0.73	8.29 ± 0.51	4.66 ± 0.33	4.92 ± 0.10	0.0001	NS	NS
20:0	0.01 ± 0.0	0 ± 0	0.11 ± 0.0	0.01 ± 0	—		
18:3	0.72 ± 0.17	0.50 ± 0.14	0 ± 0	0 ± 0	—		
20:1	0.40 ± 0.0	0 ± 0	0.28 ± 0.01	0.32 ± 0.02	—		
21:0	0.09 ± 0.04	0.08 ± 0.05	0 ± 0	0 ± 0	—		
20:2	0 ± 0	0 ± 0	0.32 ± 0.06	0.28 ± 0.02	—		
22:0	0.33 ± 0.03	0.26 ± 0.05	0.33 ± 0.06	0.36 ± 0.05	NS	NS	NS
20:5	2.28 ± 0.39	2.24 ± 0.40	5.71 ± 0.80	5.08 ± 0.51	0.05	NS	NS
22:1	0.44 ± 0.10	0.53 ± 0.06	0 ± 0	0 ± 0	—		
22:4	4.56 ± 0.50	4.10 ± 0.54	0.04 ± 0.01	0.04 ± 0.01	0.0001	NS	NS
24:0	0.21 ± 0.06	0.20 ± 0.05	0.18 ± 0.02	0.16 ± 0.01	NS	NS	NS

NS, No significant difference. —, When values were 0, statistical comparisons were not made.
[a] Designations used: number of carbons in chain followed by number of double bonds.
[b] Mean ± SE; $n = 5$.

to unsaturated fatty acids determine the relative fluidity of the membrane. In so doing it can influence the permeability or porosity of the membrane and, in turn, may influence renal filtration activity. Table VI shows that the type of dietary fat influenced the total daily urinary excretion of sodium and had a marginal effect on the excretion of potassium. The excretion of both cations was greater in the rats fed the BT diet than in those fed the MO diet. Reyes *et al.* (12) postulated that the increase in cholesterol in the renal tissue might prevent the accumulation of glycogen in the glomerular cell, and this, in turn, would affect urine volume and proteinuria. Although we did not measure glycogen accumulation, we did observe a marked diet difference in the urine volume (Table V). The BT-fed rats excreted less urine/day than the rats fed MO. We also observed a mild albuminuria in the rats fed the MO diet.

Although there is a difference between the two fats with respect to cholesterol content, they differ in other ways as well. If one examines the array of fatty acids provided in these two diets (Table I), one is struck by the presence of long chain polyunsaturated fatty acids in the MO. This difference raises several possibilities. First, the long chain polyunsaturated fatty acids in MO affect prostaglandin synthesis. These localized hormones have a variety of effects relating to blood flow and blood pressure. Knapp and Fitzgerald (24), for example, enriched the diet of 32 hypertensive men and found that the formation of the vasodilatory prostaglandins initially increased, and blood pressure fell. This study did not evaluate renal function. After the initial increase in thromboxane A_2, there was a fall. Prostaglandin E metabolites tended to decrease in the fish oil-fed men. Our data on plasma and kidney 6-keto prostaglandin $F_{1\alpha}$ and leukotriene B_4 levels, shown in Table VII, revealed that the source of dietary fat did not affect the renal levels of 6-keto prostaglandin $F_{1\alpha}$, but did result in higher levels of renal leukotriene B_4 in the rats fed the MO diet compared to those fed the BT diet. Leukotriene B_4 has been shown to elicit leukocyte accumulation and adhesion to the endothelial lining of small vessels (25) and degradation and release of hydrolytic enzymes and free oxygen radicals (26). However, it has also been shown (27) that fish oil feeding attenuates the leukocyte-endothelium interaction through effects on adherence receptors on the cell surfaces of both leukocytes and endothelium. A role for the hormone endothelin has been suggested as a component of the renal disease process (28). As endothelin release has a vasoconstrictor action, any stimulator of endothelin release would increase blood pressure. In turn, it has been reported that $PGF_{2\alpha}$ and PGE_2 rise with rising levels of endothelin (28). We did not measure endothelin release in this experiment; however, the results shown in Table VII for the plasma levels of 6-keto prostaglandin $F_{1\alpha}$ (a metabolite of the PG series) suggests that plasma endothelin levels were probably elevated. There may well have been increased vasoconstriction in the MO-fed rats, and this might have contributed to their renal injury.

Lastly, as mentioned above, free oxygen radicals can cause injury, particularly to highly vulnerable tissue

such as the kidney. Nath and Paller (29) have shown that diets deficient in antioxidants (vitamin E and selenium) can potentiate the injury inflicted on the kidney by intermittent ischemia. Although the diet was supplemented with vitamin E in addition to that provided by the AIN vitamin mix, it is possible that the difference in the incidence and severity of the renal disease in the groups of rats fed the different fats may relate to their increased need for vitamin E, which might not have been met by the diet. Calculation of the unsaturated fatty acid to vitamin E ratio revealed that the amount of vitamin provided exceeded that recommended for normal rats by experts in this field (30, 31). Nonetheless, it is possible that the genetic trait for NIDDM in these rats also dictated a greater than normal need for vitamin E. The same could be said for selenium. Perhaps the diabetic trait increases the rate at which free oxygen radicals form in these rats, and that, in turn, drives up the requirements for nutrients that serve in this capacity. Work is needed to determine whether this occurs.

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