Life Span is Shortened in BHE/cdb Rats Fed a Diet Containing 9% Menhaden Oil and 1% Corn Oil[1]

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ABSTRACT The effects of feeding 2 or 10% fat on glucose tolerance, blood lipids, life span and tissue pathology of male BHE/cdb rats were studied. Six groups of 30 rats were fed from weaning diets containing 1% corn oil plus either 1 or 9% corn oil (CO diets), menhaden oil (MO diets) or beef tallow (BT diets). Animals that became ill and died were necropsied and their tissues were examined histologically. Glucose tolerance and blood lipids were determined at 300 and 600 d of age. At 300, 500, 600 and 700 d of age subsets of rats were killed and heart, aorta, lungs, liver, pancreas and kidneys collected for histological examination. The experiment was terminated at 700 d. Feeding the high level MO or BT diet delayed the development of glucose intolerance and lipemia. Longevity was shorter in the rats fed the high MO diet. There were few differences among the groups of rats fed the 2% fat diets with respect to glucose tolerance and lipemia. Glomerulosclerosis was observed in all rats but was more severe and appeared earlier in rats fed the high MO diet than in those fed the high CO or BT diets. In rats with severe renal lesions, mineralized foci were observed in soft tissue, notably the aorta and heart. The results of this study indicate that the source and amount of the dietary fat can influence age-related tissue changes and longevity. J. Nutr. 122: 1309–1317, 1992.

INDEXING KEY WORDS:

- *aging* • *dietary fat* • *lipemia*
- *BHE/cdb rats* • *glucose tolerance*

Over the last decade considerable interest has been generated by the reports of Bang, Dyerberg and others (1–4) on the possible benefits attributed to the consumption of marine oils. These scientists reported that Greenland Eskimos had much less cardiovascular disease and lower serum lipid concentrations than did Europeans. The typical Eskimo diet includes large quantities of fish, whale, seal, walrus and other sea creatures and is relatively low in carbohydrate. What distinguishes this high protein, high fat diet from the typical diet consumed by Europeans or people in the United States is that most of the protein and fat comes from marine animals. The fat portion of the diet is rich in long-chain (*n*-3) unsaturated fatty acids and, as a result, the ratio of unsaturated to saturated fatty acids is 0.84 compared with 0.24 for the Danish diet (1, 3). Of the total unsaturated fatty acids consumed, 13.1% are (*n*-3) fatty acids. These differences in dietary fat and disease incidence have prompted many nutritionists to examine the possible efficacious effects of (*n*-3) fatty acids on a variety of diseases, including cardiovascular disease, arthritis, hypertension and diabetes mellitus (see ref. 5–9 for reviews).

Lifelong controlled intake studies on the possible efficacious effects of (*n*-3) fatty acid–rich marine oils on the development of noninsulin-dependent diabetes mellitus (NIDDM) are not possible in humans. However, it is possible to conduct controlled studies using a shorter-lived species genetically programmed to develop this disorder. Such was the purpose of the study reported in this paper. The BHE strain of rat was selected because it develops abnormal glucose tolerance as it ages and becomes lipemic, yet is not obese (10, 11). The study was designed to determine whether the type of dietary fat and the amount of dietary fat could affect the age progression of tissue changes, blood lipids and glucose tolerance. The energy intake per 100 g body wt was held constant across the diet groups so that differences in the above measurements could not be attributable to differences in energy intake. Care was also taken to assure freshness of the fat so that peroxidized lipids were not fed. We found that although feeding (*n*-3) fatty acid–rich menhaden oil delayed the onset of abnormal glucose tolerance, rats fed this diet died sooner than rats fed corn oil or beef tallow.

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METHODS AND MATERIALS

Animals and diets. A total of 180 (six groups of 30) male BHE/cdb rats[3] were used. As litters of rats were weaned at 21 d of age, they were distributed across the dietary treatments to ensure a distribution that was not skewed by the presence of full siblings at any one age. Hence the rats did not enter the study all at once, but over a 7-mo period of time. The animals were housed individually in hanging wire-mesh cages in a room controlled for temperature (21 ± 1°C), humidity (40–50%) and light (lights on, 0600–1800 h). The rats were cared for according to the standards for humane care set forth by the American Association for Accreditation of Laboratory Animal Care and by the appropriate government agencies. The Animal Care Unit is supervised by a licensed veterinarian and is regularly inspected by the University Animal Care and Use Committee. All possible consideration was given to the welfare of the animals.

The diets differed in the amount and source of fat. The fat content was either 2 or 10% (of which 1% was always corn oil). (Two other fat levels (4 and 6%) were also used. However, for the sake of brevity the data from the animals fed these diets was not included in this report.) The diets contained menhaden oil (a gift from Zapata Hayne, Reidsville, VA) (the MO diets), frying tallow (94% beef tallow and 6% safflower oil) (the BT diets) or corn oil (a gift from Best Foods) (the CO diets). The fatty acid composition (analysis courtesy of P. Koehler, Department of Food Science, University of Georgia, Athens, GA) and the results of a scan for contaminants (analysis courtesy of the Environmental Protection Agency, Athens, GA) of these fats are shown in **Table 1**. Only very small amounts of two environmental contaminants, 1,1,dichloro-2,2-di(*p*-chlorophenyl) (DDE) and polychlorinated biphenyl 1254 (PCB), were found in menhaden oil; none were found in the frying tallow or corn oil. As the level of fat increased from 2 to 10%, the amount of sucrose in the diet decreased. Sucrose was used because of its ability to hasten NIDDM development in BHE/cdb rats (10, 11). The sucrose content of the diets varied from 68 to 60 g/100 g diet. The other dietary ingredients were as follows (g/100 g diet): casein, 10; lactalbumin, 10; alphacel, 5; AIN-76 mineral mix, 4; AIN vitamin mix, 1; and additional vitamin E (all-*rac*-α-tocopherol acetate), 0.04. Except as noted the diet ingredients were purchased from U.S. Biochemicals (Cleveland, OH). The dry ingredients were mixed in large amounts and kept at 0°C. The fats were stored at −20°C under nitrogen.

Rats were weighed daily and the energy intake fixed such that all rats had the same energy intake per 100 g body wt per day. The food required for the next 24 h was then mixed under a layer of nitrogen and fed to the rats just before the onset of the dark cycle. The timing of feeding took advantage of the nocturnal feeding habit of laboratory rats. An earlier study (12) showed that rats consume nearly 80% of their food in the first 4 h of the dark period in a 12-h light:dark cycle. Uneaten food (if any) was discarded at the end of each 24-h period. These precautions were taken to minimize the peroxidation of the unsaturated fatty acids. Periodically the diets were sampled for extent of peroxidation by reaction with thiobarbituric acid. Using the above precautions with the diets, no peroxidized lipids were found in either freshly mixed diets or diets available to the rats for 6, 12 or 18 h.

TABLE 1

Fatty acid composition of, and contaminants in, the dietary fats

	Dietary fat		
	Corn oil	Beef tallow	Menhaden oil
	g/100 g fatty acids		
Fatty acid[1]			
14:0	trace	5	9
16:0	12.5	24	15
18:0	trace	29	4
16:1	—	6	13
18:1	33	29	14
18:2	51	7	2
18:3	2	—	1
18:4	—	—	7
20:4	—	—	2
22:1	—	—	3
20:5	—	—	20
22:6	—	—	11
Contaminants,[2] μg/g			
PCB 1254	—	—	1.57
DDE	—	—	0.17

[1]Fatty acid analysis courtesy of P. Koehler, Department of Foods Science, University of Georgia, Athens, GA.

[2]Contaminant analysis courtesy of the Environmental Protection Agency, Athens, GA. PCB 1254 = polychlorinated biphenyl 1254; DDE = 1,1,dichloro-2,2-di(*p*-chlorophenyl).

Procedures. Animals that appeared ill were removed from the study, killed by decapitation after anesthesia, and necropsied. Signs of illness included weight loss over a 3-d period, unusual exudates, lethargy, poor appetite, respiratory noises, unbalanced gait, unusual lumps or swellings. A few animals died before these signs were noted; their tissues were also

[3]These rats were from the 36th generation of the BHE/cdb subline of BHE rats housed at the University of Georgia. This is a closed colony of black and white rats bred to develop NIDDM and lipemia without the development of obesity. Approximately 75% of the animals have these traits at 300 d of age. The colony is maintained by a random breeding pattern with avoidance of brother-sister matings.

collected and examined. Because it became apparent that the rats would not live beyond 700 d of age, the experiment was terminated. Thus, rats were killed at 300, 500, 600 and 700 d of age. Liver, kidneys, pancreas, heart, lungs and aorta were excised, placed in phosphate-buffered 10% (v/v) formalin, and processed for histological examination (13). All tissues were coded and examined by the pathologist without knowledge of age, diet or whether the animal died spontaneously or was killed at the specified age. The tissues were ranked to indicate lesion severity, with scores from 1 (no lesions) to 5 (very severe lesions).

At 300 and 600 days of age, subsets of five rats from each diet treatment were starved for 16 h and tested for glucose tolerance. The glucose tolerance test consisted of drawing tail blood before and 30, 60 and 120 min after a glucose challenge (1 g glucose/kg body wt administered as a 250 g/L solution by gavage) and determining glucose in the serum using glucose oxidase (Sigma Kit #510, Sigma Chemical, St. Louis, MO). The same rats were anesthetized with sodium pentobarbitol (12 mg/kg) and killed 5 to 6 d later by pneumothorax. Blood was drawn by heart puncture for the determination of serum triglycerides (Sigma Kit #405) and cholesterol (Sigma Kit #352). Tissues were collected for histological examination as described above and weighed. Composition of the residual carcass (body minus gut contents and harvested tissues) was determined by gravimetric methods. A portion of the liver of the 300-d-old rats was used for the determination of the activities of the mixed-function oxidases; these data were previously reported (14).

Statistical analysis. Data are expressed as means ± SEM. One-way ANOVA coupled with Duncan's multiple range tests were used to identify significant ($P < 0.05$) differences due to fat type. Where two groups of data were compared, a Student's t test was used. Where applicable, the t test for unequal n was used; otherwise, the t test for equal n was used. All statistical analyses used SAS procedures (SAS Institute, Cary, NC).

RESULTS

This abbreviated report includes only observations made in 300- and 600-d-old rats. Rats were examined at 500 and 700 d of age, but the group sizes were too small for meaningful comparisons. (The experiment also used rats fed either 4% or 6% fat diets. For the sake of brevity these data were omitted. The glucose tolerance of the 6% fat–fed groups was reported in ref. 15.) **Figure 1** shows the survival curves of the different groups of rats. Reduction in group size was due in part to the planned sampling to assess the age-related changes in tissue histology and in part to the spontaneous deaths. Note that at 700 d of age only one rat

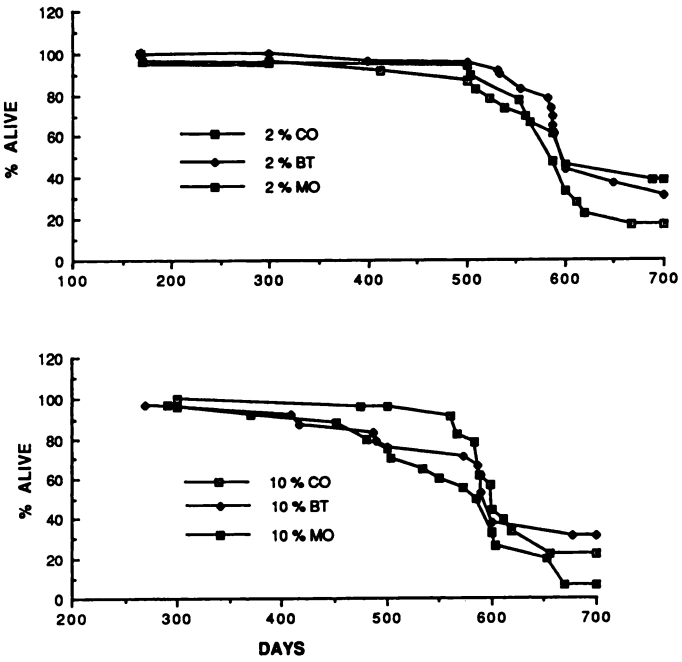

FIGURE 1 Life curves of male BHE/cdb rats fed diets containing 2% (*upper panel*) or 10% (*lower panel*) fat; in each diet 1% of the fat was corn oil and the remainder either more corn oil (CO diet), beef tallow (BT diet) or menhaden oil (MO diet). The percent alive was calculated as the number of rats alive divided by the number of rats expected to be alive at an age.

(6.6%) fed the high MO diet was alive, whereas five of the rats fed the high BT diet and four of those fed the high CO diet were alive. Among the groups fed the 2% fat diets, four of the BT-fed group, three of the CO-fed group and four of the MO-fed group were alive at 700 d of age. The percent survival was calculated as the number of rats alive divided by the number of rats expected to be alive at this age. The denominator changed as rats were removed for the planned tissue sampling.

Body and organ weight. Final body weights, liver weights and kidney weights of rats at 300, 500, 600 and 700 d of age are shown in **Table 2**. Both diet and age affected these values. Although we equalized the energy intake per 100 g of body weight, differences in final body weight were observed. Rats continued to gain weight as they aged until they reached an age at which the disease process dominated. At this point they either stopped gaining weight or lost weight. At each age, dietary fat type was without effect on final body weight. Fat level (2 vs. 10%) affected the body weight of the 700-d-old CO-fed rats (10% fat–fed rats weighed less than the 2% fat–fed rats) and the 500- and 600-d-old rats fed the BT diet (10% fat–fed rats weighed more than the 2% fat–fed rats). No differences due to fat level were observed in the MO-fed rats. In general, liver weight followed the same pattern as did the body weight with respect to age and diet. Kidney weights increased as the animals aged.

TABLE 2

Effect of age and dietary fat on final body weight, liver weight and kidney weight in male BHE/cdb rats[1]

Diet	Age	n	Final body weight	Liver weight	Kidney weight
	d			*g*	
2% Corn oil	300	5	476 ± 30[a]	13.0 ± 1.0[a]	3.23 ± 0.23[a]
	500	1	562	15.9	3.95
	600	4	513 ± 21[a]	14.5 ± 0.5[a]	4.37 ± 0.30[b]
	700	3	607 ± 27[b]	20.0 ± 1.1[b]	5.18 ± 0.33[c]
10% Corn oil	300	5	429 ± 34[a]	11.1 ± 0.9[a]	2.84 ± 0.14[a]
	500	2	560, 592	17.4, 18.2	3.80, 5.90
	600	5	531 ± 40[b]	14.4 ± 1.1[c]	3.96 ± 0.40[b]
	700	4	506 ± 57[bz]	14.4 ± 1.0[c]	6.11 ± 1.75[c]
2% Beef tallow[2]	300	5	485 ± 10[a]	13.1 ± 0.5[a]	3.13 ± 0.02[a]
	500	2	534, 551	17.1, 15.6	4.90, 3.94
	600	5	527 ± 28[b]	14.2 ± 1.3[a]	3.95 ± 0.37[b]
	700	4	522 ± 43[b]	16.0 ± 1.4[b]	4.31 ± 0.62[b]
10% Beef tallow[2]	300	5	465 ± 26[a]	11.6 ± 1.2[a]	2.83 ± 0.21[a]
	500	3	626 ± 67[bz]	16.5 ± 1.4[b]	3.71 ± 0.39[b]
	600	5	605 ± 21[bz]	15.9 ± 0.8[b]	4.36 ± 0.26[bc]
	700	4	567 ± 78[b]	14.6 ± 2.0[b]	5.52 ± 1.38[c]
2% Menhaden oil[2]	300	5	476 ± 20[a]	12.2 ± 0.8[a]	2.98 ± 0.10[a]
	500	3	551 ± 13[b]	14.9 ± 0.7[b]	4.11 ± 0.50[b]
	600	4	539 ± 21[b]	15.9 ± 2.1[b]	5.05 ± 1.18[b]
	700	4	500 ± 20[b]	12.9 ± 0.4[a]	4.56 ± 0.32[b]
10% Menhaden oil[2]	300	5	468 ± 19[a]	13.7 ± 0.9[a]	3.32 ± 0.23[a]
	500	5	602 ± 22[b]	17.5 ± 1.6[b]	4.75 ± 0.47[b]
	600	3	519 ± 14[a]	15.3 ± 0.8[ab]	4.91 ± 0.77[b]
	700	1	566	16.2	8.27

[1]Values are means ± SEM; [abc]means within a diet group not sharing the indicated letter superscripts are significantly different ($P < 0.05$) as determined by one-way ANOVA followed by Duncan's multiple range test; [z]significant ($P < 0.05$) effect of level of fat within age and type of fat as determined by Student's *t* test.

[2]Included 1% corn oil.

The age changes in kidney weight are consistent with the literature on chronic progressive nephrosis in aged (800–1000 d of age) rats (16, 17).

Pathology. As mentioned, renal weight rose as the animals aged and as their renal disease became more severe. The microscopic features of the glomerulonephropathy included hyaline casts, glomerulosclerosis, tubular degeneration and fibrosis. The disease in its earliest stage (score = 2) was characterized by focal hyalin casts and by focal, segmental glomerulosclerosis with small aggregates of mesangial cells in the peripheral glomerular capillary loops. Some of the podocytes (visceral epithelial cells) contained protein droplets. As the disease developed (score = 4) there was a further increase in the aggregation of mesangial cells and a further increase in the frequency of hyalin casts, tubular degeneration and fibrosis. In addition, some adhesion of the glomerulus to the Bowman's capsule was observed. Finally, in the most severe state (score = 5), there was global glomerulosclerosis characterized by a diffuse increase in mesangial cells and matrix and considerable adhesion of the glomerulus to Bowman's capsule. Hyalin casts were numerous as were the tubular changes and fibrosis. These observations are consistent with those reported by others studying spontaneously diabetic rodents (see ref. 18 for review). The mean scores for the renal lesions in those rats killed at 300, 500, 600 and 700 d of age are presented in **Table 3**. Note that in the group fed the high MO diet the mean score increased as the animals aged and that the number of animals decreased. As shown in Figure 1, there was a large loss of animals from this group between 400 and 500 d of age and between 500 and 600 d of age. A total of 88 rats either died spontaneously or were killed when they became moribund; 92 rats were killed at the stated ages. Kidneys harvested from the moribund rats fed the high MO diet usually scored 5 on the five-point scoring system, whereas rats from the other groups that died spontaneously scored around 3 (data not included in Table 3). In addition to the described renal lesions, lesions in the lungs, pancreas, aorta and heart were observed. These lesions included the presence of chronic respiratory

TABLE 3

Effect of diet and age on renal lesions in tissues of male BHE/cdb rats[1]

Dietary fat	Age	Dietary fat level 2%		Dietary fat level 10%	
	d	*mean lesion score*			
Corn oil	300	2.9 ± 0.3	(5)	1.2 ± 0.1	(5)
	500	3.0	(1)	2.5, 4.0	(2)
	600	2.6 ± 0.4	(5)	2.1 ± 0.3	(5)
	700	3.3 ± 0.3	(3)	3.5 ± 0.5	(4)
Beef tallow[2]	300	2.6 ± 0.3	(5)	2.2 ± 0.1	(5)
	500	1.5, 3.5	(2)	2.5 ± 0.5	(3)
	600	2.8 ± 0.5	(7)	2.7 ± 0.4	(5)
	700	2.9 ± 0.5	(8)	3.4 ± 0.8	(5)
Menhaden oil[2]	300	3.1 ± 0.1	(5)	2.5 ± 0.2	(5)
	500	3.2 ± 0.3	(3)	2.6 ± 0.4	(5)
	600	3.0 ± 0.5	(5)	3.2 ± 0.4	(3)
	700	2.3 ± 0.3	(5)	4.0	(1)

[1]Values are means ± SEM, with the number of rats in parentheses. Lesions were graded from 1 (no lesions) to 5 (severe lesions).
[2]Included 1% corn oil.

disease in the lungs. This was characterized by hyperplastic bronchial-associated lymphoid tissue, bronchiectasis and inflammatory cells in the airways. Pancreata were atrophied and inflamed. The exocrine pancreas exhibited multiple foci of interstitial fibrosis in the glandular acini, degeneration of acinar cells, atrophy of acinar tissue, and lymphocytic infiltration. The islets by comparison to the exocrine tissue appeared enlarged. Insulin stores were not assessed. Of those rats that died or were killed when they became moribund, six had malignancies of various tissues, 13 had severe respiratory disease, 27 had mineralized aortas, 18 had mineralization of the myocardium, one had myocarditis, two had epicarditis, two had vascular thrombi in the heart and 43 had severe renal disease. Most of the 43 with renal disease (score 4 or 5) were in the groups fed the MO diets. In addition to the above described lesions, we also observed in the 600- and 700-d-old rats enlarged blackened rear feet that appeared gangrenous. No cataracts or inner ear disease were observed. Although we planned to continue the experiment until all rats died spontaneously or were killed at 900 d of age, the rats at 700 d of age were, for the most part, too ill to continue. Thus, for humane reasons the study was terminated.

Carcass composition. Composition of the residual carcass (body minus gut contents and harvested tissues) is shown in **Table 4**. Only the values for the 300- and 600-d-old rats are presented. Although carcass fat increased with age, in some groups the body weight did not change appreciably. The largest change in body weight and body fat with age occurred in rats fed the high BT diet, whereas the smallest change occurred in rats fed the low MO diet. Rats fed the high MO diet also evidenced a relatively small change in body weight and body fat compared with the rats fed the other diets. Except in the low MO–fed group, as the percentage of carcass fat rose with age, the percentage of ash and protein fell. However, the age effect was not always significant, particularly with respect to the percentage of ash. Again, these findings are consistent with the voluminous literature on the age-related changes in body composition in rats.

Serum lipids. The fasting serum cholesterol, triglyceride and glucose concentrations at 300 and 600 d of age are shown in **Table 5**. In the rats fed the high CO or MO diets, serum cholesterol concentrations rose as the animals aged. In the 300-d-old rats, serum cholesterol values were affected by the dietary fat source in the 10% dietary fat groups. The rats fed the high BT diet had higher cholesterol concentrations at 300 d of age than did the rats fed either high CO or MO diets. Cholesterol concentrations in rats fed the low fat diets did not differ. By 600 d of age, diet was without effect on serum cholesterol concentrations. The triglyceride concentrations fell in MO-fed rats as they aged. The type of fat as well as the level of fat had some effect on serum triglyceride concentrations. Fat level affected only the serum triglyceride levels in the 600-d-old BT-fed rats. Those fed the high BT diet had higher triglyceride concentrations than those fed the low BT diet. Triglycerides in 100-d-old fasted rats ranged from 0.53 to 1.02 mmol/L. These values were well within the expected normal range for rats of this age and were not affected by fat type or amount. In the 300-d-old rats, fat level did not affect the fasting blood glucose concentrations of the rats fed either the CO or BT diets. Those rats fed the high MO diet had lower fasting glucose concentrations than those fed the low MO diet. At 600 d of age, dietary fat level affected only the CO-fed rats. Rats fed the high CO diet had lower fasting glucose levels than those fed the low CO diet. Age affected the fasting glucose concentrations of rats fed the low CO diet and those fed the BT diets. In each instance the older rats had higher fasting blood glucose concentrations than did the younger rats.

Glucose tolerance. Glucose tolerances are shown in **Table 6**. The type and level of fat influenced the degree of intolerance as well as the age at which it appeared. Those rats fed the low and high MO and BT diets did not evidence glucose intolerance (failure to return to prechallenge blood glucose concentrations) at 300 d of age. Rats fed both CO diets were intolerant at this age. The rats fed the high BT diet had a glucose tolerance similar to that of rats fed the high MO diet. By 600 d of age all groups of rats showed abnormal glucose tolerance. At 600 d of age, the glucose tolerances of rats fed the low CO and high MO diets had deteriorated to a greater extent than those of rats fed the other diets.

TABLE 4

Effect of diet on body composition in male BHE/cdb rats at 300 and 600 d of age[1]

Diet	Age	n	Lipid	Ash	Protein	Water
	d		*g/100 g body wt*			
2% Corn oil	300	5	16.7 ± 1.8^{a}	2.8 ± 0.3^{a}	25.5 ± 2.9^{a}	56.6 ± 1.9^{a}
	600	4	25.5 ± 1.1^{xa}	1.6 ± 0.3^{xa}	18.3 ± 0.2^{xa}	54.6 ± 1.5^{a}
10% Corn oil	300	5	19.2 ± 2.5^{ab}	2.9 ± 0.2^{a}	22.6 ± 1.3^{a}	55.8 ± 2.4^{a}
	600	5	26.4 ± 2.1^{xa}	2.4 ± 0.6^{a}	18.1 ± 0.5^{xa}	53.1 ± 2.0^{a}
2% Beef tallow[2]	300	5	18.3 ± 3.3^{ab}	2.7 ± 0.2^{ab}	22.7 ± 2.2^{a}	57.1 ± 2.4^{a}
	600	5	25.7 ± 2.9^{xa}	1.9 ± 0.1^{xa}	18.4 ± 0.6^{xa}	54.0 ± 2.2^{a}
10% Beef tallow[2]	300	5	21.3 ± 1.0^{bc}	2.4 ± 0.4^{a}	21.2 ± 0.9^{a}	55.0 ± 0.6^{a}
	600	5	32.4 ± 2.4^{xbz}	1.8 ± 0.2^{a}	17.0 ± 0.4^{xa}	48.9 ± 2.0^{xza}
2% Menhaden oil[2]	300	5	25.9 ± 2.9^{c}	2.6 ± 0.2^{b}	17.0 ± 2.8^{b}	54.6 ± 2.5^{a}
	600	4	23.4 ± 2.4^{a}	3.1 ± 0.8^{b}	19.8 ± 1.1^{a}	53.7 ± 1.8^{a}
10% Menhaden oil[2]	300	5	18.9 ± 1.0^{az}	3.2 ± 0.2^{bz}	21.7 ± 0.6^{a}	56.4 ± 0.7^{a}
	600	3	23.5 ± 1.3^{a}	2.3 ± 0.4^{xaz}	19.8 ± 0.3^{a}	54.4 ± 0.9^{a}

[1]Values are means ± SEM; ^{x}significant effect of age within diet ($P < 0.05$) as determined by Student's *t* test; ^{ab}significant ($P < 0.05$) effect of fat type within fat level and age as determined by one-way ANOVA followed by Duncan's multiple range test; ^{z}significant ($P < 0.05$) effect of fat level within age group and fat type as determined by Student's *t* test.
[2]Included 1% corn oil.

DISCUSSION

The results of this work are of interest for several reasons: *1*) This is the first time a group of BHE rats of this substrain has been studied over a lifetime with respect to histology, body composition, glucose tolerance and serum lipids. Presented here are measurements and observations on some of these rats fed purified diets from weaning (data from rats fed 4 and 6% fat diets were not included). Data such as these have not been published previously. *2*) This is also the first time that an examination of the long-term (lifelong) effects of feeding different fats, and in par-

TABLE 5

Effect of dietary fat on fasting serum lipid and glucose concentrations in male BHE/cdb rats at 300 and 600 d of age[1]

Diet	n	Age	Percent alive[2]	Cholesterol	Triglycerides	Glucose
		d			*mmol/L*	
2% Corn oil	5	300	100	2.66 ± 1.47^{a2}	3.00 ± 0.85^{a}	5.4 ± 0.5^{a}
10% Corn oil	5	300	100	1.78 ± 0.23^{a}	3.62 ± 0.60^{a}	5.7 ± 0.6^{a}
2% Corn oil	4	600	33	3.36 ± 1.06^{a}	2.22 ± 1.18^{a}	7.6 ± 0.8^{xa}
10% Corn oil	5	600	44.4	4.01 ± 1.45^{xa}	2.09 ± 1.15^{ab}	6.1 ± 0.3^{za}
2% Beef tallow[3]	5	300	100	2.40 ± 0.31^{a}	2.96 ± 1.12^{a}	5.2 ± 0.6^{a}
10% Beef tallow[3]	5	300	95.8	2.30 ± 0.23^{b}	2.92 ± 0.27^{a}	5.1 ± 0.6^{a}
2% Beef tallow[3]	5	600	43.8	2.74 ± 1.27^{a}	1.50 ± 0.60^{a}	6.3 ± 0.5^{xab}
10% Beef tallow[3]	5	600	37.5	2.56 ± 0.02^{a}	2.69 ± 0.58^{za}	6.3 ± 0.1^{xa}
2% Menhaden oil[3]	5	300	94	2.15 ± 0.31^{a}	3.03 ± 0.37^{a}	7.2 ± 0.9^{b}
10% Menhaden oil[3]	5	300	100	1.45 ± 0.21^{a}	3.10 ± 0.47^{a}	5.9 ± 0.4^{za}
2% Menhaden oil[3]	4	600	46.2	3.26 ± 1.42^{a}	1.47 ± 0.45^{xa}	6.1 ± 0.4^{b}
10% Menhaden oil[3]	3	600	33	3.39 ± 0.91^{xa}	1.72 ± 0.01^{xb}	5.9 ± 0.1^{a}

[1]Values are means ± SEM; ^{x}significant effect of age within the same diet group as determined by Student's *t* test; ^{ab}values within the same age group and fat level not sharing an a or b superscript are significantly different as determined by a one-way ANOVA; ^{z}significant effect of fat level within the same diet fat source and age group as determined by *t* test.
[2]Percent alive = number of rats alive/number of rats expected alive at this age.
[3]Included 1% corn oil.

TABLE 6

Effect of dietary fat on glucose tolerance in male BHE/cdb rats at 300 and 600 days of age[1]

Diet	n	Age	Minutes after glucose challenge			
			−15	30	60	120
		d	Serum glucose (mmol/L)			
2% Corn oil	5	300	5.4 ± 0.5[a]	10.9 ± 1.6[a]	10.4 ± 0.7[a]	9.2 ± 1.2[a]
10% Corn oil	5	300	5.7 ± 0.6[a]	10.8 ± 0.7[a]	11.2 ± 1.7[a]	10.7 ± 2.9[a]
2% Corn oil	4	600	7.6 ± 0.8[xa]	20.6 ± 2.9[xa]	21.3 ± 3.7[xb]	18.5 ± 3.4[xa]
10% Corn oil	5	600	6.1 ± 0.3[a]	13.4 ± 1.2[xaz]	15.9 ± 1.8[xa]	14.4 ± 1.3[a]
2% Beef tallow[2]	5	300	5.2 ± 0.6[a]	8.9 ± 0.5[a]	8.8 ± 0.4[a]	6.4 ± 0.3[a]
10% Beef tallow[2]	5	300	5.1 ± 0.6[a]	8.7 ± 0.9[a]	8.6 ± 1.0[a]	6.4 ± 1.4[b]
2% Beef tallow[2]	5	600	6.3 ± 0.5[xab]	10.9 ± 1.2[xb]	12.2 ± 1.2[xb]	14.8 ± 2.2[xab]
10% Beef tallow[2]	5	600	6.3 ± 0.1[xa]	12.2 ± 0.4[a]	13.1 ± 0.8[xa]	13.2 ± 1.4[xa]
2% Menhaden oil[2]	5	300	7.2 ± 0.9[b]	8.8 ± 0.4[a]	8.5 ± 0.3[a]	6.6 ± 0.4[b]
10% Menhaden oil[2]	5	300	5.9 ± 0.4[a]	9.3 ± 0.6[a]	8.1 ± 0.3[a]	6.4 ± 0.4[b]
2% Menhaden oil[2]	4	600	6.1 ± 0.4[b]	11.8 ± 1.2[xb]	12.8 ± 1.6[xb]	13.8 ± 2.2[xb]
10% Menhaden oil[2]	3	600	5.9 ± 0.1[a]	12.1 ± 0.9[xa]	15.5 ± 2.3[xa]	15.6 ± 3.0[xa]

[1]Values are means ± SEM; [x]significant ($P < 0.05$) age effect within diet group as determined by Student's t test; [ab]values within age group and fat level not sharing an a or b superscript are significantly different as determined by one-way ANOVA; [z]significant ($P < 0.05$) fat level effect within the same age and fat source as determined by Student's t test.

[2]Included 1% corn oil.

ticular fish oil, on a rat strain genetically programmed to develop age-related impaired glucose tolerance and lipemia has been reported. To the best of our knowledge no long-term (life-long) feeding studies of this type have been conducted. Many reports of short-term (2–6 wk) feeding experiments exist. Feeding (*n*-3) fatty acid–rich fish oils has been shown to increase the unsaturated fatty acid content of a variety of tissues (21), decrease hepatic fatty acid synthesis (19–22), decrease hepatic triglyceride output (22), increase the efficiency of hepatic mitochondrial oxidative phosphorylation (23, 24) and change a number of other metabolic processes that in turn may affect other aspects of life. The issue in question, however, is whether one can assume that the responses to short-term exposures to marine oils will be similar to those elicited in animals fed these oils over their lifetime. The results of the present work were unexpected. When the experiment was designed, we expected to find an amelioration by menhaden oil of the lipogenic and glycemic traits in the rats selected for use. We observed a delay in the appearance of abnormal glucose tolerance; in this respect, our hypothesis proved correct. That is, feeding (*n*-3) fatty acid–rich menhaden oil in the 10% fat diet affected glucose utilization, such that the 300-d glucose tolerance was normal. By 120 min post challenge, the blood glucose level had returned to its prechallenge level. We also observed this tolerance in the high BT–fed rats. These results were in contrast with those usually found in our stock colony animals.[4] Routinely, every generation of breeding animals is screened for glucose tolerance at 300 d of age; 75% of the rats are usually abnormal. Had we evaluated glucose tolerance at 100-d intervals in the present work, we might have been able to also evaluate the relationship of glucose tolerance to the development of glomerulosclerosis. Studies on this relationship are needed because humans with diabetes are far more likely to develop renal disease, particularly glomerulosclerosis, than humans without diabetes. More than 25% of all patients on dialysis are diabetics. Hence there is a clear need for more research in this area.

Several hypotheses have been advanced to explain the secondary renal complication of diabetes. It is a complication of both insulin-dependent diabetes mellitus and NIDDM. Thus, its development is probably due to one or more derangements in metabolism caused by the diabetic state, rather than due to glucose intolerance per se. High concentrations of blood glucose indicate that glucose synthesis is increased and/or that glucose utilization by peripheral and central tissues is impaired. High concentrations

[4]Animals in the colony are routinely surveyed for their serum triglyceride concentrations and glucose tolerance. The colony is maintained on a commercially available pelleted nonpurified diet (Purina Laboratory Animal Chow, Ralston Purina, St. Louis, MO). At 300 d of age their average triglyceride levels are 1.80 mmol/L and their serum glucose values are 5.2 ± 0.1 (prechallenge), 8.6 ± 0.3, 8.8 ± 0.3 and 8.7 ± 0.3 mmol/L at 30, 60, 120 min post challenge.

of serum triglycerides indicate that fatty acid synthesis and transport are greater than normal. Both these features of diabetes have been implicated in the pathophysiology of diabetic nephropathy (18), which includes a gradual deterioration of the mesangial cells in the glomerulus, similar to what we observed here.

In humans and rats, serum lipid concentrations have been shown to rise with the onset of renal disease (25–29). It has been suggested that their high blood lipid concentrations in obese Zucker rats may be responsible for their glomerular injuries (28, 29). Treatment of the lipemia in the Zucker obese rats, through either energy restriction or drugs, reduced their glomerular injury (29). In the present work, the serum triglyceride concentrations in rats fed the high MO diet generally were not as great as those reported for the Zucker rats, nor as great as those in rats fed the high CO or BT diets. Yet, the MO-fed rats had more renal disease earlier in life, and more MO-fed rats than BT- or CO-fed rats became moribund and were killed or died spontaneously with high renal lesion scores. Thus, a causal relationship of blood lipids to renal injury seems unlikely in the present work.

Goldfarb et al. (30) and others (31–33) have suggested that myoinositol may play a role in the pathophysiology of diabetic nephropathy via its importance in the phosphatidylinositol cycle. Seyer-Hansen (31) reported a reversal of diabetes-induced increased glomerular filtration rate with a seven- to 10-fold increase in dietary levels of myoinositol. Increased glomerular filtration rate is an early indicator of renal disease. Bergh et al. (32) and Kennington et al. (33) reported that inositol turnover is substantially increased in acute diabetes. However, neither inositol status nor glomerular filtration rates were evaluated, so this explanation of accelerated renal disease in rats fed the high MO diet cannot be substantiated.

Nath and Salahuden (34) reported that normal Sprague-Dawley rats fed a vitamin E– and selenium-deficient diet had enlarged kidneys and decreased glomerular filtration rates. They suggested that this dietary deficiency stimulated ammoniagenesis, which in turn induced the increase in renal volume, tubular volume and glomerular volume. Perhaps, in the present work, the MO-fed rats were insufficiently nourished with vitamin E and selenium. This seems unlikely because additional vitamin E was supplied and the most recent AIN formulations of the vitamin and mineral mixes were used. On the other hand, BHE rats may have abnormally high requirements for these nutrients, and the high MO diet may have further raised their requirements. The renal disease observed in this study might have been due to this aspect of their unique genetic heritage. Parinandi et al. (35), using streptozotocin-treated diabetic rats, showed that hearts and kidneys from diabetic rats were more resistant to damage by oxidative stress than tissues from nondiabetic rats. Thus, the hypothesis that a relative dietary deficiency of vitamin E and selenium might have induced an increase in free radicals, which in turn was responsible for the greater incidence of renal disease in the MO-fed rats, does not seem very strong. Nonetheless, it warrants further investigation. Although these rats were shorter lived and died with advanced renal disease, we cannot assume that feeding menhaden oil over the long term caused this disease to develop more rapidly. Regardless of the reasons for the earlier than expected death of these rats, the results of the present work show that the results of lifelong studies with a dietary fat variable can be quite different than what might be expected based on results from short-term studies. This long-term study provided a quite different perspective on the attributes of corn oil, menhaden oil and beef tallow and their effects on health and longevity.

LITERATURE CITED

1. Bang, H. O., Dyerberg, J. & Hjorne, N. (1976) The composition of food consumed by Greenland Eskimos. Acta Med. Scand. 200: 69–73.
2. Bang, H. O. & Dyerberg, J. (1972) Plasma lipids and lipoproteins in Greenlandic West Coast Eskimos. Act Med. Scand. 192: 85–94.
3. Bang, H.O., Dyerberg, J. & Sinclair, H. M. (1980) The composition of the Eskimo food in northwestern Greenland. Am. J. Clin. Nutr. 33: 2657–2661.
4. Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S. & Vane, J. R. (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet 1: 117–119.
5. Kinsella, J. E., Lokesh, B. & Stone, R. A. (1990) Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52: 1–28.
6. Axelrod, L. (1989) Omega-3 fatty acids in diabetes mellitus. Diabetes 38: 539–543.
7. Berry, E. M. & Hirsch, J. (1982) Does dietary linoleic acid influence blood pressure? Am. J. Clin. Nutr. 44: 336–342.
8. Kremer, J. M., Jubiz, W., Michalek, A., Rynes, R. I., Bartholomew, L. E., Bigaouette, J., Timchalk, M., Beeler, D. & Lininger, L. (1987) Fish oil fatty acid supplementation in active rheumatoid arthritis. Ann. Intern. Med. 106: 497–503.
9. Carroll, K. K. (1986) Biological effects of fish oils in relation to chronic diseases. Lipids 21: 731–736.
10. Berdanier, C. D. (1984) Evidence for a membrane error in maturity onset diabetes. In: Lessons from Animals Diabetes (Shafrir, E. & Renold, A. E., eds.), pp. 210–221. John Libby, London, U.K.
11. Berdanier, C. D. (1991) The BHE rat: an animal model for the study of non-insulin-dependent diabetes mellitus. FASEB J. 5: 2139–2144.
12. Berdanier, C. K., Kaul, L. & Odland, D. (1973) Time of food intake and the enzyme activities of the meal-fed and ad libitum-fed rats. Nutr. Rep. Int. 10: 291–297.
13. Noll-Herndon, J. A., Berdanier, C. D. & Crowell, W. A. (1986) Influence of dietary casein and sucrose levels on urea cycle enzyme activities and renal histology in young BHE rats. Nutr. Rep. Int. 34: 403–411.
14. Wade, A. E., Bunce, O. R., Abou-El-Ela, S., Kim, H., Mahdy, M., Barber, J. & Berdanier, C. D. (1989) Mixed function oxidase activities in 300-day-old male BHE rats fed different fats. Biochem. Arch. 5: 201–210.

15. Pan, J-S. & Berdanier, C. D. (1991) Dietary fat saturation affects glucose metabolism without affecting insulin receptor number and affinity in adipocytes from BHE rats. J. Nutr. 121: 1811–1819.
16. Goldstein, R. S., Tarlof, J. B. & Hook, J. B. (1988) Age-related nephropathy in laboratory rats. FASEB J. 2: 2241–2251.
17. Grone, H-J., Walli, A., Grone, E., Nedmann, P., Thiery, J., Seidel, D. & Helmchen, U. (1989) Induction of glomerulosclerosis by dietary lipids. Lab. Invest. 60: 433–446.
18. Velasquez, M. T., Kimmel, P. L. & Michaelis, O. E. (1990) Animal models of spontaneous diabetic kidney disease. FASEB J. 4: 2850–2859.
19. Berdanier, C. D., Johnson, B. J. & Buchanon, M. (1989) Interacting effects of menhaden oil and sucrose on the lipogenic response to starvation-refeeding. Nutr. Res. 9: 1167–1176.
20. Pan, J-S. & Berdanier, C. D. (1990) Thyroxine effects on parameters of glucose turnover in BHE rats fed menhaden oil. J. Nutr. Biochem. 2: 262–266.
21. Huang, Y. S., Nassar, B. A. & Horrobin, D. F. (1986) Changes of plasma lipids and long-chain n-3 and n-6 fatty acids in plasma, liver, heart and kidney phospholipids of rats fed variable levels of fish oil with or without cholesterol supplementation. Biochim. Biophys. Acta 879: 22–27.
22. Herzberg, G. R. & Rogerson, M. (1988) Hepatic fatty acid synthesis and triglyceride secretion in rats fed fructose- or glucose-based diets containing corn oil, tallow, or marine oil. J. Nutr. 118: 1061–1067.
23. Blomstrand, R. & Svensson, L. (1983) Effects of partially hydrogenated marine oils on the mitochondrial function and membrane phospholipids fatty acids in rat heart. Lipids 18: 151–170.
24. Kim, M-J. & Berdanier, C. D. (1989) Influence of menhaden oil on mitochondrial respiration in BHE rats. Proc. Soc. Exp. Biol. Med. 192: 242–247.
25. Bagdade, J. D., Porte, D., Jr. & Bierman, E. L. (1968) Hypertriglyceridemia: a metabolic consequence of chronic renal failure. N. Eng. J. Med. 269: 181–186.
26. Gregg, R., Mondon, C. E., Reaven, E. P. & Reaven, G. M. (1976) Effect of acute uremia on triglyceride kinetics in the rat. Metabolism 25: 1557–1565.
27. Bagdade, J. D., Yee, E., Wilson, D. E. & Shafrir, E. (1978) Hyperlipidemia in renal failure: studies of plasma lipoproteins, hepatic triglyceride production and tissue lipoprotein lipose in a chronically uremic rat model. J. Lab. Clin. Med. 91: 176–186.
28. Shimamura, T. (1982) Relationship of dietary intake to the development of glomerulosclerosis in obese Zucker rats. Exp. Mol. Path. 36: 423–434.
29. Kasiske, B. L., O'Donnell, M. P., Cleary, M. P. & Keane, W. F. (1988) Treatment of hyperlipidemia reduces glomerular injury in obese Zucker rats. Kidney Int. 33: 667–672.
30. Goldfarb, S., Simmons, D. A. & Kern, E.F.O. (1986) Amelioration of glomerular filtration in acute experimental diabetes mellitus by dietary myoinositol supplementation and aldose reductase inhibition. Trans. Assoc. Am. Phys. 99: 67–72.
31. Seyer-Hansen, K. (1983) Renal hypertrophy in experimental diabetes. Kidney Int. 23: 643–646.
32. Bergh, C. H., Hjalmarson, A., Sjogen, K. G. & Jacobson, B. (1988) Effect of diabetes on phosphatidyl inositol turnover and calcium influx in myocardium. Horm. Metab. Res. 20: 381–386.
33. Kennington, A. S., Hill, C. R., Craig, J., Bogardus, C., Raz, I., Ortmeyer, H. K., Hansen, B. C., Romero, G. & Larner, J. (1990) Low urinary chiro inositol excretion in noninsulin-dependent diabetes mellitus. N. Eng. J. Med. 323: 373–378.
34. Nath, K. A. & Salahuden, A. K. (1990) Induction of renal growth and injury in the intact rat kidney by dietary deficiency of antioxidants. J. Clin. Invest. 86: 1179–1192.
35. Parinandi, N. L., Thompson, E. W. & Schmid, H.H.O. (1990) Diabetic heart and kidney exhibit increased resistance to lipid peroxidation. Biochim. Biophys. Acta 1047 63–69.