Dietary Response of Two Genetically Different Lines of Inbred Rats: Lipids in Serum and Liver (34085)

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(Introduced by Mildred Adams)

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Our laboratory strain of rats (BHE) has been shown in previous nutrition studies to differ in their lipid metabolism from Wistar rats fed the same diets under identical conditions (1–4). In the reports cited, BHE rats fed a semipurified diet with 25% cooked, whole egg developed larger livers with more fat and cholesterol, excreted more protein, had higher serum cholesterol, and more total lipid in the serum and liver than Wistar rats fed this diet. Further, BHE rats have demonstrated a sensitivity to type of dietary carbohydrate. Under certain conditions of sucrose-feeding, accumulation of excess fat in blood and carcass occurs especially with certain types of fat in the diet (5, 6). Increased body fat in individual BHE rats was not invariably seen and, it was recognized that some of the variations in lipid metabolism were associated with the spontaneous occurrence of nephrosis in the BHE stock. Therefore, pairs of BHE rats from families with or without kidney damage were selected from the stock colony and inbred by continuing full-sib matings of offspring in successive generations until two distinct inbred lines were produced. A recent report of a part of the study dealt with differences in the inbred lines in kidney defects and the effect of diet on these (7). The present report relates to observations on levels of cholesterol and total lipid in serum and liver of the same rats in the two inbred lines. Nephrotic rats are known to be less economical with protein than are non-nephrotic ones so the synthetic diets, low in fat and cholesterol, provided protein at two levels. A main goal of the study was to separate genetic responses from nutritional responses. There is a need to understand the great variability in food needs of individuals. One of the many ways of studying this problem is to characterize genetically different metabolic pathways in animals which may be involved in governing nutritional requirements. This study is a part of an attempt in this laboratory to understand strain differences in lipid metabolism.

Experimental. A description of the rats used, composition of the diets fed, procedures followed, and statistical treatment of the data reported herein are the same as outlined in the earlier report (7). A few details are repeated to provide necessary information to aid in interpreting present results. A low-fat, low-cholesterol stock diet (Animal Laboratory Foundation Diet, made by Standard Brands, Inc.), or purified diets containing one of two levels of defatted egg or lactalbumin was fed *ad libitum* from weaning until the rats were killed at 300 days of age[2]. The stock diet used in this study should not be considered the control diet for the purified diets fed, but it was fed to all animals in the stock colony. Both sexes of the two lines of rats were divided at weaning, housed individually, and fed one of the three diets as follows: line 1, pelleted stock, 23.72 or 47.43% defatted egg; line 2, pelleted stock, 23.72 or 47.43% lactalbumin. Some litters of line 2 received defatted egg as the source of protein instead of lactalbumin. Other litters of both lines were kept in the stock colony as environmental control groups; both males and females of these litters were fed only the stock diet. In addition to the two levels of

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[2] Data from sick animals were excluded.

protein, all of the purified diets and intakes thereof, previously described (7), contained 43% sucrose and 5% corn oil; cornstarch replaced protein when the lower level was fed. Sucrose was fed at the same level in all purified diets because the BHE rat is "carbohydrate-sensitive" and produces more lipid when fed sucrose-containing diets than when fed corresponding diets containing cornstarch. Adequate vitamins and minerals were supplied.

On the day of autopsy, rats were anesthetized, without previous fasting, with sodium amytal solution. The body cavity was opened and 3 ml of blood removed by heart puncture. All organs were weighed immediately thereafter. The process of removing blood and organs, weighing them, and placing tissues in formalin required not more than 5 min per rat. Livers were kept frozen until analyzed for nitrogen, fat, and cholesterol. Fat was determined by acid hydrolysis (AOAC procedure) on homogenates of the livers. Cholesterol was determined in the liver homogenates by the method of Koval (8) after extraction of the lipid by the method of Folch *et al.* (9); it was determined in the serum by a modification of the Sperry and Webb procedure after precipitation with digitonin.

Analysis of variance was by the method of least squares (10). Models were used which evaluated effects of line, litters in lines, sex, and line × sex interaction (between lines); diet, sex, and the interaction of diet and sex (within lines), further, the variables were adjusted for final body weight. Body weight was taken into account to determine whether the differences observed between the two lines were primarily the result of differences in body size. The analysis took into account differences between litters for some variables. Because of small numbers in some groups of line 2 rats fed defatted egg, separate analyses were done and significance of differences evaluated by the t test. All data in the tables are unadjusted.

Results. Differences between lines. Table I shows that serum cholesterol of line 2 rats was almost double that of line 1 rats and total lipids of line 2 rats were almost three times the levels of line 1 rats. Although there was little difference in average liver size of the two lines fed stock diet, livers of line 2 rats were a significantly larger percentage of their final body weight than those of line 1 rats. Line 1 rats had a slightly higher but significantly different liver fat content, though not considered abnormal, than line 2 rats. Mean percentage of liver nitrogen was

TABLE I. Serum Lipids and Liver Composition of Two Genetically Different Lines of Inbred Rats Fed a Pelleted Stock Diet.

	Serum (mg/100 ml)		Liver, wet basis						
				Wt			Cholesterol		
Source of variation	Cholesterol	Total lipid (pooled)	Wt (g)	Body wt (%)	Fat (%)	N (%)	%	% Liver fat	No. rats
Males									
Line 1	159[a]	666[a]	21.6	4.0	4.82	3.39	0.46	9.4	57
Line 2	297	1555	20.6	4.6	4.08	3.20	0.45	11.1	61
Females									
Line 1	119[a]	571[a]	13.2	4.3	4.63	3.46	0.34	7.4	72
Line 2	226	1960	13.8	5.0	4.10	3.24	0.35	8.5	47
Significance of difference[b]									
Line	.05	—	NS	.01	.01	.01	NS	.01	—
Sex	.01	—	.01	NS	NS	.01	.01	.01	—

[a] One less rat than total number.

[b] NS = not significant; $p <.05$ or $<.01$. Line × sex interaction for all measurements in this table, NS.

significantly lower in line 2 rats than in line 1 rats. Again, the differences though small, were quite consistent. No significant genetic differences were observed in either percentage or total liver cholesterol. But cholesterol as percentage of liver fat was significantly higher in line 2 rats. Differences in body weight could account for differences between lines in liver fat but could not account for any other differences in liver composition.

As expected, male rats of the two lines had larger livers than females with significantly more total fat, nitrogen, and cholesterol. Differences in body weight of the sexes could explain the differences in percentage and total liver cholesterol but could not explain any other differences due to sex.

Differences within lines. Tables II and III show data for males and females fed the stock diet and one of two levels of defatted egg or lactalbumin. Line 1 rats (both male and female) showed no significant differences in serum cholesterol due to type of diet. But a significant sex × diet interaction was observed because females had the highest level when fed 24% defatted egg while males had the highest level when fed 47% defatted egg or the stock diet. All of these levels were in the range usually observed in stock-fed BHE rats at this age. When line 2 rats were fed the same defatted egg diet, serum cholesterol was almost three times that of line 1 rats. Serum lipids of line 2 rats greatly exceeded those of line 1 rats on all diets. Because the differences between lines were larger than the differences due to protein level, the responses were related more to heredity of cholesterol level than to diet. The increase in serum cholesterol of male rats when the higher level of defatted egg was fed, though not significant, as well as larger content of serum lipids of females with increase in defatted egg, indicates an influence of some component of egg other than fat or cholesterol or some interaction of dietary ingredients.

Serum cholesterol of line 2 rats fed lactalbumin was significantly influenced by diet. Rats (both sexes) fed 47% lactalbumin had higher levels than those fed 24%. This finding may be related to the higher degree

TABLE II. Serum and Liver Lipids of Two Genetically Different Lines of Male Rats Fed Different Diets.

	Line 1			Line 2				
Males	Stock	24% DFE	47% DFE	24% DFE	47% DFE	Stock	24% Lact.	47% Lact.
No. rats	21	19	17	5	22	20	19	14
Serum cholesterol, mg/100 ml	172 ± 12[a]	135 ± 7	169 ± 12	294 ± 81	464 ± 50	315 ± 22	257 ± 32	339 ± 28
Total lipid (pooled), mg/100 ml	870	530	670	1020	1760	1780	1700	1790
Liver cholesterol								
% Wet wt	0.57 ± 0.03	0.40 ± 0.02	0.42 ± 0.02[b]	0.34 ± 0.02	0.39 ± 0.01[b]	0.58 ± 0.03	0.61 ± 0.05	0.63 ± 0.04
% Liver fat	10.2 ± 0.3	7.9 ± 0.4	8.8 ± 0.5	8.5 ± 0.5	9.6 ± 0.3	13.2 ± 0.6	11.8 ± 0.9	14.3 ± 0.9

[a] Standard error.

[b] One rat less than total number.

TABLE III. Serum and Liver Lipids of Two Genetically Different Lines of Female Rats Fed Different Diets.

Females	Line 1			Line 2				
	Stock	24% DFE	47% DFE	24% DFE	47% DFE	Stock	24% Lact.	47% Lact.
No. rats	18	12	14	29	5	15	12	10
Serum cholesterol, mg/100 ml	138 ± 14[a]	160 ± 14	148 ± 7	393 ± 44	377 ± 91	263 ± 26	223 ± 26	298 ± 55
Total lipid (pooled), mg/100 ml	780	1010	1090	2830	5190	1960	4140	3300
Liver cholesterol								
% Wet wt	0.35 ± 0.01	0.33 ± 0.01	0.37 ± 0.01	0.41 ± 0.03	0.42 ± 0.04[b]	0.36 ± 0.02	0.47 ± 0.03	0.40 ± 0.02
% Liver fat	6.8 ± 0.3	6.5 ± 0.3	7.0 ± 0.5	7.7 ± 0.3	7.6 ± 0.3	8.2 ± 0.5	6.5 ± 0.5	7.9 ± 0.4

[a] Standard error.
[b] One rat less than total number.

of nephrosis in the line 2 rats fed 47% lactalbumin.

Questions arise about the nature of the association of the high serum cholesterol values and the high incidence of nephrosis in line 2, for it is well known that rapid losses of protein in urine bring about accelerated synthesis also of serum lipoprotein and an altered partition of fat and cholesterol between liver and serum. Line 2 female rats fed stock diet and which did not exhibit gross nephrosis had 194 mg cholesterol per 100 mg serum while those with nephrosis had 273 mg/100 ml. Since the level of 197 mg exceeded the average level of line 1 rats, the difference between the two lines appeared to be related more to heredity of cholesterol level than to nephrosis though the occurrence of nephrosis was associated with a further increase in serum cholesterol level. Genetic correlation of serum cholesterol level with occurrence of nephrosis is under investigation in this laboratory. It should be pointed out also that type of stock diet influences the cholesterol level of rats (unpublished data). The stock diet fed in the present study produced invariably higher cholesterol levels and more severe nephrosis than another tested.[3]

No significant effects of diet were observed in liver weights or composition within either line of rats fed either of the three diets except in liver cholesterol. Within line 1 rats (both sexes) fed 24% defatted egg had a significantly lower level of cholesterol in the liver, both percentage and total, than rats fed 47% defatted egg or the stock diet.

Within line 2, rats fed either level of lactalbumin had a higher percentage of liver cholesterol than those fed the stock diet. Within either line, rats (both sexes) fed 24% protein had significantly less cholesterol in their liver fat than those fed 47% protein or the stock diet. Lactalbumin-fed female rats of both lines had significantly less liver cholesterol than male rats. Significant sex by diet interaction in total liver cholesterol was due to the increase in cholesterol in livers of males fed 47% of either protein.

In view of the established relationship be-

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[3] Purina Laboratory Chow.

TABLE IV. Levels of Probability and Significance of F Values from Analysis of Variance of Unadjusted Data Presented in Tables II and III.

		Liver				Cholesterol	
			Wt				
Source of variation[a]	Serum cholesterol	Wt	Body wt	Fat	N	%	% Liver fat
Line 1							
Sex (1)	NS	.05	NS	NS	NS	.01	.01
Diet (2)	NS	NS	NS	NS	NS	.01	.01
Sex × diet (2)	.01	NS	NS	NS	NS	NS	NS
Residual	(161)	(84)	(84)	(84)	(84)	(161)	(161)
Line 2							
Sex (1)	.01	.05	NS	NS	NS	NS	.01
Diet (2)	.05	NS	NS	NS	NS	.01	.01
Sex × diet (2)	NS	NS	NS	NS	NS	NS	.01
Residual	(140)	(76)	(76)	(76)	(76)	(137)	(137)

[a] Degrees of freedom in parentheses. Litter differences removed; lines analyzed separately.

tween liver size and degree of nephrosis in rats, liver weights of both lines and sexes fed the stock diet were correlated with liver components. As expected, total liver cholesterol and total liver fat increased slightly with liver weight, but all of the values were within the range usually observed for rats of this age. Correlation coefficients for liver weight vs. total liver cholesterol or liver cholesterol vs. liver fat in rats of the various liver weight ranges were about the same for females in both lines. However, the males of the two lines differed; these components were more highly correlated in line 1 males than in line 2 males. There was a significant correlation between total liver cholesterol and serum cholesterol in the individual rats within the groups fed the various diets, but the line 2 male rats which had the highest serum cholesterol had the lowest correlation ($r = .34$).

Discussion. One of the great needs in human nutrition research is to define requirements of nutrients for individuals and to determine why some individuals need different amounts from others. A genetic basis for differences in nutritional requirements was proposed (11) and the concept was supported by animal studies (12). Although heredity of blood cholesterol levels was reported in humans (13–15) and in mice and rats (16, 17) there is a paucity of information on how, or indeed whether, genetically high levels vary in sensitivity to dietary intake. More recently, many inborn errors of lipid metabolism in humans (18–20) were shown to be influenced by diet. Some carbohydrate- and fat-sensitive individuals with hyperlipidemia of various origins are being identified with atherogenesis (21). Rat strains which respond to cholesterol feeding or those which are sensitive to various dietary carbohydrates have been identified including some insensitive ones (22, 23). By studying different rat strains with known genetic characteristics, basic differences in lipid or cholesterol metabolism can be evaluated. From such studies, the types of diets that can best meet the various needs of individuals can be determined.

Rats in the present study with high levels of serum cholesterol had nephrosis in varying degrees but some did not, particularly some of the females of line 2. Within the BHE parent stock of the inbred lines studied herein, a bimodal distribution of cholesterol levels is seen (1). The genetically obese rats of Zucker and Zucker (24) are prone to nephrosis and have high blood lipids. Individual animals of the Sprague-Dawley strain were classified as "hyper- or hypo-responders" according to a rise or lack of it in their serum cholesterol levels on eating a diet high in cholesterol and fat (25).

One question may be raised whether the lower serum cholesterol in line 2 rats fed

24% defatted egg protein was due to the presence of cornstarch in the diet. The hypothesis is rejected, however, because the same diet did not produce a significant reduction in serum cholesterol in line 1 rats. The sparing effect of the lower protein diet on the kidneys of the nephrotic line 2 rats is a more likely explanation.

The high serum lipids of experimentally nephrotic rats have been associated with increased synthesis of low-density lipoproteins (26), but these rats had normal liver cholesterol and fat content (27) although the livers were usually hypertrophied. Nephrotic BHE rats fed 25% whole cooked dried egg had fatty livers with high serum and liver cholesterol (1, 2). Nephrotic line 2 inbred rats in the present study fed defatted whole egg had high serum cholesterol but relatively normal liver fat and cholesterol content. Zucker's obese rats had increased synthesis of liver protein which decreased with cholesterol feeding (23). Inbred line 2 rats herein had a decreased content of liver protein accompanied by high circulating cholesterol. Although it is not yet clear whether there is a cause and effect relationship between the defect in lipid metabolism in these rats and in their susceptibility to nephrosis, the present findings support the concept of a defect in a regulatory mechanism not yet identified which influences the production and channeling of cholesterol between liver and serum.

An interesting finding early in the present study may be pertinent here. Several male rats of line 1 ("normal" cholesterol) exhibited a typical biotin deficiency after receiving the diet containing 47% defatted egg for 4 to 6 weeks even though the diets contained 100 μg biotin/kg diet. The latter amount of biotin had been adequate under all previous dietary conditions. Those rats developing the symptoms were given 4 μg biotin rat/day orally for 2 weeks and all symptoms disappeared thereafter, 400 μg biotin/kg diet were incorporated in all purified diets. After administration of biotin, the rats returned to a normal appearance and growth. It should be emphasized that none of the rats of line 2 (high cholesterol) or the females of either line fed the same defatted egg diet exhibited symptoms of biotin deficiency. A recent study (28) showed an increase in accumulation of cholesterol in carcasses of biotin-deficient rats when compared with biotin-fed controls. Evidence was presented to indicate that the malonyl CoA pathway may not be the major pathway of cholesterol synthesis. Alterations in the lipogenic pattern and in glucose oxidation (29) of biotin-deficient rats are similar to those found in the diabetic state. Our BHE parent stock was developed from a strain of rats once characterized as having a diabetic trait (30) and subject to genetic obesity (31). The question arises whether we are dealing with an animal whose primary defect relates to the functional role of biotin in cholesterol synthesis. Current attempts to answer this question are underway in this laboratory.

Summary. Responses of two inbred lines of rats to a stock diet or to diets (all low-fat, low-cholesterol) containing one of two levels of defatted egg or lactalbumin as the source of protein differed depending upon the genetic characteristics of the rats. One line with serum cholesterol levels in the range usually considered normal for the parent (BHE) stock of the same age showed no significant changes in levels with diet. The other line, with abnormally high levels, had levels above "normal" regardless of diet; yet an additional rise was seen in these rats when the protein was increased from 24% to 47%. The elevated serum cholesterol of this "high" line was accompanied by approximately the same amount of cholesterol in the livers and a smaller concentration of lipid than in the "normal" line. The "high" line was particularly susceptible to nephrosis and/or abnormal lipid metabolism. Although the defective control mechanism is not yet identified, it clearly is related to the partition of cholesterol between serum and liver and the error is heritable.

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