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High and low responding strains of laboratory opossums differ in sterol 27-hydroxylase and acyl-coenzyme A:cholesterol acyltransferase activities on a high cholesterol diet

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Abstract

Two partially inbred strains of laboratory opossums exhibit extremely high or low levels of VLDL+LDL cholesterol concentrations, respectively, when challenged with a high cholesterol and high fat diet. The present studies were conducted to determine whether the high and low responding strains differ in activities of important enzymes that have been shown to affect lipemic responsiveness to diet. We measured plasma 27-hydroxycholesterol and hepatic activities of 27-hydroxylase and 7α-hydroxylase in high and low responding opossums while consuming the basal diet and cholesterol-enriched diets. Plasma 27-hydroxycholesterol concentration and 27-hydroxylase activity in liver did not differ between groups on the basal diet, but both were significantly higher in low responders than in high responders on the cholesterol-enriched diet with unsaturated fat (10.79 $\pm$ 0.56 in low vs. 7.31 $\pm$ 0.50 μg/dl in high responders for 27-hydroxycholesterol and 14.14 $\pm$ 0.79 in low vs. 10.07 $\pm$ 0.80 pmol/mg protein/min in high responders for 27-hydroxylase activity). On the other hand, 7α-hydroxylase activity was significantly higher in high responding opossums (75.72 $\pm$ 6.81 pmol/mg protein/min) than in low responding opossums (51.39 $\pm$ 6.18 pmol/mg protein/min) on the basal diet, but it did not differ on the high cholesterol and high fat diet. We measured hepatic ACAT and extrahepatic hepatic 27-hydroxylase activities in high and low responding opossums on the cholesterol enriched diet. Hepatic ACAT activity was significantly higher in high responding opossums (137.00 $\pm$ 18.33 pmol/mg protein/min) than in low responding opossums (47.67 $\pm$ 2.71 pmol/mg protein/min), whereas extrahepatic 27-hydroxylase activity was higher in low responding opossums (33.00 $\pm$ 2.10 pmol/mg protein/min in lungs and 3.69 $\pm$ 0.20 in kidneys) than in high responding opossums (21.17 $\pm$ 1.54 pmol/mg protein/min in lungs and 2.82 $\pm$ 0.31 in kidneys). We also compared the composition of bile between high and low responders. The concentration of taurine conjugates of cholic acid in bile of both groups was similar, but concentration of taurine conjugates of chenodeoxycholic acid in bile of low responding animals was higher than in high responding animals (124.9 $\pm$ 17.3 in low vs. 59.2 $\pm$ 13.2 μmol/ml in high responders). The results of these studies suggest two enzymes may affect the lipemic response to diet in laboratory opossums: sterol 27-hydroxylase and ACAT. Each of these enzymes may influence diet-induced hyperlipidemia at a different step of lipoprotein metabolism. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Diet-induced hyperlipidemia; Laboratory opossum; *Monodelphis domestica*; Lipoproteins; Sterol 27-hydroxylase; Acyl-coenzyme A:cholesterol acyltransferase; ACAT

1. Introduction

Dietary cholesterol and saturated fatty acids increase plasma cholesterol levels in humans and most experimental animals, but there are wide variations in the responsiveness of plasma lipoproteins among animal species and among the individuals of any one species [1]. This variability in plasma cholesterol response to dietary cholesterol and fat has led to the recognition of high and low responders within many species, including humans. High responding individuals within the same species have a greater increase in plasma LDL and/or HDL cholesterol levels than low responding individuals. The differences in high and low plasma cholesterol responses to dietary lipids may be due largely to a variation in a single gene (exons or regulatory sequences) or due to interactions among a number of genes. Animal models for diet-induced hyperlipidemia have been developed to identify metabolic or genetic markers for high and low

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responsiveness [1]. Such markers may be useful in identifying individual humans who are sensitive to dietary cholesterol and fat. Although no marker for dietary induced hyperlipidemia has been located, it is anticipated that the use of existing models and the development of new models will make such markers available in the future.

Laboratory opossums have quite uniform plasma and lipoprotein cholesterol levels on a low cholesterol and low fat basal diet, but exhibit extreme variability in plasma cholesterol levels when challenged with a high cholesterol and high fat (HCHF) diet [2]. Genetic analyses have indicated that the regulation of VLDL+LDL cholesterol concentration after the dietary challenge is primarily determined by a single gene; this gene is responsible for 80% of the variability in VLDL+LDL cholesterol on the challenge diet [3]. Thus, laboratory opossums are a unique model for identifying genetic markers for responsiveness to dietary lipids. The present studies used two partially inbred strains of laboratory opossums, which have been selectively developed as high and low responding lines, respectively. The intent of the studies was to determine the differences in activities of enzymes involved in lipid metabolism between high and low responding strains of laboratory opossums.

2. Materials and methods

2.1. Experimental animals

The term "laboratory opossum" refers to domestically bred stocks and partially inbred strains of the gray short-tailed opossum (*Monodelphis domestica*), which is a nocturnal marsupial native to Brazil and adjacent countries. They are small (80–120 g), docile animals that breed throughout the year and produce large litters [4,5]. This is the only marsupial species that has been produced in captivity in large numbers. At the Southwest Foundation for Biomedical Research (SFBR), an inbreeding program has led to the development of 18 partially inbred strains with inbreeding coefficients >0.6. Among them are 3 high responding and 3 low responding lines that have been selectively bred specifically for responsiveness to dietary lipids. Two of these stocks have been designated as ATHE (a hyporesponding line) and ATHH (a hyperresponding line), respectively. High and low responding animals from these stocks were used for the present studies. The animals were maintained in polycarbonate rodent cages under laboratory conditions that have been standardized for this species [5].

The protocol of these experiments was approved by the Institutional Animal Care and Use Committee of the SFBR. The SFBR is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International and is registered with the US Department of Agriculture.

Table 1
Fat and sterol contents (% dry weight basis) of diets

Ingredient	Basal diet	HCHF[a]	HCLF[b]
Basal fat[c]	3.0	2.7	3.0
Lard	4.7	14.0	4.6
Corn oil	2.3	2.1	3.2
Cholesterol	0.16	0.71	0.63
Plant sterols	0.05	0.04	0.05

[a] High cholesterol, high fat.
[b] High cholesterol, low fat.
[c] Basal fat is derived mainly from dairy products.

2.2. Experimental diets

The standard laboratory diet, a commercial pelleted fox food, was provided *ad libitum* (Reproduction Diet, Nutritionally Complete Fox Food Pellets, Milk Specialties Co., New Holstein, WI). This diet contains 10% fat by weight (Table 1, basal diet). The cholesterol content of the diet is relatively low (0.16% by dry weight basis). Special diets are prepared from the fox chow by adding 7.5 L tap water to 22.7 kg of pellets, together with fat (liquified) and for some diets, crystalline cholesterol. The ingredients are mixed in a Hobart food mixer until all of the liquids are absorbed. Then the blend is passed through a mist grinder to produce soft pellets, and frozen at –20°C to prevent spoilage and oxidation. Table 1 summarizes the fat and sterol contents of the diets.

2.3. Experimental design

We conducted these studies by using 39 high responding and 41 low responding young adult male and female opossums. Based on our preliminary results, opossums develop a maximal response to a HCHF diet in 4 weeks and the response is due primarily to dietary cholesterol and not fat. Therefore, to measure cholesterol responsive enzymes, we challenged animals with high cholesterol diets with saturated or unsaturated fats for at least 4 weeks. Animals were also challenged with a high cholesterol diet to confirm their phenotype and were fed subsequently a basal diet for 12 weeks to ensure that their hepatic lipid metabolism has returned to basal levels. Some experiments were designed on the basis of the results of previous experiments. In all the experiments, the animals were maintained on a 14:10, light: dark cycle, with the light coming on at 7:00 a.m. The animals were fasted (12 to 14 hr) before blood drawings and necropsies, which took place at 9:00–11:30 a.m.

To determine plasma 27-hydroxycholesterol concentrations and hepatic activities of sterol 27-hydroxylase and cholesterol 7α-hydroxylase in high and low responding opossums on the basal diet, we used 10 high and 10 low responding opossums. To determine the effect of diet-induced hyperlipidemia on plasma 27-hydroxycholesterol concentration and hepatic activities of sterol 27-hydroxylase

and 7α-hydroxylase in high and low responding opossums, we used 15 high and 14 low responding animals. These 29 animals were necropsied after 8 weeks of dietary challenge with a high cholesterol and low fat (HCLF) diet in which cholesterol was dissolved with a small amount of corn oil (Table 1). To determine if sterol 27-hydroxylase activity also differs in extrahepatic tissue we used 6 high and 9 low responding opossums. These 15 animals were necropsied after 4 weeks of dietary challenge of HCHF diet in which cholesterol was dissolved in lard (Table 1). We also measured hepatic ACAT activities in this experiment. To determine if differences in hepatic and extrahepatic sterol 27-hydroxylase activities were associated with differences in bile acid composition we used 8 high and 8 low responding opossums. These animals were necropsied after 8 weeks of dietary challenge with the HCHF diet (Table 1), and gall bladder bile was obtained. We also used livers of 3 of these high and 3 low responding animals to measure hepatic cholesterol concentration.

2.4. Blood sampling

Blood (0.8–1.0 ml) was collected by cardiac puncture of animals anesthetized with methoxyfluorane [6] and placed in a 3 ml vacutainer tube coated with EDTA. Cardiac puncture can be conducted on an animal repeatedly over time without ill effects [7]. Plasma was obtained by centrifugation and cholesterol and 27-hydroxycholesterol levels were determined immediately.

2.5. Tissue collection

For tissue collection, animals were exsanguinated by cardiac puncture under methoxyfluorane anesthesia. The liver, lungs, kidneys, and brain were removed, placed in vials, and frozen in liquid nitrogen. Samples were stored at –80°C prior to their use.

2.6. Plasma and HDL cholesterol analysis

Total plasma cholesterol was measured by enzymatic methods using commercial kits. When the cholesterol level in a sample exceeded the value of the highest calibrator, 358 mg/dl, the sample was diluted with saline to bring it into the range of the calibrators and analyzed again to obtain an accurate measurement. HDL cholesterol was measured after precipitation of VLDL and LDL by heparin-manganese chloride according to the Lipid Research Clinics procedure [8]. The VLDL+LDL cholesterol concentration was calculated as the difference between the total plasma cholesterol and HDL cholesterol concentrations. For samples that were diluted for the total cholesterol assay, the same dilutions were used for the precipitation procedure.

2.7. Measurement of 27-hydroxycholesterol in plasma

27-Hydroxycholesterol was separated and quantified by high performance liquid chromatography (HPLC) as described [9] with some modifications. Briefly, 0.4 ml plasma was incubated in potassium phosphate buffer, pH 7.4, with 2 units of cholesterol esterase (Boehringer Mennheim Corp. Indianapolis, IN) and 2 units of cholesterol oxidase (Cal-Biochem-Nova-Biochem Intl., La Jolla, CA) at 37°C for 20 min. 7β-Hydroxycholesterol (0.2 μg, Research Plus, Bayonne, NJ) was added as an internal standard. The reaction was terminated by adding 1.5 ml methanol followed by 0.5 ml saturated potassium chloride. The mixture was extracted 3 times with 3 ml of hexane. The combined extracts were evaporated to dryness under nitrogen at 37°C and redissolved in 400 μl of hexane. Oxysterols (ketone derivatives) were separated by HPLC (Waters, Millford, MA) using a Spherisorb® silica column (4.6 × 25 cm, 5 μm particle size, Alltech Associate Inc., Deerfield, IL). The mobile phase was isopropanol:hexane (5:95, HPLC grade, Fisher Scientific, Houston, TX). Flow rate was kept at 1 ml/min and the absorbency was monitored by UV detector at 240 nm. The individual peaks were identified by their retention times compared with the known standards. The area ratio method was used to quantify 27-hydroxycholesterol as previously described [10]. In our system, cholesterol (Sigma Chemical Co., St. Louis, MO) eluted approximately at 4 min, 7α-hydroxycholesterol (Research Plus) at 10 min, 27-hydroxycholesterol (Research Plus) at 12 min, and 7β-hydroxycholesterol (Research Plus) at 20 min.

2.8. Measurement of hepatic and extrahepatic mitochondrial sterol 27-hydroxylase and hepatic microsomal 7α-hydroxylase activity

Mitochondria and microsomes were prepared from liver samples stored at –80°C by differential centrifugation [11]. In short, 10% homogenate was prepared with 10 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid], pH 7.4, Sigma Chemical Co.) containing 0.25 M sucrose (Sigma Chemical Co.), 1 mM EDTA (Sigma Chemical Co.), and 0.1 mM PMSF (phenylmethylsulfonyl fluoride; Sigma Chemical Co.). The homogenate was centrifuged at 600 × *g* for 10 min in a refrigerated centrifuge to remove the nuclear fraction and cell debris, and the supernatant was centrifuged again at 8,000 × *g* for 20 min. The pellet of hepatic mitochondria was washed twice at 8,000 × *g* and resuspended in the same buffer. After obtaining mitochondria, the original supernatant was centrifuged at 20,000 × *g* for 10 min and the pellet was discarded. The resulting supernatant was ultracentrifuged at 105,000 × *g* in a Beckman Ti rotor for 1 h. The microsome pellet was washed once and resuspended. The mitochondrial and microsomal final suspension were stored at –80°C. The frozen preparations retained full enzyme activity for 2–3 weeks. Protein concentrations were determinated by the method of

Table 2
Plasma lipoprotein 27-hydroxycholesterol concentration and hepatic activities of sterol 27-hydroxylase and cholesterol 7α-hydroxylase in 10 high and 10 low responding opossums consuming the basal diet

Group	Plasma 27-hydroxy-cholesterol	Hepatic cholesterol	Hepatic sterol 27-hydroxylase	Hepatic cholesterol 7α-hydroxylase
	μg/dl	*mg/liver*	*pmol/mg protein/min*	*pmol/mg protein/min*
Low responding	4.61 ± 0.61[a]	2.59 ± 0.16	8.50 ± 0.85	51.39 ± 6.18[b]
High responding	4.42 ± 0.65	2.89 ± 0.39	8.37 ± 0.78	75.72 ± 6.81

[a] Mean ± SE.
[b] Significantly different from values for high responding opossums ($P < 0.017$).

Bradford (protein assay kit, Bio-Rad, Richmond, CA) using bovine plasma albumin as a standard [12].

For routine assay [9,13], mitochondria or microsomes (0.2 mg protein) were incubated at 37°C for 30 min in a total volume of 1 ml containing 100 mM phosphate buffer, pH 7.4, 1 mM DL-dithiothreitol, 0.2 mM EDTA, 1.2 mM NADPH, 5 mM D,L-isocitric acid, 0.2 units isocitrate dehydrogenase, and 200 nM cholesterol in 2-hydroxypropyl-β-cyclodextrin (Sigma Chemical Co.). The reaction was initiated with the addition of D,L-isocitric acid/NADPH. At the same time, control experiments were conducted in which reaction mixtures were incubated at 4°C for 30 min. The reaction was stopped by adding 50 μl of 40% cholic acid after which 20 μl of 7β-hydroxycholesterol (10 ng/ml methanol) was added as an internal standard. After the addition of 2 units of cholesterol oxidase, the tubes were incubated at 37°C for 20 min. The reaction was terminated with 1.5 ml methanol, followed by 0.5 ml of saturated potassium chloride. The rest of the extraction and HPLC procedure were the same as described for the measurement of 27-hydroxycholesterol in plasma.

2.9. Composition of bile

We compared bile acid composition with bile obtained from these animals maintained on the HCHF diet for another experiment. At the time of necropsy, the bile was drawn from the gall bladder with a syringe. The bile acid composition was determined by HPLC using a reverse-phase column as described by Jackson et al. [14]. Testosterone acetate was used as an internal standard. The concentration of each bile acid conjugate was calculated by linear regression from the standard curves analyzed with the samples.

2.10. Hepatic cholesterol concentration

Liver (100–500 mg) was homogenized and extracted with chloroform-methanol as described by Folch et al. [15]. The chloroform extract was evaporated to dryness and dissolved in 200 μl of isopropanol. The cholesterol concentrations in hepatic lipid extracts were measured by an enzymatic method using a kit (Wako Pure Chemicals USA, Inc., Richmond, VA) and expressed as mg/g liver.

2.11. Data analysis

Values in tables are expressed as mean ± SEM. The values for high and low responding opossums were compared by a standard *t*-test. Associations among the bile acid concentrations and plasma lipoprotein cholesterol concentrations were determined by using Pearson's correlation. Significance was set at $P \leq 0.05$.

3. Results

3.1. Differences in plasma 27-hydroxycholesterol concentration and hepatic activities of sterol 27-hydroxylase and cholesterol 7α-hydroxylase between high and low responding opossums

To determine metabolic differences between the two lines, first we measured plasma 27-hydroxycholesterol concentration and hepatic activities of sterol 27-hydroxylase and 7α-hydroxylase in high and low responding opossums on the basal diet. Plasma 27-hydroxycholesterol concentration and hepatic sterol 27-hydroxylase activities did not differ between high and low responding opossums (Table 2). However, hepatic 7α-hydroxylase activities were higher in high responding opossums than low responding opossums on the basal diet even though the hepatic cholesterol concentration did not differ between the two groups (Table 2).

3.2. Differences in hepatic activities of sterol 27-hydroxylase and 7α-hydroxylase on high cholesterol diet

To determine the effect of diet-induced hyperlipidemia on plasma 27-hydroxycholesterol and hepatic activities of sterol 27-hydroxycholesterol and cholesterol 7α-hydroxylase, we challenged 15 high (ATHH strain) and 14 low (ATHE strain) responding opossums with the HCHF diet (Table 1). Plasma 27-hydroxycholesterol and hepatic enzymes were measured in high and low responding animals after 8 weeks of dietary challenge. As presented in Table 3, plasma 27-hydroxycholesterol concentration and hepatic sterol 27-hydroxylase activities were significantly higher in low responding animals by comparison with those in high responding animals. Hepatic cholesterol 7α-hydroxylase ac-

Table 3
Plasma 27-hydroxycholesterol concentration and hepatic activities of sterol 27-hydroxylase and cholesterol 7α-hydroxylase in 15 high and 14 low responding opossums consuming the HCLF diet for 8 weeks

Group	Plasma 27-hydroxycholesterol	Hepatic sterol 27-hydroxylase	Hepatic cholesterol 7α-hydroxylase
	μg/dl	*pmol/mg protein/min*	*pmol/mg protein/min*
Low responding	10.79 ± 0.56[a,b]	14.14 ± 0.79[c]	112.2 ± 17.0[d]
High responding	7.31 ± 0.50	10.07 ± 0.80	80.2 ± 11.6

[a] Mean ± SE.
[b] Significantly different from values for high responding opossums ($P \leq <0.001$).
[c] Significantly different from values for high responding opossums ($P = 0.001$).
[d] Not significantly different from values for high responding opossums ($P = 0.117$).

tivities were 40% higher in low responding animals than in high responding animals but the difference was statistically not significant.

3.3. Differences in extrahepatic and hepatic activities of cholesterol-responsive enzymes

To determine the effect of dietary cholesterol on extrahepatic activities of sterol 27-hydroxylase in high and low responding opossums, we challenged 6 high and 9 low responding animals with the HCHF diet (Table 1) for 4 weeks. Afterwards, the animals were necropsied, and sterol 27-hydroxylase activities were measured in kidneys and lungs. As presented in Table 4, sterol 27-hydroxylase activities both in kidneys and in lungs of low responders were significantly higher than in high responders.

In this experiment, we also measured ACAT activities in high and low responding opossums challenged with a HCHF diet for 4 weeks. As presented in Table 4, hepatic ACAT activity in high responding opossums was much higher (2.87-fold) than in low responding opossums.

In this experiment we also measured plasma total and VLDL+LDL cholesterol concentrations on the basal diet and on the HCHF diet in high and low responding laboratory opossums. As presented in Table 5, total and VLDL+LDL cholesterol concentrations did not differ between high and low responding opossums. However, total and VLDL+LDL cholesterol concentrations were much higher in high responding opossums than in low responding opossums.

Table 4
Hepatic activities of ACAT and extrahepatic activities of sterol 7α-hydroxylase in 9 high and 6 low responding opossums consuming the HCHF diet for 4 weeks

Group	Lung sterol 27-hydroxylase	Kidney sterol 27-hydroxylase	Hepatic ACAT
		pmol/mg protein/min	
Low responding	33.00 ± 2.10[a,b]	3.69 ± 0.20[c]	47.67 ± 2.71[d]
High responding	21.17 ± 1.54	2.82 ± 0.31	137.00 ± 18.33

[a] Mean ± SEM.
[b] Significantly different from values for high responding opossums ($P = 0.001$).
[c] Significantly different from values for high responding opossums ($P = 0.026$).
[d] Significantly different from values for high responding opossums ($P = 0.004$).

3.4. Differences in bile acid and hepatic cholesterol concentration between high and low responding opossums

In another experiment, 8 high and 8 low responding opossums were necropsied after 8 weeks of consuming the HCHF diet. At the time of necropsy, we collected bile from the gall bladders and measured the bile acid composition. Plasma cholesterol and VLDL+LDL cholesterol concentrations in high responding animals were several fold higher than those in low responding animals (Table 6). The major bile acids of opossum bile were taurine conjugates of cholic acid (TC), chenodeoxycholic acid (TCDC), and deoxycholic acid (TDC). As presented in Table 6, total bile acid concentration was significantly higher in bile of low responding animals than in bile of high responding animals. There was no significant difference in the concentration of TC in bile of high and low responding animals. TCDC was significantly higher in bile of low responding animals than in high responding animals. TDC was a minor component of the bile from opossums, and the percentage of TDC in the bile of low responding animals was significantly higher than in high responding animals. The percent composition of bile acids in the bile is presented in Fig. 1. The concentration of taurine conjugates of cholic acid in the bile was positively correlated with plasma ($r = 0.545$, $P = 0.029$) and LDL ($r = 0.564$, $P = 0.023$) cholesterol concentrations in opossums maintained on the HCHF diet for 8 weeks. However, concentrations of TCDC were negatively correlated with plasma ($r = -0.575$, $P = 0.020$, $n = 16$) and LDL ($r = -0.594$, $P = 0.015$) cholesterol concentrations. TC concentration in bile was not correlated with HDL cholesterol concentrations. However, TCDC concentration in bile was positively correlated with HDL cholesterol concentrations ($r = 0.488$, $P = 0.055$).

To determine the effect of diet-induced hyperlipidemia on hepatic cholesterol concentrations, we measured hepatic cholesterol concentration in 3 high and 3 low responding opossums maintained on the HCHF diet for at least 8 weeks.

Table 5
Plasma and LDL cholesterol concentrations (mg/dl) in 9 high and 6 low responding laboratory opossums on a basal and a high cholesterol high fat diet

Group	Basal diet[a]		HCHF diet	
	Total cholesterol	VLDL + LDL cholesterol	Total cholesterol	VLDL + LDL cholesterol
Low responding	59 ± 3.8[a]	17 ± 3.6	92 ± 4.4[b]	32 ± 2.0[c]
High responding	57 ± 2.7	21 ± 0.9	707 ± 76.6	631 ± 77.3

[a] Mean ± SE.
[b] Significantly different from high responding group ($P = 0.0001$).
[c] Significantly different from high responding group ($P = 0.001$).

At necropsy, plasma cholesterol concentration in high responding animals (1,246 ± 290 mg/dl) was 10-fold higher than in low responding animals (113 ± 15 mg/dl, $P = 0.005$). Hepatic cholesterol concentration in high responding animals (16.277 ± 2.24 mg/g liver) was also much higher than in low responding animals (3.537 ± 0.413 mg/g liver, $P = 0.018$).

4. Discussion

The expression of a number of lipid responsive genes is affected by increased delivery of cholesterol to the liver. Variations in any of these cholesterol responsive genes will affect hepatic and extrahepatic lipoprotein metabolism and in turn, will affect responsiveness to diet. Genetic variations in apo E [16], apo B [17,18] and apo A-IV [19,20] have been shown to affect responsiveness to diet, but the effects of these variants are small. Our studies in baboons [21,22] have suggested that sterol 27-hydroxylase is induced by dietary cholesterol in hepatic and extrahepatic tissues, and the ability of baboons to induce sterol 27-hydroxylase affects the responsiveness to diet. The present studies in opossums suggest that there are two major differences between high and low responding opossums. First, the sterol 27-hydroxylase activity in hepatic and extrahepatic tissues and plasma 27-hydroxycholesterol is higher in low responders than in high responders; and second, hepatic ACAT activity is lower in low responders than in high responders. Both of these enzymes may affect diet-induced hyperlipidemia in opossums.

High responding opossums have a higher activity of hepatic 7α-hydroxylase than low responding opossums on the basal diet. Cholesterol feeding increases hepatic 7α-hydroxylase activity in low responding opossums and there was no difference in hepatic 7α-hydroxylase activity between high and low responding opossums on the cholesterol-enriched diet. Thus, as in baboons, hepatic 7α-hydroxylase level does not seem to associate with diet-induced hyperlipidemia in opossums [22].

It is well established that hepatic LDL receptor is primarily responsible for the removal of plasma LDL cholesterol [23]. We did not measure hepatic LDL receptor activity in high and low responding strains of laboratory opossums and it is possible that the differences between strains in hepatic LDL receptor activities may play an important role in influencing activities of ACAT and sterol 27-hydroxylase. However, hepatic LDL receptor is down regulated by a cholesterol enriched diet in all animals and the LDL receptor activity is not related to diet-induced hyperlipidemia in any animal species [1]. Therefore, it is unlikely that the LDL receptor activity influences diet-induced hyperlipidemia in laboratory opossums. Thus, the differences in ACAT and sterol 27-hydroxylase activities between high and low responding strains may be due to polymorphisms in gene that control the expression of these enzymes either directly or indirectly.

Increased bile acid synthesis has been suggested to be the

Table 6
Bile acid composition of the gall bladder bile from 8 high and 8 low responding opossums maintained for 8 weeks on the HCHF diet

Group	Plasma cholesterol	Bile acids			
		Total	TC	TCDC	TDC
	mg/dl	*umol/ml*			
Low responding	125.6 ± 22.9[a]	243.6 ± 15.0[b]	97.8 ± 8.9[c]	124.9 ± 17.3[d]	20.9 ± 3.2[e]
High responding	710.8 ± 189.5	193.6 ± 18.5	126.7 ± 16.9	59.2 ± 13.2	9.1 ± 2.1

[a] Mean ± SE.
[b] Significantly different from values for high responding opossums ($P = 0.050$).
[c] Not significantly different from values for high responding opossums ($P = 0.153$).
[d] Significantly different from values for high responding opossums ($P = 0.009$).
[e] Significantly different from values for high responding opossums ($P = 0.008$).

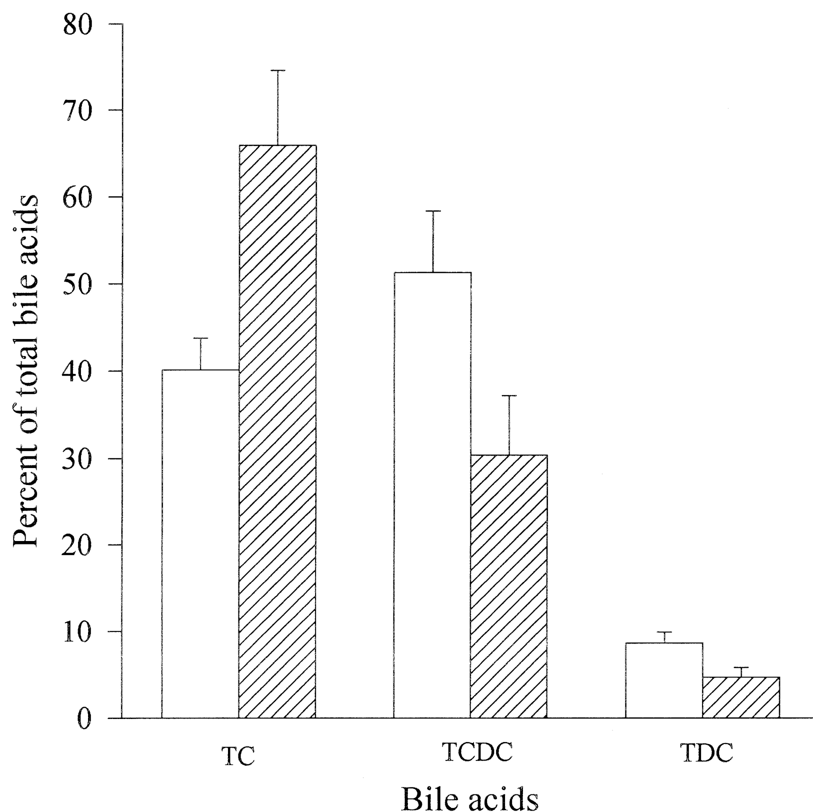

Fig. 1. Bile acid composition (%) of gall bladder bile from high and low responding opossums maintained on the HCHF diet. Major bile acids of the gall bladder bile of opossums are TC, TCDC, and TDC. Clear bars are for low responding opossums and crossed bars are for high responding opossums. Bile acids were expressed as μmol/ml of bile.

mechanism responsible for lower lipemic responsiveness to dietary cholesterol and fat in several species of animals and in humans [1]. Administration of bile acid-binding resins that interrupt the enterohepatic circulation of bile acids lowers plasma LDL cholesterol levels and the risk of coronary heart disease in humans [24]. Miettinen and Gylling [25] reported low bile acid synthesis as an independent risk factor for the incidence of coronary artery disease, and the bile acid synthetic capacity was significantly correlated with cardiac mortality in subjects heterozygous for familial hypercholesterolemia. Because bile acid metabolism plays an important role in lipoprotein metabolism, we measured total bile acid concentration and bile acid composition in the gall bladder bile of high and low opossums maintained on the HCHF diet. There were significant differences in bile acid concentration and composition between high and low responding opossums. The major bile acid of bile from high responding opossums was cholic acid (65.44%), whereas the major bile acid of bile from low responding opossums was chenodeoxycholic acid (51.27%). Cholic acid concentrations in the bile of opossums were associated positively with total plasma and VLDL+LDL cholesterol concentrations, whereas chenodeoxycholic acid concentrations in the bile of opossums were associated negatively with total plasma and VLDL+LDL cholesterol concentrations.

Recent studies by several investigators suggest that bile acid synthesis occurs by two different pathways in the liver [26]. In the first pathway, the initial step is the conversion of cholesterol into 7α-hydroxycholesterol; this product is catalyzed by cholesterol 7α-hydroxylase. The other pathway starts with the synthesis of 27-hydroxycholesterol catalyzed by the enzyme sterol 27-hydroxylase. Duane and Javitt [27] have reported that relatively only a small amount of total bile acid synthesis takes place by the 27-hydroxylase pathway. However, as in opossums, cholesterol feeding increases hepatic mitochondrial sterol 27-hydroxylase activity in rats, guinea pigs and rabbits [28], and baboons [18]. The sterol 27-hydroxylase has been reported to be present in extrahepatic tissues and thus the products of this enzyme in extrahepatic tissues is utilized for bile acid synthesis in the liver [29–31]. It has been reported that the second pathway for bile acid synthesis mainly produces chenodeoxycholic acid [32]. Thus, the increased concentration of chenodeoxycholic acid in the bile of low responding opossums suggests that low responding opossums have a higher level of synthesis of chenodeoxycholic acid than high responding opossums. Thus, low responding opossums synthesize a larger amount of bile acids by the alternate (second) pathway than the high responding opossums. The increased synthesis of chenodeoxycholate by the alternate pathway is consistent with the presence of higher amounts of 27-hydroxycholesterol in the plasma and higher activities of hepatic and

extrahepatic sterol 27-hydroxylase in low responding opossums than in high responding opossums. It is likely that increased formation of 27-hydroxycholesterol in the hepatic and extrahepatic tissues due to the increased activities of sterol 27-hydroxylase in low responding opossums as compared with high responding opossums influences the rate of bile acid synthesis by the alternate pathway in low responding opossums.

High and low responding opossums have a similar hepatic cholesterol concentration on the basal diet. However, high responding opossums have much higher hepatic cholesterol concentration than low responding opossums on the cholesterol enriched diet. These observations suggest that low responding opossums regulate their hepatic cholesterol concentrations more efficiently than high responding opossums. Increased cholesterol absorption or decreased bile acid synthesis may increase hepatic cholesterol concentration in high responding opossums. Our studies in baboons also suggest that the increase in hepatic cholesterol was associated with increased cholesterolemic response to diet [1]. In baboons, the increase in cholesterol absorption was not responsible for increased hepatic cholesterol concentration [1].

The major apolipoprotein of VLDL and LDL is apolipoprotein B, which is regulated at the translational level [33]. It has been suggested that the availability of lipid components affects the degradation of apo B in HepG2 cells [34], and the availability and secretion of apoB in isolated perfused liver [35]. Because most of the cholesteryl ester present in VLDL is produced by the ACAT reaction in the liver, ACAT plays an important role in the synthesis and secretion of VLDL [36,37]. Hepatic ACAT activity has been shown to be highly correlated with plasma LDL cholesterol enrichment in African green monkeys [38]. A partial inhibition of hepatic ACAT activity decreased the apolipoprotein B secretion from the liver of African green monkeys [39]. Thus, hepatic ACAT activity may also play an important role in the diet-induced hyperlipidemia. The present studies demonstrate that hepatic ACAT activity differs between high and low responding opossums. High responding opossums have much higher hepatic ACAT activity than low responding opossums. The increased activity of ACAT in high responding opossums may be due to increased hepatic cholesterol concentration, or due to a differential regulation and thus, influences diet-induced hyperlipidemia in this species.

Bile acid composition plays an important role in regulating cholesterol absorption. Cholesterol absorption in mice increased more with cholic acid than chenodeoxycholic acid [40]. If the intestinal bile acid composition is similar to gall bladder bile, the presence of high amounts of cholic acid in the bile of high responders will make them efficient absorbers of cholesterol from the intestine. Thus, the differences in cholesterol absorption may play an important role in influencing diet-induced hyperlipidemia in the opossums. It is also possible that the increased activity of ACAT in the intestine may be responsible for the increase in cholesterol absorption in high responding animals [41]. Recently a different form of ACAT (ACAT2) has been reported to be expressed predominately in the liver and intestine [42,43]. Joyce et al. [44] suggest that ACAT2 may be responsible for the secretion of cholesteryl ester into apolipoprotein B-containing lipoprotein from liver and intestine. It is likely that ACAT2 may have genetic variability in opossums and may influence diet-induced hyperlipidemia. Further studies are needed to evaluate these possibilities.

The results of these studies suggest that there are two enzymes that may be affecting the lipemic response to diet in laboratory opossums: sterol 27-hydroxylase and ACAT. Both of these enzymes may influence diet-induced hyperlipidemia at different steps of lipoprotein metabolism. Since 27-hydroxycholesterol is a potent inhibitor of cholesterol synthesis, increased activity of sterol 27-hydroxylase may down-regulate cholesterol synthesis in extrahepatic tissues and attenuate diet-induced hyperlipidemia. Since VLDL is the precursor for LDL, decreased activity of ACAT may affect VLDL secretion from the liver and may decrease LDL formation. However, it is possible that ACAT activity is influenced by the availability of the cholesterol in the hepatocytes and thus may play a secondary role in diet-induced hyperlipidemia. Further studies are needed to determine whether sterol 27-hydroxylase is the major determinant of diet-induced hyperlipidemia in opossums and to determine the mechanisms responsible for differences in sterol 27-hydroxylase and ACAT activities between high and low responding opossums.

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