Effect of dietary cholesterol with or without saturated fat on plasma lipoprotein cholesterol levels in the laboratory opossum (*Monodelphis domestica*) model for diet-induced hyperlipidaemia

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Laboratory opossums (*Monodelphis domestica*) show extreme genetic variability in their responsiveness to dietary lipids; a great proportion of the genetic variability in responsiveness is due to a single major gene. To determine whether the major gene for dietary response detected by genetic analysis in opossums is responsive to dietary cholesterol or dietary saturated fat, or a combination of both, we used males and females of susceptible and resistant lines of laboratory opossums that were 5 to 7 months old. The animals were challenged with three different experimental diets (high-cholesterol diets with or without high saturated fat from lard or coconut oil) and plasma lipoproteins were measured. Plasma and VLDL+LDL-cholesterol concentrations increased several-fold when the animals were fed the diet containing elevated cholesterol ($P<0.001$) or elevated cholesterol and fat ($P<0.001$) and differed between the two lines when they were fed high-cholesterol diets with or without fat ($P<0.001$). Plasma HDL-cholesterol concentrations were higher ($P<0.05$) in animals of the resistant line than in the susceptible line when they were fed the basal diet (550 (SEM 30) *v.* 440 (SEM 20) mg/l) and when they were fed the low-cholesterol and high-fat diet (600 (SEM 30) *v.* 490 (SEM 30) mg/l). Dietary coconut oil and lard had similar effects on plasma lipoprotein cholesterol concentrations in the susceptible line of opossums. A reduction in dietary cholesterol by 50 % with either the lard or coconut oil blunted the plasma cholesterol response. The results from the present studies suggest that the major gene for dietary response previously detected by genetic analysis in laboratory opossums affects the response to dietary cholesterol but not to saturated fat.

Cholesterol: Lipoproteins: Susceptible and resistant lines of opossums: High-density lipoproteins: Very low-density lipoproteins+low-density lipoproteins

Laboratory opossums (*Monodelphis domestica*) show extreme variability in their plasma cholesterol levels when challenged with a high-cholesterol and high-fat (HCHF) diet (Rainwater & VandeBerg, 1992). The plasma cholesterol variability is due to VLDL+LDL-cholesterol only. Low-responding animals show a slight increase in VLDL+LDL-cholesterol, whereas high-responding animals show up to a 100-fold increase in VLDL+LDL (mainly in LDL) cholesterol. Lipoprotein phenotype data from a large number of animals fed two diets were previously analysed by complex segregation analysis (Rainwater *et al.* 2001). The results suggested that VLDL+LDL-cholesterol concentrations in animals fed the basal and the fat- and cholesterol-enriched diets are affected by two major genes (i.e. single genes with a large effect). The major gene detected for VLDL+LDL-cholesterol concentration whilst feeding the basal diet

explains 24 % of the variability in VLDL+LDL-cholesterol concentration but does not influence VLDL+LDL-cholesterol concentration when feeding the HCHF diet. The major gene locus for VLDL+LDL-cholesterol when feeding the HCHF diet explains 80 % of the variability in VLDL+LDL-cholesterol concentrations in animals fed the HCHF diet. Since a single major gene is responsible for most of the genetic variability in VLDL+LDL-cholesterol response, the laboratory opossum is an excellent model for obtaining a genetic marker for responsiveness to diet. This genetic marker may be applicable to man or it may be useful in designing drugs to manage human diet-induced hyperlipidaemia. However, it is not known whether the differences in plasma lipoprotein cholesterol responses to diet between susceptible and resistant lines of opossums are due to the dietary fat or dietary cholesterol or a combination of both. It is generally believed that the

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Abbreviations: ACAT, acyl-coenzyme A:cholesterol acyltransferase; HCHF, high-cholesterol and high-fat; SFBR, Southwest Foundation for Biomedical Research.

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genes that regulate lipoprotein metabolism in response to dietary fat (saturated fatty acids) may be different from those that regulate lipoprotein metabolism in response to dietary cholesterol. Since plasma cholesterol concentration whilst being fed the HCHF diet is inherited as a major gene, we tested the hypothesis that the major gene is responsive to dietary cholesterol, to dietary fat, or to a combination of both. Therefore, the aim of the present study was to identify the lipid component of the diet to which the major gene responds in controlling VLDL+ LDL-cholesterol level in laboratory opossums. We also compared the effect of the type of saturated fatty acids (from lard and coconut oil) on plasma lipoprotein cholesterol response in animals from a susceptible line of opossums.

Methods

Animals

For these studies, we used partially inbred strains of the grey short-tailed opossum, a marsupial native to Brazil and adjoining countries. At the Southwest Foundation for Biomedical Research (SFBR), a breeding programme has led to the development of eleven partially inbred strains of laboratory opossums with inbreeding coefficients >0·6. Among these inbred strains, one high- and three low-responding strains were developed specifically for their responsiveness to dietary lipids. Two of these lines have been designated as ATHH (the high-responding or susceptible line) and ATHE (the low-responding or resistant line), respectively. For both experiments, we used eighty opossums from the susceptible line (ATHH stock) and forty opossums from the resistant line (ATHE stock). The selected animals were 5–7 months old. Initially, the animals were challenged with a HCHF diet, which contains 188 g fat/kg (140 g lard/kg) and 7·1 g cholesterol/kg. After 6 weeks of consuming the HCHF diet, 1·0 ml blood was collected from each animal. Fasting plasma was used for the measurement of total plasma cholesterol and triacylglycerols, and lipoprotein cholesterol concentrations. On the basis of cholesterol response data, the animals were assigned to experiments.

Experimental diets

The basal diet for the laboratory opossums was a commercial pelleted fox food (Reproduction Diet, Nutritionally Complete Fox Food Pellets; Milk Specialties Co., New Holstein, WI, USA). This diet contained 100 g fat/kg (Table 1; basal diet). The cholesterol content of the diet was 1·6 g/kg. Special diets were prepared from the fox basal diet by adding 7·5 litres tap water to 22·7 kg of pellets, together with fat and/or crystalline cholesterol (USB Corp., Cleveland, OH, USA). The ingredients were mixed in a Hobart food mixer (Hobart Corporation, Troy, OH, USA), until all of the liquids were absorbed. Then the blend was passed through a meat grinder (Torrey open bowl grinder; Rodriguez Butcher Supplier, San Antonio, TX, USA) to produce soft pellets, and frozen at −20°C to prevent spoilage. Table 1 summarises the fat and sterol contents of the diets.

Experimental design

Long-term experiment. We selected twenty-four animals from the susceptible line and twenty-six from the resistant line. This selection process ensured the availability of at least twenty animals that responded typically for their respective lines, and that survived this first stage of the experiment. However, during the course of the experiment, some animals died and there were at least ten animals in each group fed the last diet. Therefore, data for ten animals in each group are presented. However, the data for differences in the response of males and females are presented using all animals.

The selected laboratory opossums were returned to the basal diet and maintained on this diet for 10 weeks. By this time, plasma cholesterol levels had reached baseline levels, and the animals were started on the HCHF diet. This diet contained 188 g fat/kg (140 g lard/kg) and 7·1 g cholesterol/kg. After 6 weeks of consuming the HCHF diet, the animals were bled, and plasma and lipoprotein cholesterol concentrations were measured. Afterwards, animals were fed the basal diet for 10 weeks, after which time the plasma cholesterol levels had again reached baseline levels. Then the animals were challenged with a low-cholesterol and high-fat diet. This diet contained 188 g fat/kg (140 g lard/kg) and 1·4 g cholesterol/kg. After 6 weeks of consuming the low-cholesterol and high-fat diet, the animals were bled, and plasma and lipoprotein cholesterol concentrations were measured. Animals were then fed the basal diet for 10 weeks, after which the animals were fed a high-cholesterol and low-fat diet. This diet contained 108 g fat/kg and 7·1 g cholesterol/kg. After

Table 1. Fat and sterol content of opossum (*Monodelphis domestica*) diets (g/kg; dry weight basis)

Ingredient	Basal diet*	High-cholesterol and high-fat diet†	Low-cholesterol and high-fat diet†	High-cholesterol and low-fat diet*
Basal fat‡ (dairy products)	30	27	27	30
Lard§	47	140	140	46
Maize oil	23	21	21	32
Cholesterol	1·6	7·1	1·4	7·1
Plant sterols	0·5	0·4	0·4	0·5

* Low-fat diets provided approximately 20 % of the total energy from fat.
† High-fat diets provided approximately 40 % of the total energy from fat.
‡ Other ingredients of the basal diet included (g/kg): protein, 310; crude fibre, 45; simple carbohydrates, 530; vitamins and minerals, <20.
§ For coconut-oil diets, lard was replaced with coconut oil.

6 weeks of dietary challenge with the high-cholesterol and low-fat diet, the animals were bled, and plasma and lipoprotein cholesterol and triacylglycerol levels were measured. The dietary treatment has been summarised in Table 2.

Short-term experiment. We selected forty animals from the susceptible line and divided them into four groups with ten animals in each group. These animals were challenged for 4 weeks with four diets to determine the effect of reduced level of cholesterol with lard or coconut oil. We also compared the effect of type of saturated fat from coconut oil and lard on the plasma cholesterol response in this experiment. The first diet was low-cholesterol and high-lard (3·55 g cholesterol/kg, 140 g lard/kg). The second diet was low-cholesterol and high-coconut-oil (3·55 g cholesterol/kg, 140 g coconut oil/kg). The third diet was high-cholesterol and high-lard (7·1 g cholesterol/kg, 140 g coconut oil/kg). The fourth diet was high-cholesterol and high-lard, the same as the HCHF diet (7·1 g cholesterol/kg, 140 g lard/kg). The dietary treatment of animals has been summarised in Table 2.

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 and is registered with the US Department of Agriculture.

Blood sampling

Animals were fasted overnight and blood (1·0 ml) was collected by cardiac puncture from animals anaesthetised with halothane (Halocarbon Laboratory, River Edge, NJ, USA), and placed in a 2 ml EDTA-vacutainer tube (Becton Dickinson Labware, Franklin Lakes, NJ, USA). Experienced technical staff collecting blood routinely are able to obtain 0·8–1·0 ml blood with a death rate of <1 % (Robinson & VandeBerg, 1994; VandeBerg, 1999). Cardiac puncture can be conducted repeatedly over time on an animal without ill effects (Manis *et al.* 1992). Plasma was obtained by centrifugation.

Plasma and lipoprotein cholesterol analysis

Total plasma cholesterol was measured enzymically on a Ciba-Corning Express Analyser (Infolab, Round Rock, TX, USA) with reagents supplied by Boehringer Mannheim Diagnostics (Indianapolis, IN, USA). Samples having cholesterol levels that exceeded the highest calibrator (Polestar Laboratory, Escondido, CA, USA) were diluted with saline and were repeated. The assay for HDL-cholesterol was performed on the diluted sample also. The within-run CV was 2·0 % and the between-run CV was 3·0 %. HDL-cholesterol was determined by precipitation of Apo B-containing lipoproteins (VLDL+LDL) by heparin– (Elkins-Sinn, Inc., Cherry Hill, NJ, USA) manganese chloride (Sigma Chemical Co., St Louis, MO, USA) according to a procedure specified by the Lipid Research Clinics Program (1974). The VLDL+LDL-cholesterol concentration was calculated as the difference between the total plasma cholesterol and HDL-cholesterol concentration.

Measurement of triacylglycerols

Plasma triacylglycerols were measured enzymically on a Ciba-Corning Express Analyser (Infolab) with reagents supplied by Stanbio Laboratory (San Antonio, TX, USA).

Data analysis

Values in tables and figures are expressed as means and standard errors of the mean. The values for high- and low-responding opossums were compared using ANOVA with repeated measures. Significance was set at $P < 0.05$.

Results

Effect of dietary cholesterol and/or fat on plasma cholesterol

Plasma cholesterol concentrations of susceptible and resistant lines of laboratory opossums in response to dietary

Table 2. Dietary treatment of laboratory opossums (*Monodelphis domestica*) in long- and short-term experiments*

Diet	Duration of diet (weeks)	Number of animals completing the diet	
		Susceptible	Resistant
Long-term experiment			
Basal	10	24	26
High-cholesterol and high-fat	6	23†	21
Basal	10	17	21
Low-cholesterol and high-fat	6	15	19
Basal	10	15	19
High-cholesterol and low-fat	6	10	12
Short-term experiment			
Low-cholesterol and high-lard	4	10	
Low-cholesterol and high-coconut-oil	4	10	
High-cholesterol and high-coconut-oil	4	10	
High-cholesterol and high-lard	4	10	

* For details of diets and procedures, see Table 1 and p. 64.
† The animals died due to procedures for repeated bleedings. The body weight and feed consumption did not differ between the animals of the two lines.

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cholesterol and/or fat are shown in Fig. 1 (A). Plasma cholesterol concentrations increased significantly in high-responding animals fed diets containing elevated levels of cholesterol alone ($P < 0.001$), or cholesterol and fat ($P < 0.001$), but not fat alone. The increase in plasma cholesterol in animals of the susceptible line when fed the HCHF diet was more than 10-fold (8290 (SEM 920) mg/l) compared with when they were fed the basal diet (640 (SEM 40) mg/l). However, the increase in plasma cholesterol when the susceptible animals were fed the high-cholesterol and low-fat diet (3980 (SEM 930) mg/l) was 6-fold. Similarly, plasma cholesterol concentration increased significantly in resistant opossums when they
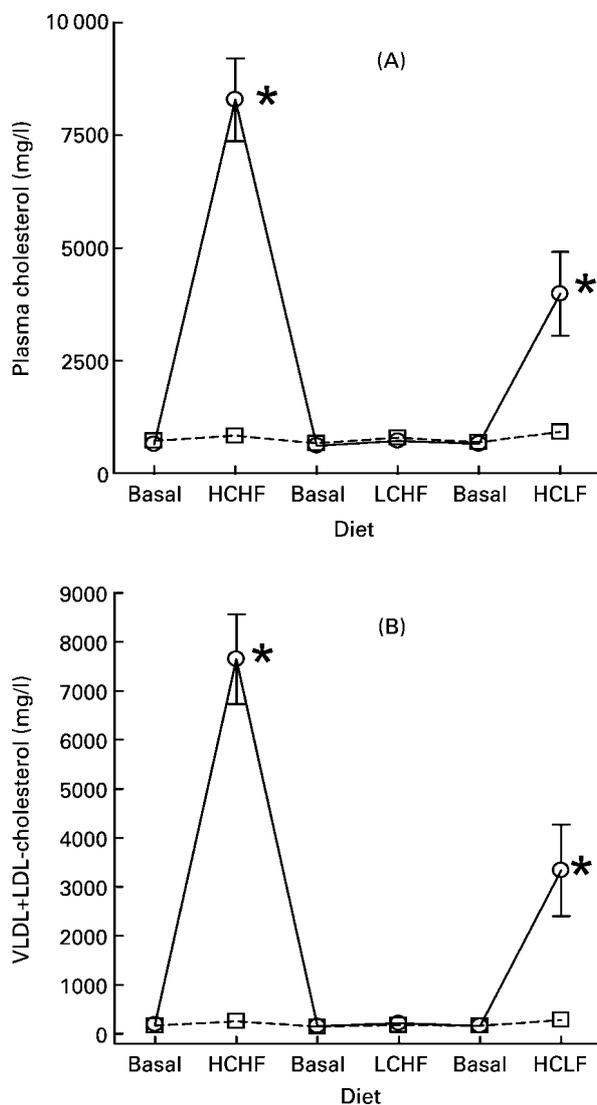

Fig. 1. Plasma cholesterol (A) and VLDL + LDL-cholesterol (B) concentrations of susceptible ($-\bigcirc-$) and resistant ($-\square-$) lines of laboratory opossums (*Monodelphis domestica*; ten in each group) in response to diets containing elevated cholesterol and/or fat. A basal diet was fed between each experimental diet. The experimental diets were: high-cholesterol and high-fat (HCHF); low-cholesterol and high-fat (LCHF); high-cholesterol and low-fat (HCLF) (see Table 1). Mean values are shown, with standard errors of the mean represented by vertical bars. * Mean values for susceptible and resistant lines were significantly different ($P < 0.01$).

were fed diets containing elevated cholesterol levels ($P < 0.05$). However, the extent of increase was much less, ranging from 1·2-fold (720 (SEM 40) mg/l when fed the basal diet *v.* 830 (SEM 50) mg/l when fed the HCHF diet) in response to elevated dietary cholesterol and fat to 1·4-fold (920 (SEM 60) mg/l) in response to elevated dietary cholesterol alone. Plasma cholesterol concentration did not differ between animals of the susceptible and resistant lines when they were fed the basal diet or the diet containing a high level of fat alone.

Effect of dietary cholesterol and/or fat on plasma very low-density lipoprotein+low-density lipoprotein-cholesterol

The VLDL+LDL-cholesterol-concentrations of laboratory opossums in response to diets containing high amounts of cholesterol and/or fat are shown in Fig. 1 (B). Plasma cholesterol in animals of the susceptible line increased significantly when they were fed the HCHF diet ($P < 0.001$), and the high-cholesterol diet ($P < 0.001$), but not when they were fed the high-fat and low-cholesterol diet ($P = 0.15$). VLDL+LDL-cholesterol concentrations of the susceptible line fed the basal, HCHF, high-cholesterol and low-fat, and low-cholesterol and high-fat diets were 200 (SEM 20), 7650 (SEM 910), 3340 (SEM 930) and 220 (SEM 20) mg/l, respectively. The increase in plasma VLDL+LDL in susceptible animals when fed the HCHF diet was approximately 38-fold, whereas the increase in plasma VLDL+LDL-cholesterol when fed the high-cholesterol diet was approximately 17-fold. In response to the HCHF diet, and the high-cholesterol and low-fat diet, more than 85 % of the total plasma cholesterol existed in the form of VLDL+LDL in high-responding opossums. Similarly, plasma VLDL+LDL-cholesterol increased significantly in the low-responding opossum line when fed the HCHF diet ($P < 0.001$), and the high-cholesterol and low-fat diet ($P < 0.001$), but not when fed the low-cholesterol and high-fat diet. VLDL+LDL-cholesterol concentrations of the resistant line when fed the basal, HCHF, high-cholesterol and low-fat, and low-cholesterol and high-fat diets were 170 (SEM 10), 260 (SEM 30), 280 (SEM 40) and 180 (SEM 20) mg/l, respectively. The increase in plasma VLDL+LDL-cholesterol in the resistant line was much less by comparison with the susceptible line (1·5- to 1·8-fold). As a result, there was very little increase in the proportion of VLDL+LDL-cholesterol in the plasma. Plasma VLDL+LDL-cholesterol concentrations differed significantly between animals of the susceptible and resistant lines when they were fed the HCHF diet ($P < 0.001$) and the high-cholesterol and low-fat diet ($P < 0.001$).

Effect of dietary cholesterol and/or fat on plasma high-density lipoprotein-cholesterol

As shown in Fig. 2, resistant animals have significantly higher ($P < 0.05$) HDL-cholesterol (530 (SEM 19) mg/l) than susceptible animals (463 (SEM 13) mg/l) when fed the basal diet. There was an increase in plasma HDL-cholesterol in both the susceptible and resistant lines for all three experimental diets ($P < 0.05$). The increases in plasma HDL-cholesterol concentrations in susceptible
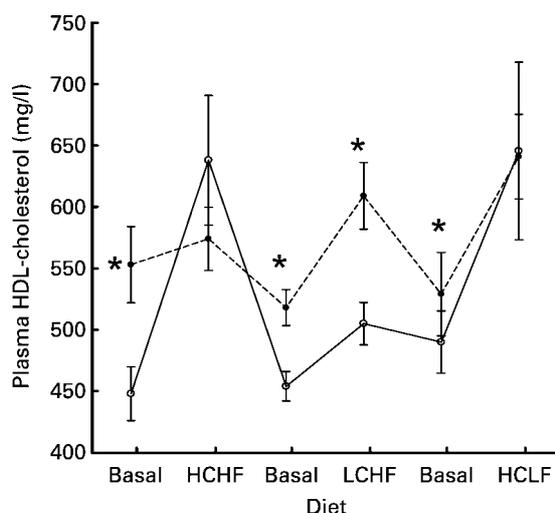
Fig. 2. Plasma HDL-cholesterol concentrations of susceptible (−○−) and resistant (−●−) lines of laboratory opossums (*Monodelphis domestica*; ten in each group) in response to diets containing elevated cholesterol and/or fat. The experimental diets were: high-cholesterol and high-fat (HCHF); low-cholesterol and high-fat (LCHF); high-cholesterol and low-fat (HCLF) (see Table 1). Mean values are shown, with standard errors of the mean represented by vertical bars. * Mean values for susceptible and resistant lines were significantly different ($P<0.05$).

animals were more than in resistant animals when fed the HCHF diet (37 % increase in high-responding animals *v.* 8 % in low-responding animals; $P<0.05$) and the differences in HDL-cholesterol between the high and low lines observed at baseline were eliminated during the feeding of these diets. However, the differences in HDL-cholesterol between the two lines when they were fed the high-fat and low-cholesterol diet were significant (528 (SEM 19·4) mg/l in susceptible animals *v.* 630 (SEM 18) mg/l in resistant animals) ($P<0.01$; Fig. 2).

Effect of dietary cholesterol and/or fat on plasma triacylglycerols

Plasma triacylglycerol concentrations did not differ between animals of the susceptible and resistant lines

when fed the basal diet (200 (SEM 10) mg/l in the susceptible line *v.* 220 (SEM 10) mg/l in the resistant line). The values for plasma triacylglycerols varied considerably after the experimental diets. Plasma triacylglycerols in animals of the susceptible line fed the HCHF and low-cholesterol and high-fat diets were 350 (SEM 40) and 276 (SEM 30) mg/l, respectively. Similarly, plasma triacylglycerols in animals of the resistant line fed the HCHF and low-cholesterol and high-fat diets were 214 (SEM 20) and 300 (SEM 20) mg/l, respectively. However, there were no significant differences between the susceptible and resistant lines when feeding the experimental diets.

Effect of sex on the diet-induced hypercholesterolaemia in susceptible and resistant lines of opossums

Table 3 presents plasma and lipoprotein lipid levels for male and female opossums when feeding the basal diet and the HCHF diet. We used seven males and seventeen females from the susceptible line and ten males and sixteen females from the resistant line on the basis of availability. Plasma and lipoprotein cholesterol and plasma triacylglycerol concentrations did not differ between male and female opossums within the same line when fed either the basal or the HCHF diet. Plasma and VLDL+LDL-cholesterol concentrations of both male and female opossums of the susceptible line were significantly higher ($P<0.001$) than plasma and VLDL+LDL-cholesterol concentrations of both male and female opossums of the resistant line. Males in the susceptible line tended to have higher plasma and lipoprotein cholesterol concentrations than females. The difference in HDL-cholesterol concentration between males and females in the susceptible line group was significant ($P=0.05$).

Effect of type of dietary fat with reduced levels of cholesterol on diet-induced hypercholesterolaemia in susceptible opossums

Table 4 presents the data for effect of type of dietary fat on plasma cholesterol concentrations in opossums of the susceptible line. The animals consumed these diets for 4

Table 3. Effect of sex on diet-induced hypercholesterolaemia in high- and low-responding opossums (*Monodelphis domestica*)†
(Mean values and standard errors of the mean)

Diet	Group	Sex	Plasma cholesterol (mg/l)		Plasma triacylglycerols (mg/l)		HDL-cholesterol (mg/l)		VLDL+LDL-cholesterol (mg/l)	
			Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Basal	High	Male (*n* 17)	660	24	206	07	449	14	211	13
		Female (*n* 7)	679	61	203	22	497	40	181	22
	Low	Male (*n* 10)	681	23	230	11	500	14	181	16
		Female (*n* 16)	721	35	189	09	549	30	171	12
HCHF	High	Male (*n* 17)	7688*	947	351	33	677*	38	7011*	967
		Female (*n* 7)	5491*	1060	294	49	526	69	4966*	1022
	Low	Male (*n* 10)	817	43	248	11	557	26	260	26
		Female (*n* 16)	861	36	195	14	595	18	273	23

HCHF, high-cholesterol and high-fat.
* Mean values were significantly higher ($P<0.001$) than those for males and females in the low responding group fed the HCHF diet. Mean values for HDL-cholesterol in males were significantly higher than females in the high-responding group ($P=0.05$).
† For details of diets and procedures, see Table 1 and p. 64.

Table 4. Effect of type of dietary fat with reduced levels of cholesterol on diet-induced hypercholesterolaemia in high-responding opossums (*Monodelphis domestica*)†

(Mean values and standard errors of the mean)

Diet	Plasma cholesterol (mg/l)		HDL-cholesterol (mg/l)		VLDL+LDL-cholesterol (mg/l)	
	Mean	SEM	Mean	SEM	Mean	SEM
Basal (*n* 50)	654	14	548	12	107	06
Low-cholesterol and high-lard (*n* 10)	970	61	530	31	440	36
Low-cholesterol and high-coconut-oil (*n* 10)	726	35	528	34	198	39
High-cholesterol and high-coconut-oil (*n* 10)	6347*	583	843*	120	5503*	703
High-cholesterol and high-lard (HCHF) (*n* 10)	7004*	1120	885*	83	6110*	1143

HCHF, high-cholesterol and high-fat.
* Mean values were significantly different from cholesterol values for other diets ($P < 0.01$).
† For details of diets and procedures, see Table 1 and p. 64.

weeks. The type and amount of saturated fatty acids (lard or coconut oil) had similar effects on the plasma and lipoprotein cholesterol concentrations in susceptible opossums. When dietary cholesterol was fed at 7·1 g/kg with either the coconut oil or lard, the animals responded with extreme increases in plasma and VLDL+LDL-cholesterol concentrations ($P < 0.05$). However, feeding a lower concentration of cholesterol (3·55 g/kg) with coconut oil or lard eliminated the response in plasma and lipoprotein cholesterol completely.

Discussion

There is considerable variability in the plasma cholesterol response to dietary cholesterol and fat among species and among individuals within the same species (Kushwaha & McGill, 1997). The high-responding individuals within the same species have a greater increase in plasma cholesterol levels than low-responding individuals. The metabolic mechanisms responsible for the differences in plasma cholesterol responses to dietary lipids differ among various species of animals (McGill & Kushwaha, 1995). The differences in dietary responses are generally due to dietary cholesterol, dietary fat, or a combination of both. As a consequence of the results reported in the present paper, we are using the laboratory opossum model to identify genetic and metabolic markers for individual variability in plasma cholesterol response to dietary lipids. The uniqueness of this model is that it has extreme genetically controlled variability in responsiveness to dietary lipids, and a great proportion of the genetic variability in responsiveness is due to a single major gene (Rainwater *et al.* 2001). The present studies were conducted to determine whether the major gene for dietary response detected by genetic analysis in opossums is responsive to dietary cholesterol, dietary fat, or a combination of both. The results demonstrated that the major gene is responsive to dietary cholesterol but not to fat. However, it responds more consistently and more extremely to dietary cholesterol when a high level of fat is present in the diet.

Cholesterol feeding in human subjects also causes an increase in VLDL+LDL-cholesterol, and this increase in LDL is much higher in some individuals than in others (Beynen *et al.* 1989). Selective breeding in baboons has also produced high and low lines that differ in plasma cholesterol response to dietary cholesterol and fat (McGill *et al.* 1988). Baboons from a high-responding line increased their VLDL+LDL-cholesterol much more than baboons from a low-responding line when challenged with a HCHF diet (McGill *et al.* 1988). Dietary cholesterol increased plasma HDL-cholesterol to a small extent, but fat with saturated fatty acids consistently increased HDL-cholesterol in these lines of baboons (McGill *et al.* 1988). Similarly, in laboratory opossums the dietary cholesterol with or without saturated fat increased HDL-cholesterol levels, but the increase in VLDL+LDL-cholesterol was considerably more than the increase in HDL-cholesterol. Dietary fat selectively increased HDL-cholesterol in both high- and low-responding opossums; however, the increase in HDL-cholesterol in low-responding opossums was higher than in high-responding opossums. Our studies of baboons have also suggested that dietary cholesterol and dietary saturated fat have different effects on metabolic processes (Kushwaha *et al.* 1991, 1994). In addition, Mott *et al.* (1992) have suggested that cholesterol and saturated fat have different effects on the hepatic cholesterol and bile acid metabolism in baboons. According to their results, dietary cholesterol elevated hepatic cholesterol content and bile acid secretion, whereas saturated fat decreased hepatic cholesterol content and bile acid secretion. These studies have led to the conclusion that the genes that regulate the cholesterolaemic response to dietary cholesterol are different from those that regulate response to dietary fat in baboons. We have not conducted metabolic studies in opossums, but since their plasma lipoprotein cholesterol responses to dietary fat and dietary cholesterol are similar to those in baboons, it is probable that plasma cholesterol responses to dietary cholesterol and dietary fat are regulated by different genes.

In previous studies we examined metabolic differences between susceptible and resistant opossums (Kushwaha *et al.* 2001). These studies suggested that susceptible and resistant strains differ in hepatic acyl-coenzyme A:cholesterol acyltransferase (ACAT) and sterol 27-hydroxylase activity. The activity of ACAT was higher in the susceptible line, whereas the activity of sterol 27-hydroxylase was higher in the resistant strain and was induced in response to dietary lipids. Differences in ACAT activity may affect cholesterol absorption and hepatic lipoprotein secretion (Kushwaha & McGill, 1997). Similarly,

differences in sterol 27-hydroxylase may affect bile acid synthesis and cholesterol metabolism in the liver and extra-hepatic tissues and may modulate diet-induced hyperlipidaemia. Further studies are needed to determine whether ACAT or sterol 27-hydroxylase induces these differences in response to diet between susceptible and resistant opossums.

In the present study, the addition of fat to the diet exacerbated the plasma cholesterol response to dietary cholesterol in high-responding but not in low-responding opossums. However, fat alone did not have any effect on VLDL+LDL-cholesterol concentrations in either stock of opossums. Two types of saturated fats (lard and coconut oils) had similar effects on plasma cholesterol response in high responders. The reduction in dietary cholesterol to one-half that of the HCHF diet blunted the response to diets containing lard or coconut oil. These results are consistent with those from other animals and human subjects. In hamsters and other animal species, when dietary fat is added without added cholesterol, there is little effect on plasma and VLDL+LDL-cholesterol. However, in the presence of dietary cholesterol and fat, plasma and VLDL+LDL-cholesterol concentrations are elevated (Spady & Dietschy, 1988; Dietschy *et al.* 1993). In hamsters, excess dietary cholesterol also increases HDL-cholesterol (McConihay *et al.* 2001; Souidi *et al.* 2001). These studies are also consistent with results from human studies, in which the effect of dietary cholesterol on plasma cholesterol concentrations was not modified by the nature and amount of dietary fat in normolipidaemic human subjects (Kestin *et al.* 1989). In other human studies, the lipaemic effect of saturated fat was variable. Clifton *et al.* (1990) demonstrated that diet-sensitive human subjects had a greater response to dietary cholesterol, dietary fat or both as compared with modestly hypercholesterolaemic subjects. Fielding *et al.* (1995) observed considerable variability in plasma cholesterol response to dietary fat and cholesterol, but an increase in saturated fat in place of polyunsaturated fat had no or little effect on the cholesterolaemic effect of diet in human subjects. The presence of a mixed population of high, moderate and low human responders may be a major contributor to the variable results.

In summary, the present results demonstrate that the major gene detected by previous genetic analysis in laboratory opossums is responsive to dietary cholesterol and not to fat. However, dietary fat amplifies the effect of dietary cholesterol, especially in high-responding opossums. Dietary fat increases plasma HDL-cholesterol in both high- and low-responding opossums. The variable effects of dietary cholesterol and dietary fat in laboratory opossums are consistent with the hypothesis that genetic mechanisms that modulate the effect of dietary cholesterol on plasma lipoprotein cholesterol concentrations are different from those that modulate the effect of dietary fat. The many-fold difference in non-HDL-cholesterol concentrations between high- and low-responding opossums, and the existence of a single major gene responsible for these genetic differences in non-HDL-cholesterol concentrations whilst being fed the high-cholesterol diet establishes the laboratory opossum as a unique model for investigating the metabolic and genetic basis for dietary cholesterol responsiveness.

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