Metabolic mechanisms for responses to dietary cholesterol and fat in high and low LDL responding baboons (*Papio* sp.)

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Abstract These studies were conducted to determine how plasma low density lipoprotein (LDL) cholesterol levels respond to dietary cholesterol, fed in increasing amounts with either corn oil or coconut oil diets, in high as compared to low LDL responding baboons; and to determine how apolipoprotein (apo) B transcription levels are modulated in response to dietary lipids. Eight high and eight low LDL responding pedigreed adult baboons, balanced for sire, age, sex, and weight, were challenged for successive 7-week periods with increasing levels of dietary cholesterol combined with either coconut oil or corn oil. At the end of each dietary period, plasma and lipoprotein lipids, apoB, apoA-I, and hepatic mRNA levels for apolipoproteins were measured. As dietary cholesterol increased, plasma cholesterol concentrations (mostly LDL cholesterol) increased in both phenotypes and with both types of fat, but phenotypic differences were greater with coconut oil. There was not a consistent dose–response relationship of plasma or LDL cholesterol levels to increasing intakes of dietary cholesterol. Neither dietary cholesterol, type of dietary fat, nor LDL phenotype affected hepatic apoB or apoE mRNA levels. In a second experiment to resolve the inconsistent dose–response to dietary cholesterol, we fed the animals varying levels of dietary cholesterol combined with coconut oil, and separated the challenge periods with intervening 12-week chow periods. Plasma and LDL cholesterol and apoB concentrations rose consistently with increasing dietary cholesterol, and the slope of the increase diminished at the higher doses. The results suggest that genetic differences in the initial response of LDL cholesterol to dietary cholesterol and saturated fatty acids are not due to the differences in hepatic transcription of apoB, and that the preceding dietary intake of cholesterol and saturated fatty acids is a major determinant of the response of plasma lipids and the associated metabolic processes to a dietary challenge. The response of baboon plasma LDL cholesterol concentrations to dietary cholesterol, when fed with saturated fatty acids, is similar to that of humans.—**Kushwaha, R. S., C. A. Reardon, G. S. Getz, D. S. Lewis, K. S. Rice, K. D. Carey, and H. C. McGill, Jr.** Metabolic mechanisms for response to dietary cholesterol and fat in high and low LDL responding baboons (*Papio* sp.). *J. Lipid Res.* 1994. **35:** 633–643.

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Elevated levels of low density lipoproteins (LDL) and reduced levels of high density lipoproteins (HDL) are associated with atherosclerosis in humans and experimental animals (1–4), and are regulated by both genetic and dietary factors, including dietary cholesterol (5–8). Dietary cholesterol increases LDL concentration, alters HDL size and density, and usually (in high responding animal species) increases very low (VLDL) and intermediate (IDL) density lipoprotein concentrations (6). Dietary cholesterol combined with saturated fatty acid also increases the concentrations of HDL-containing apoE in many animals, particularly in dogs (6).

High performance liquid chromatographic lipoprotein profiles of selectively bred baboons identified families of baboons with low or high LDL responses to a challenge high cholesterol and high saturated fat (HCHF) diet (9). Comparison of responses of high and low line baboons indicated that the phenotypic difference is mainly in the VLDL + LDL cholesterol response to dietary cholesterol (10). To identify the genetic basis of the differences in VLDL + LDL cholesterol responses between high and low lines, it is necessary to determine the metabolic mechanism(s) responsible for the contrasting phenotypes. The present studies were conducted to compare the dose–response relationship of plasma LDL cholesterol levels to dietary cholesterol between high and low LDL responding baboons; and to determine whether the higher plasma apoB concentration in high LDL responding baboons was related to increased apoB transcription in the liver.

Abbreviations: apo, apolipoprotein; HCHF, high cholesterol and high fat; HDL, high density lipoproteins; HMG-CoA, hydroxymethylglutaryl coenzyme A; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

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METHODS

Animals

Eight low and eight high responding adult (6- to 11-year-old) baboons (*Papio* sp.), matched for sex and age, were selected (**Table 1**). The low responding baboons were progeny of two low responding sires and eight low responding dams; and the high responding baboons were progeny of two high responding sires and eight high responding dams. High LDL responding baboons had VLDL+LDL cholesterol >125 mg/dl (187.38 ± 16.55, mean ± SE) on the HCHF challenge diet, whereas low LDL baboons had VLDL+LDL cholesterol <70 mg/dl (47.33 ± 3.36, mean ± SE) on the same diet. The baboons were housed in indoor-outdoor cages and were fed once daily ad libitum. They had access to water at all times. Liver samples from two baboons (with average phenotype) obtained at necropsy and stored in a freezer (−70°C) were used for the measurement of hepatic mRNA levels. The protocol of this experiment was approved by the institutional Animal Research Committee. The Southwest Foundation for Biomedical Research is accredited by the American Association for Accreditation of Laboratory Animal Care and is registered with the U.S. Department of Agriculture.

Design of experiment 1

Four low and four high responding baboons (matched for sex) were randomly assigned to either group I or to group II. All animals were first fed the chow diet for 7 weeks, after which group I was fed the coconut oil diet and group II was fed the corn oil diet, both with increasing levels of cholesterol as described under Diets. Each diet was fed for 7 weeks. After completion of the diet containing the highest level of cholesterol (1.35 mg/kcal), the animals were again fed chow for 7 weeks. Groups were then crossed over and group I was fed the corn oil diet while group II was fed the coconut oil diet. Fasting blood samples and a liver punch biopsy were obtained from each baboon after 6 weeks on each diet.

Design of experiment 2

We designed this experiment after examining the results of experiment 1, in which we found a biphasic response of plasma and LDL cholesterol levels with increasing doses of dietary cholesterol. The purpose of experiment 2 was twofold: first, to separate the effect of the lowest dietary cholesterol intake (0.05 mg/kcal) from that of the saturated fatty acid substitution for carbohydrate; and second, to determine whether the responses to various doses of dietary cholesterol are influenced by the intake of dietary cholesterol and saturated fatty acid intake immediately preceding the challenge diet. We used the same eight high and eight low LDL responding baboons used in the crossover study. The animals were fed the chow diet for 7 weeks, followed by the coconut oil diet without cholesterol for 6 weeks. After consuming the chow diet again for 12 weeks, animals were fed the coco-

TABLE 1. Selected characteristics of baboons used in the study

Phenotype and Number	Sex	Age	Body Weight	Cholesterol					
				Chow			HCHF[a]		
				Total	VLDL + LDL	HDL	Total	VLDL + LDL	HDL
		yr	*kg*		*mg/dl*			*mg/dl*	
Low LDL									
1862	M	10.8	33.7	85	18	67	134	44	90
2000	F	10.6	20.7	63	6	57	97	41	56
2384	F	9.8	17.2	78	36	42	122	41	81
2822	M	9.4	37.3	83	18	65	140	47	93
3195	F	9.0	21.1	67	19	48	110	36	74
3938	M	8.1	32.0				146	62	84
3980[b]	M	7.3	27.3	88	34	54	125	66	59
4056	F	6.9	13.0	92	45	47	126	42	84
4266	M	6.0	21.7	91	43	48	120	47	73
High LDL									
3218	F	8.9	16.0	115	63	52	257	190	67
3310	M	8.8	30.2	125	59	66	256	149	107
3834	M	7.8	28.0	114	67	47	219	128	91
4038	F	7.0	21.5	105	46	59	316	189	127
2712	F	9.6	15.6				333	235	98
3001	M	9.2	34.0	155	83	72	367	269	98
4258	M	6.1	27.3	171	95	76	254	151	103
4278	F	5.9	15.5	133	55	78	286	188	98

[a]HCHF, high cholesterol, high fat diet fed for 7–8 weeks.
[b]Animal 3980 died halfway through experiment and was replaced by 3938.

nut oil diet with a cholesterol level of 0.15 mg/kcal for 6 weeks. After a chow period of 12 weeks, the baboons were fed the coconut oil diet with a cholesterol level of 0.45 mg/kcal for 6 weeks. After another chow period of 12 weeks, the animals were fed the coconut oil diet with a cholesterol level of 1.35 mg/kcal for 6 weeks. At the end of each chow and each coconut oil diet period, the baboons were bled, and plasma, LDL and HDL cholesterol, and LDL apoB concentrations were measured. We did not take liver biopsies in this experiment.

Diets

Prior to the experiment, baboons were maintained on a chow diet (Monkey Diet, Purina, St. Louis, MO) low in cholesterol (0.03 mg/kcal) and fat (10% of total calories). High fat diets provided 40% of their total calories from either corn oil or coconut oil (**Table 2**). Four levels of cholesterol (0.05, 0.15, 0.45, and 1.35 mg/kcal) were used for experiment 1 and four levels of cholesterol (0.00, 0.15, 0.45, and 1.35 mg/kcal) were used for experiment 2. The ingredients for the experimental diets were mixed with water and pelleted, and the feed was stored in a freezer (−20°C). Coconut oil and corn oil were each used from the same commercial batch. By gas-liquid chromatographic analysis, the cholesterol concentrations (mg/kcal) of the coconut oil diets were 0.055 ± 0.014, 0.143 ± 0.015, 0.371 ± 0.0375, and 1.33 ± 0.045; and of the corn oil diets, 0.064 ± 0.008; 0.134 ± 0.010, 0.374 ± 0.032, and 1.356 ± 0.050 (mean ± SE, six determinations). The fatty acid compositions of the corn oil and coconut oil are given in the footnote to Table 2.

Blood sampling and liver punch biopsies

After a fast of approximately 20 h, venous blood was drawn while the animals were immobilized with ketamine hydrochloride (10 mg/kg body weight, intramuscular). At each blood sampling, animals were weighed and three 25-mg cores of liver were obtained by punch biopsy. The liver cores for each animal were pooled, wrapped in aluminum foil, quick frozen in liquid nitrogen, and stored at −70°C. Liver samples from two other baboons were obtained at necropsy, frozen, and stored at −70°C in small pieces to be used as controls.

Cholesterol, triglyceride, and phospholipid measurements

Cholesterol and triglycerides in plasma and lipoproteins were measured by enzymatic methods (Sigma Chemical Co., St. Louis, MO). Cholesteryl esters were determined by subtracting free from total cholesterol. Phosphorus in lipoproteins was measured by the method of Ames and Dubin (11) and multiplied by 25 to estimate phospholipids.

Separation of plasma lipoproteins

Blood samples were centrifuged at low speed to obtain plasma. Lipoproteins were separated by density gradient ultracentrifugation using an SW 41 Ti rotor. The density gradient procedure was a modification of Redgrave, Roberts, and West (12) described in detail previously (13). The refractive index was measured and the fractions were pooled on the basis of their densities as described previously (13). The densities of fractions pooled corresponded to VLDL+IDL (d < 1.019 g/ml), LDL (d 1.020–1.045 g/ml), HDL_2 (d 1.046–1.090 g/ml), and HDL_3 (d 1.10–1.21 g/ml).

Apolipoprotein measurements

ApoB and apoA-I were measured in plasma and apoB was measured in lipoprotein fractions separated by ultracentrifugation by the electroimmunoassay procedure of Laurell (14) as modified by Mott et al. (15). Antisera were purchased commercially from Boehringer Mannheim Corp. (Indianapolis, IN) and were monospecific. The concentrations of apoA-I and apoB were well within the linear ranges of the assays. Two duplicate serum controls in each row of 28 samples assessed the day-to-day variation in the assay. The sample values were calculated by the average peak heights using a standard curve pre-

TABLE 2. Nutritional composition of baboon chow and experimental diets

Nutrients	Chow Diet	Coconut Oil Diet	Corn Oil Diet
Carbohydrate (% cal)	62	40	40
Protein (% cal)	28	20	20
Fat (% cal)	10	40	40
Energy (kcal per 100 g diet)	329	360	360
Cholesterol (mg/kcal)	0.03	0.05–1.35	0.05–1.35

Coconut oil and corn oil diets were prepared by mixing 81.4% (dry weight basis) of Purina monkey meal 5-5046-6 (a special mix with no added fat, dehydrated alfalfa, sodium chloride, ascorbic acid, or retinyl acetate) with coconut or corn oil (16.5%), sodium chloride (1.1%), retinyl acetate (0.005%), ascorbic acid (0.2%), a vitamin mixture (1.0%), and cholesterol (0.05–1.35 mg/kcal). The major fatty acids of the corn oil diet were C16:0, 13.6%; C18:0, 2.2%; C18:1, 24.5%; C18:2, 57.5%; C18:3, 1.4% and in the coconut oil diet these were C8:0, 9.3%; C10:0, 7.0%; C12:0, 45.4%; C14:0, 15.0%; C16:0, 8.0%; C18:0, 2.0%; C18:1, 6.0%; C18:2, 7.0%. The major fatty acids of the monkey chow were C16:0, 17%; C18:1, 19.7%; and 18:2, 58%. An adult baboon consumes approximately 75 kcal/kg body weight per day.

pared with standard serum on the same plate. The coefficients of variation for apoB and apoA-I assays were below 5%.

Measurements of hepatic mRNA levels

The hepatic mRNA levels were measured for each animal separately only in experiment 1. Liver samples (50 mg for experimental animals and 200 mg for the control animals) were extracted with guanidine thiocyanate for measurement of total cellular RNA (16). The mRNA levels were measured by the slot blot procedure (17). To confirm the results of the slot blot analysis, some of the same samples were also measured by an RNase protection assay, which yielded similar values. The liver samples from control animals were obtained at the time of necropsy and frozen at −70°C. Liver RNA samples from control baboons were included in each slot blot to allow for normalization between blots.

Statistical analysis

The effects of phenotype (high vs. low), fat (coconut oil vs. corn oil), and cholesterol (dose) and their two factor interactions on plasma lipids and mRNA levels were analyzed by analysis of variance with repeated measures using cholesterol dose (0.05, 0.15, 0.45, and 1.35 mg cholesterol/kcal) and fat as trial factors. Chow values were not included in the analyses of cholesterol and fat effects. Prior to analysis, data were logarithmically transformed to satisfy better the assumptions underlying the analysis. The effects of phenotype and diet sequence were tested against a between-animal error term. For analysis of data obtained during the experimental dietary periods, the effects of fat, cholesterol, diet sequence by fat, diet sequence by cholesterol, phenotype by cholesterol, phenotype by fat, and fat by cholesterol were tested against a within-animal error term. Significance was set at $P \leq 0.05$, but we also report differences at $P \leq 0.10$ to balance between Type I and Type II statistical errors.

RESULTS

Experiment 1. Effects of diet and phenotype on plasma lipids and apolipoproteins

The mean values for plasma lipids and apolipoproteins in high and low responders fed corn and coconut oil based diets containing increasing amounts of cholesterol are reported in **Table 3.** High responders had higher ($P = 0.006$) plasma cholesterol levels than low responders on each diet. Regardless of phenotype, dietary cholesterol raised ($P = 0.000$) plasma cholesterol levels; cholesterol with coconut oil caused a greater increase than cholesterol with corn oil (fat and cholesterol interaction, $P = 0.04$). Cholesteryl esters and free cholesterol in the plasma were affected similarly (data not shown).

Phenotype had no effect on plasma triglycerides or phospholipids. Dietary cholesterol increased plasma phospholipid levels ($P = 0.000$), but had no effect on plasma

TABLE 3. Plasma lipid and apolipoprotein concentrations (mean ± SE) by diet and phenotype

Type of Fat and Dietary Cholesterol (mg/kcal)	Cholesterol		Triglycerides		Phospholipids		ApoA-I		ApoB	
	High[a,b]	Low[a]	High	Low	High	Low	High	Low	High	Low
	mg/dl									
Chow 1										
0.03	96 ± 11	71 ± 5	46 ± 10	40 ± 3	166 ± 19	140 ± 14	68 ± 7	44 ± 3	30 ± 5	17 ± 2
Corn oil										
0.05[c]	126 ± 8	85 ± 7	44 ± 4	48 ± 6	152 ± 18	145 ± 15	80 ± 8	69 ± 9	34 ± 3	26 ± 3
0.15	134 ± 9	103 ± 6	41 ± 3	34 ± 4	240 ± 30	198 ± 19	115 ± 8	93 ± 6	28 ± 4	22 ± 3
0.45	117 ± 10	85 ± 6	34 ± 4	44 ± 6	201 ± 35	181 ± 29	107 ± 2	91 ± 4	36 ± 4	26 ± 5
1.35	150 ± 11	108 ± 5	35 ± 5	39 ± 4	180 ± 14	160 ± 10	92 ± 11	85 ± 9	36 ± 5	20 ± 2
Chow 2										
0.03	89 ± 7	77 ± 5	48 ± 8	46 ± 5	128 ± 15	136 ± 14	52 ± 4	50 ± 6	32 ± 3	27 ± 3
Coconut oil[d,e]										
0.05	171 ± 13	137 ± 7	49 ± 4	52 ± 6	239 ± 31	223 ± 17	94 ± 6	91 ± 6	34 ± 2	28 ± 2
0.15	230 ± 24	169 ± 15	51 ± 8	51 ± 6	254 ± 18	253 ± 22	96 ± 8	95 ± 6	31 ± 3	25 ± 3
0.45	212 ± 22	152 ± 11	54 ± 5	55 ± 7	300 ± 11	253 ± 8	114 ± 6	112 ± 6	42 ± 34	32 ± 3
1.35	261 ± 28	165 ± 11	56 ± 5	60 ± 11	313 ± 17	263 ± 15	86 ± 9	84 ± 6	50 ± 8	30 ± 4

[a]High and low phenotypes.
[b]Phenotype had significant effects on plasma cholesterol ($P = 0.006$) and apoB ($P = 0.059$) concentrations.
[c]Dietary cholesterol had significant effects on plasma cholesterol ($P < 0.001$), phospholipids ($P < 0.001$), apoA-I ($P < 0.001$), and apoB ($P < 0.001$) concentrations.
[d]Dietary fat had significant effects on plasma cholesterol ($P < 0.001$), triglycerides ($P = 0.001$), phospholipids ($P < 0.001$), apoA-I ($P = 0.045$), and apoB ($P = 0.001$) concentrations.
[e]Dietary fat and cholesterol had significant interactions on plasma cholesterol ($P = 0.040$), triglycerides ($P = 0.006$), phospholipids ($P = 0.027$), apoA-I ($P < 0.001$), and apoB ($P < 0.008$) concentrations.

triglyceride levels. Coconut oil increased plasma triglycerides (P = 0.001) and phospholipids (P = 0.000) in both high and low LDL responders. Cholesterol increased phospholipids on the coconut oil diet, but did not increase phospholipids to the same extent on the corn oil diet (fat and cholesterol interaction, P = 0.040). Cholesterol increased triglycerides only on the coconut oil diet (Table 3), but the increase in triglyceride was not remarkable.

Dietary cholesterol increased plasma apoA-I levels (P = 0.000) on both coconut oil and corn oil diets, and high responders tended to have higher levels (phenotype effect, P = 0.078). On the coconut oil diet, the highest apoA-I concentration in the plasma was attained with 0.45 mg cholesterol/kcal, but on the corn oil diet the highest apoA-I concentration in the plasma was attained with 0.15 mg cholesterol/kcal. Dietary cholesterol and fat increased plasma apoB levels in both high and low responders (cholesterol effect, P = 0.000 and fat effect, P = 0.001) and high responders had higher plasma apoB levels than low responders (P = 0.059). Dietary cholesterol increased plasma apoB levels more with coconut oil diet than with corn oil diet (fat and cholesterol interaction, P = 0.008).

Overall, there was a biphasic response in plasma cholesterol levels to increasing doses of dietary cholesterol regardless of the type of fat. Plasma cholesterol levels increased when animals were changed from chow with 0.03 mg/kcal cholesterol and 10% of calories from fat to diets with 0.05 mg/kcal cholesterol and 40% of the calories from fat; increased more with 0.15 mg/kcal cholesterol combined with both types of fat; declined slightly with 0.45 mg/kcal cholesterol and both types of fat; and increased again, but only moderately, with 1.35 mg/kcal cholesterol combined with both types of fat. Thus, there was no consistent dose-response relationship with increasing amounts of dietary cholesterol with either corn oil or coconut oil.

Effects of diet and phenotype on plasma apoB-containing lipoproteins

VLDL+IDL cholesterol (**Fig. 1A**) and apoB (Fig. 1C) did not increase in high and low responding baboons fed increasing amounts of dietary cholesterol in corn oil. In contrast, both cholesterol and apoB in VLDL+IDL increased in baboons fed increasing amounts of cholesterol in coconut oil (fat and cholesterol interaction, $P \leq 0.011$).

LDL cholesterol (Fig. 1B) and LDL apoB (Fig. 1D) were higher (P = 0.04 and P = 0.055, respectively) in high responding baboons than in low responding baboons on both diets and at each level of cholesterol. There was no appreciable increase in LDL cholesterol or apoB in high and low responders fed increasing amounts of dietary cholesterol in the corn oil diet. However, when baboons were fed increasing amounts of dietary cholesterol with coconut oil, LDL cholesterol and apoB increased in both phenotypes, but the increase was much greater in high responders than in low responders (cholesterol and phenotype interaction, $P \leq 0.012$).

There was no difference between high and low responders in VLDL+IDL (**Fig. 2A**) and LDL (Fig. 2B) triglyceride concentrations and VLDL+IDL (Fig. 2C) and LDL (Fig. 2D) phospholipid concentrations. However, VLDL+IDL and LDL triglyceride concentrations increased when baboons were fed increasing doses of dietary cholesterol ($P \leq 0.014$), and the increase was greater on the coconut oil diet than on the corn oil diet (fat and cholesterol interaction, P = 0.000).

Effect of diet and phenotype on hepatic mRNA levels

Hepatic mRNA levels for apoB (**Fig. 3A**), apoE (Fig. 3B), and apoC-II (data not shown) were not affected by phenotype, dietary cholesterol, or type of fat. There was a fat by cholesterol interaction for hepatic apoE mRNA levels (P = 0.118), an observation suggesting that hepatic apoE mRNA levels were increased by dietary cholesterol with one type of fat (coconut oil). Hepatic mRNA levels for HMG-CoA synthase increased on the corn oil diet, and decreased on the coconut oil diet (P = 0.001) in both phenotypes (data not shown). Hepatic LDL receptor mRNA levels did not change on the corn oil diet in both phenotypes. On the coconut oil diet, however, hepatic LDL receptor levels increased at the lower dietary cholesterol loads in low responding baboons, but then declined to baseline and to the same level as in high LDL responders after 14 weeks (data not shown).

Experiment 2. Dose-response for dietary cholesterol with chow intervals

The inconsistent response of plasma and LDL cholesterol levels to successively increasing doses of dietary cholesterol—that is, the initial peak after 0.15 mg/kcal, followed by a decline after 0.45 mg/kcal, and followed by an increase after 1.35 mg/kcal—raised the possibility that there may have been a carryover effect of feeding cholesterol and saturated fatty acids on the responses to increasing dietary cholesterol. We designed the second experiment to determine the response of plasma and LDL cholesterol to increasing doses of dietary cholesterol with each dose administered following a prolonged (12 week) basal (washout) period on chow.

The results of this second experiment, in which chow intervals were interspersed between each challenge diet, are illustrated in **Fig. 4.** At the end of each 12-week chow period, plasma and LDL cholesterol and apoB levels returned to expected baseline levels in both phenotypes. Plasma cholesterol concentrations increased significantly (P = 0.001, Fig. 4A) in both high and low responding phenotypes after the animals consumed the fat-enriched diet with no added cholesterol, and the high responding phenotype increased more than the low responding phe-

notype (P = 0.02). The addition of 0.15 mg/kcal cholesterol did not increase plasma cholesterol further, but the addition of 0.45 and 1.35 mg/kcal increased the plasma cholesterol concentrations of both phenotypes. The high responding phenotype consistently maintained higher plasma cholesterol levels than the low phenotype.

LDL cholesterol levels (Fig. 4B) responded as did plasma cholesterol levels, except that LDL cholesterol increased (P < 0.05) with the addition of 0.15 mg/kcal cholesterol to coconut oil in high responders. Further increases in dietary cholesterol increased LDL cholesterol significantly (P = 0.009), and high responders tended to have a greater (P = 0.09) increase than did low responders. High responding baboons consistently had

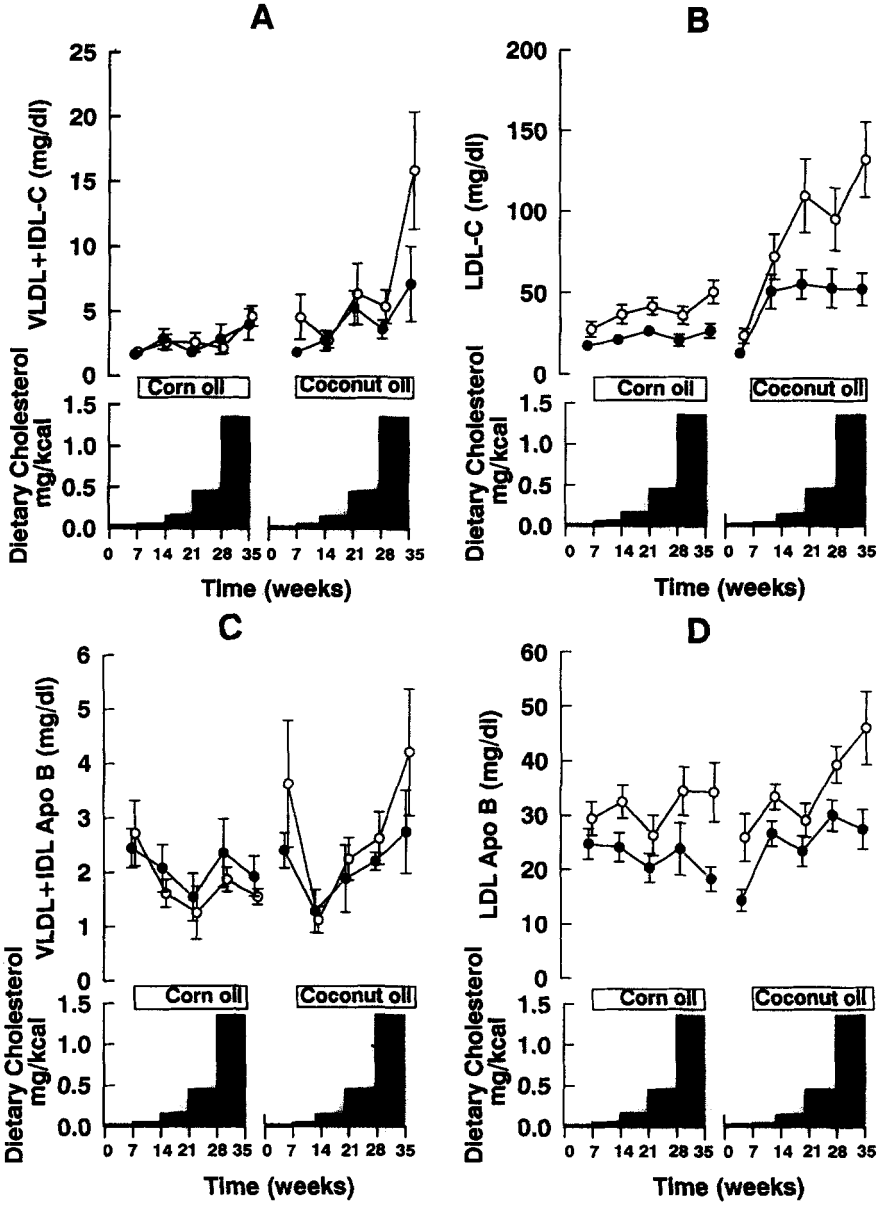

Fig. 1. Means and standard errors for VLDL+IDL cholesterol (A), LDL cholesterol (B), VLDL+IDL apoB (C), and LDL apoB (D) concentrations for high (○) and low (●) responding baboons after 6 weeks on each diet. The baboons were fed a low cholesterol, low fat chow during the first 7 weeks; thereafter, they were fed diet with either corn oil or coconut oil and increasing doses of cholesterol as shown in the lower part of the graph. The phenotype had significant effects on LDL cholesterol and apoB concentrations ($P \leq 0.055$). Dietary cholesterol had significant effects on VLDL+IDL and LDL cholesterol and apoB concentrations (P < 0.001). Dietary fat had significant effects on VLDL+IDL cholesterol (P = 0.001), and LDL cholesterol and apoB concentrations ($P \leq 0.001$). There were significant phenotype and cholesterol interactions on VLDL+IDL and LDL cholesterol ($P \leq 0.012$), and LDL apoB (P = 0.006) concentrations. There were fat and cholesterol interactions on VLDL+IDL cholesterol and apoB ($P \leq 0.011$), and LDL apoB (P = 0.025).

higher LDL cholesterol levels at each dose of dietary cholesterol (including no cholesterol) than did low responding baboons ($P < 0.016$).

LDL apoB concentrations also tended to increase with each increase in dietary cholesterol ($P = 0.057$) (Fig. 4C), and the two phenotypes had similar increases with each increase in dietary cholesterol. The coconut oil diet without added cholesterol did not raise apoB concentrations in either phenotype, but the addition of 0.15 mg/kcal cholesterol produced a substantial rise in both phenotypes.

DISCUSSION

Summary of results

These studies were conducted to assess the dose-response relationship of plasma LDL cholesterol levels to

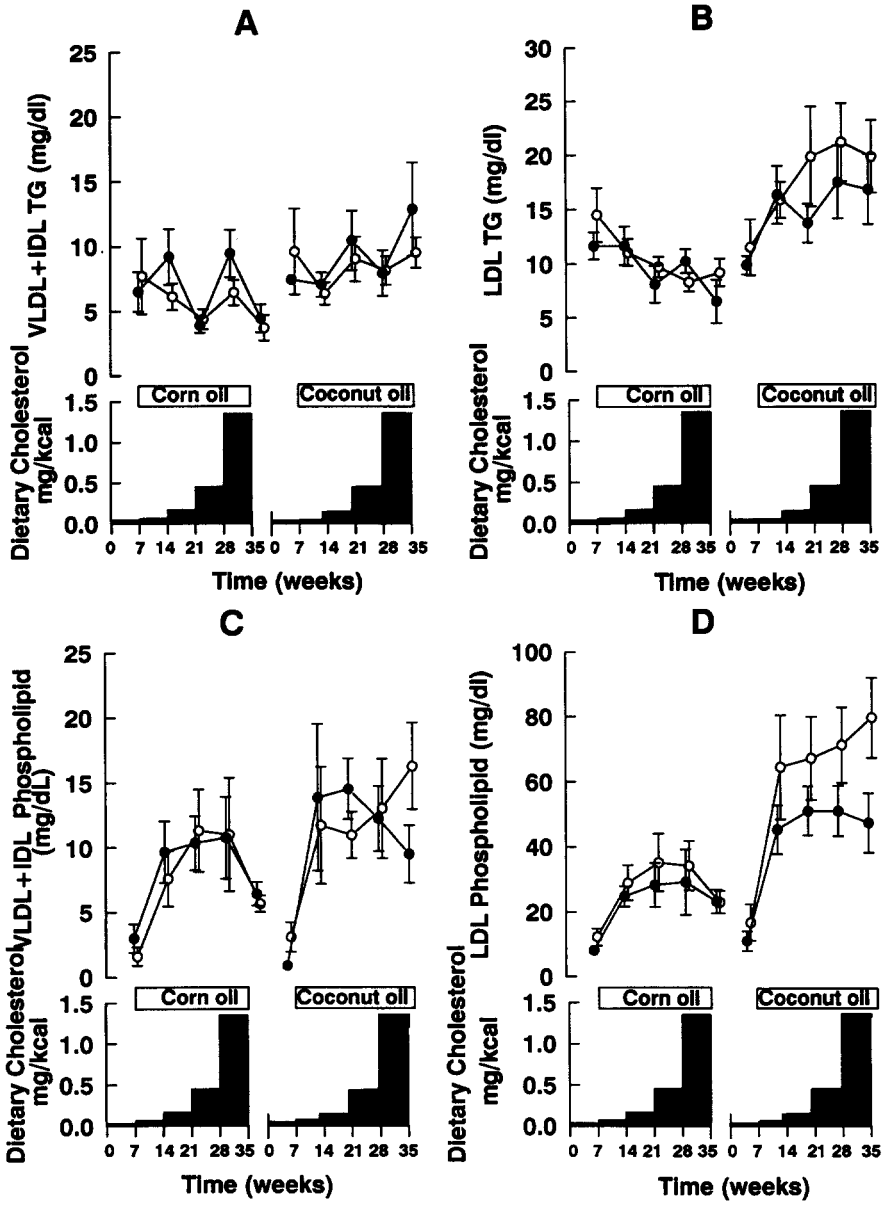

Fig. 2. Means and standard errors for VLDL+IDL triglyceride (A), LDL triglyceride (B), VLDL+IDL phospholipid (C), and LDL phospholipid (D) concentrations for high (○) and low (●) responding baboons. Diets and diet sequence are identical to those in Fig. 1. Dietary cholesterol had significant effects on VLDL+IDL and LDL triglycerides ($P \leq 0.014$) and VLDL+IDL phospholipid ($P = 0.071$) concentrations. Dietary fat had significant effects on VLDL+IDL and LDL triglyceride and phospholipid concentrations ($P \leq 0.018$). There was a phenotype by cholesterol interaction of LDL triglyceride concentration ($P = 0.009$). There were fat by cholesterol interactions on VLDL+IDL and LDL triglyceride concentrations ($P < 0.001$).

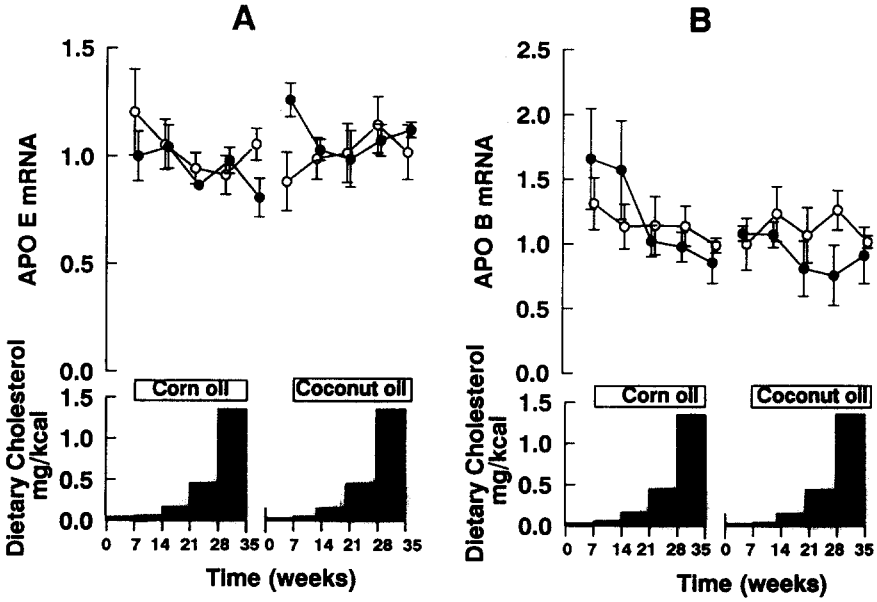

Fig. 3. Means and standard errors for hepatic mRNA levels (relative units) for apoE (A), apoB (B) in high (○) and low (●) responding baboons. Diets and diet sequence are identical to those in Fig. 1. There was a fat by cholesterol interaction on hepatic apoE mRNA levels (P = 0.118).

dietary cholesterol intake, and the interactions between dietary cholesterol and type of dietary fat in selectively bred high and low LDL responding baboons.

When both high and low LDL responding baboons, previously maintained on a high carbohydrate, low fat, low cholesterol (0.03 mg/kcal) chow diet, were initially challenged with a high saturated fatty acid, similarly low cholesterol (0.05 mg/kcal) diet, LDL cholesterol levels increased, but slightly more in the high responders. A threefold increase in dietary cholesterol (0.15 mg/kcal) with the same level of saturated fatty acids further increased LDL cholesterol concentrations only in high responders but not in low responders. Paradoxically, with two successive threefold increments in dietary cholesterol (to 0.45 and 1.35 mg/kcal) added to the same level of saturated fatty acids, LDL cholesterol remained almost stable in both high and low responders, though at different absolute levels.

The lack of consistent response in Experiment 1 to increasing doses of dietary cholesterol with the same level of saturated fatty acids suggested that responses to a change in dietary cholesterol intake depend on prior exposure to dietary cholesterol and fat. When a 12-week baseline period on chow (a low cholesterol and low fat diet) was inserted between each dose of dietary cholesterol in Experiment 2, there was a consistent response of plasma and LDL cholesterol levels to the dose of dietary cholesterol in both high and low responders.

High and low LDL responding baboons did not differ in response of hepatic mRNA levels for apolipoproteins B, E, or C-II or for HMG-CoA synthase. Hepatic LDL receptor mRNA levels unexpectedly increased in low LDL responding baboons on coconut oil diet with the lower dietary cholesterol supplements, but returned to baseline levels at higher dietary cholesterol loads, equal to those in high LDL responding baboons. The significance of this paradoxical increase in hepatic LDL receptor message in low LDL responding baboons on coconut oil diet cannot be ascertained in the absence of measures of LDL transport, which was not a part of the current investigation.

Role of apolipoprotein production in dietary response

Dietary cholesterol elevated plasma levels of apoB when fed with saturated fat, but the mRNA levels for apoB were not increased in either high or low LDL responding baboons. These results are consistent with those reported earlier for another pair of contrasting phenotypes, high and low HDL_1 baboons (17), in which the increase in plasma and LDL apoB was not associated with increased apoB message. These observations suggest that the increase in plasma and LDL apoB concentrations when cholesterol is fed with saturated fatty acids may be due to decreased catabolism or to the regulation of apoB production at the posttranscriptional level. Hepatic apoE transcript levels were modestly raised by dietary cholesterol with saturated fatty acids, but not by dietary cholesterol with polyunsaturated fatty acids. These observations are consistent with studies in other nonhuman primate species (18). Thus, the increase in apoE levels in plasma from baboons on a saturated fatty acid and cholesterol enriched

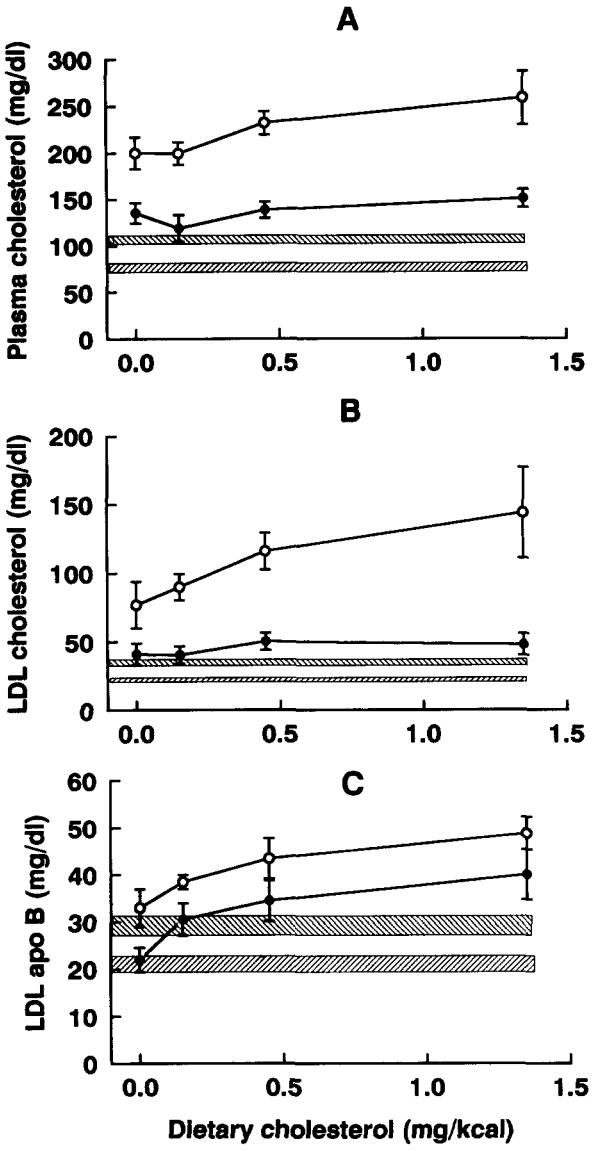

Fig. 4. Means and standard errors for plasma cholesterol (A), LDL cholesterol (B), and LDL apoB (C) with increasing levels of dietary cholesterol with coconut oil in high (○) and low (●) responding baboons. During chow periods (between high fat diets with increasing cholesterol levels), plasma and LDL cholesterol, and LDL apoB returned to baseline levels (mean ± SE shown by bar graph) in both high ▧ and low ▨ responding baboons.

diet reported earlier (13) may be due to an increase in apoE message. In the present study apoC-II and apoA-I messages in liver were not affected by cholesterol in either the coconut oil or the corn oil diet.

Mechanisms involved in response to dietary lipids

Hepatic mRNA levels for apolipoproteins, LDL receptor, and HMG-CoA synthase did not differ between high and low LDL responding baboons after 14 weeks. These observations suggest either that the differing responses to dietary fat and cholesterol are unrelated to the transcription or stability of these transcripts or complex adaptations in intracellular lipid metabolism attenuate differences in hepatic lipid loading. Such adaptations may be mediated by changes in hepatic cholesterol excretion through the bile. We have observed a marked increase in hepatic sterol 27-hydroxylase activity, and in plasma and liver 27-hydroxycholesterol levels, in low LDL responding baboons as compared to high LDL responding baboons when challenged with HCHF diet (19). Sterol 27-hydroxylase, which degrades cholesterol in extrahepatic tissues as well as in the liver, is regulated independently of 7α-hydroxylase (20) and may represent an alternate pathway for hepatic removal of cholesterol via bile acid synthesis (21).

Overturf et al. (22) described a strain of rabbits, resistant to diet-induced hypercholesterolemia and atherosclerosis, in which secretion of bile acids was greater than that of normal rabbits. Low responding baboons may be similar to rabbits that are resistant to diet-induced hypercholesterolemia and atherosclerosis and may have increased bile acid secretion mediated by 27-hydroxylase in the liver. Further studies are needed to test this hypothesis.

Implications of results for human dietary cholesterol effects

The ultimate goal of these and other experiments with selectively bred baboons is to provide insight into the controversial issue of dietary cholesterol as a contributor to diet-induced hypercholesterolemia in humans. There is considerable individual variability in the responses of individuals to dietary cholesterol and saturated fatty acids, and it would be useful to find a simple marker by which to identify such persons.

The results indicate that the nature and duration of the preceding diet, that is, the baseline diet from which responses are assessed, influences the magnitude of the response and may also influence the physiological mechanisms involved in the response. Experiments in humans that used zero or very low cholesterol diets as a baseline (23–25) usually found responses of plasma cholesterol to dietary cholesterol; while those that increased cholesterol intake above moderate levels often found little or no effect (26–28). A diet rich in saturated fatty acids, even with small amounts of cholesterol, may stimulate the adaptive mechanisms controlling cholesterol homeostasis so that increased dietary cholesterol produces little or no effect on plasma cholesterol levels, as seen in these baboons in the first experiment. This phenomenon may account for the paradoxical inverse relationship between responsiveness to dietary cholesterol and prior habitual intake of cholesterol found in humans by Katan and Beynen (29).

One of the incongruities between diet-induced hyperlipidemia in humans and that in experimental animals is the dominant effect of dietary saturated fatty acids in humans as contrasted with the dominant effect of dietary

cholesterol in experimental animals. Hegsted et al. (30) recently reevaluated the experimental human data, revised the predictive equation for the effects of fatty acids and cholesterol on plasma cholesterol concentrations, and developed a predictive equation for LDL cholesterol concentrations.

With the change from chow to zero cholesterol, coconut oil diet, equation 3 from Hegsted et al. (30) predicts an increase in plasma cholesterol concentration of 72 mg/dl, about midway between the increase observed in high responding baboons (94 mg/dl) and the increase observed in low responding baboons (59 mg/dl). Equation 8 (ref. 30) predicts an increase in LDL cholesterol concentrations about 40% higher than the increase observed in high responding baboons. Thus, baboon plasma cholesterol concentrations respond to saturated fatty acids as human plasma cholesterol concentrations respond, but the baboon carries less cholesterol in LDL than the human.

Equation 3 from the same reference (30) predicts that increasing dietary cholesterol from zero to 0.45 mg/kcal increases plasma cholesterol concentration by 30 mg/dl, about the same amount as that observed in the high responding baboons (32 mg/dl); and equation 8 predicts that the same increase in dietary cholesterol increases LDL cholesterol concentrations by 20 mg/dl, about midway between the increase observed in high responding baboons (36 mg/dl) and that observed in low responding baboons (9 mg/dl). Thus, as found in a previous experiment (10), selective breeding based on total plasma cholesterol levels produced a greater divergence between high and low lines in the response of LDL cholesterol to dietary cholesterol than in the response of LDL cholesterol to saturated fatty acids.

Responsiveness to dietary cholesterol in humans is linear at low to moderate levels of intake, and plateaus at high cholesterol intakes (30). The same phenomenon is apparent in the responsiveness of the baboon, as illustrated in Fig. 4.

Genetically determined individual variability is likely to have contributed to the widely varying effects of dietary cholesterol on plasma cholesterol levels among individual humans (29, 31, 32). The results of this experiment with selectively bred divergent phenotypes illustrate the potential for genetically mediated variability among individuals. The differing responses to saturated fatty acids between high and low LDL responding baboons also is consistent with the variability observed in responses of humans to saturated fatty acids (33); and with the correlation between responses to saturated fatty acids and dietary cholesterol observed in humans (34, 35).

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