Influence of dietary lipids on hepatic mRNA levels of proteins regulating plasma lipoproteins in baboons with high and low levels of large high density lipoproteins

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Abstract Selective breeding of baboons has produced families with increased plasma levels of large high density lipoproteins (HDL_1) and very low (VLDL) and low (LDL) density lipoproteins when the animals consume a diet enriched in cholesterol and saturated fat. High HDL_1 baboons have a slower cholesteryl ester transfer, which may account for the accumulation of HDL_1, but not of VLDL and LDL. To investigate the mechanism of accumulation of VLDL+LDL in plasma of the high HDL_1 phenotype, we selected eight half-sib pairs of baboons, one member of each pair with high HDL_1, the other member with little or no HDL_1 on the same high cholesterol, saturated fat diet. Baboons were fed a chow diet and four experimental diets consisting of high and low cholesterol with corn oil, and high and low cholesterol with lard, each for 6 weeks, in a crossover design. Plasma lipids and lipoproteins and hepatic mRNA levels were measured on each diet. HDL_1 phenotype, type of dietary fat, and dietary cholesterol affected plasma cholesterol and apolipoprotein (apo) B concentrations, whereas dietary fat alone affected plasma triglyceride and apoA-I concentrations. HDL_1 phenotype and dietary cholesterol alone did not influence hepatic mRNA levels, whereas dietary lard, compared to corn oil, significantly increased hepatic apoE mRNA levels and decreased hepatic LDL receptor and HMG-CoA synthase mRNA levels. Hepatic apoA-I message was associated with cholesterol concentration in HDL fractions as well as with apoA-I concentrations in the plasma or HDL. However, hepatic apoB message level was not associated with plasma or LDL apoB levels. Total plasma cholesterol, including HDL, was negatively associated with hepatic LDL receptor and HMG-CoA synthase mRNA levels. However, compared with low HDL_1 baboons, high HDL_1 baboons had higher concentrations of LDL and HDL cholesterol at the same hepatic mRNA levels. These studies suggest that neither overproduction of apoB from the liver nor decreased hepatic LDL receptor levels cause the accumulation of VLDL and LDL in the plasma of high HDL_1 baboons. These studies also show that, in spite of high levels of VLDL+LDL and HDL_1, the high HDL_1 baboons had higher levels of mRNA for LDL receptor and HMG-CoA synthase. This paradoxical relationship needs further study to understand the pathophysiology of VLDL and LDL accumulation in the plasma of animals with the high HDL_1 phenotype.—**Kushwaha, R. S., C. A. McMahan, G. E. Mott, K. D. Carey, C. A. Reardon, G. S. Getz, and H. C. McGill, Jr.** Influence of dietary lipids on hepatic mRNA levels of proteins regulating plasma lipoproteins in baboons with high and low levels of large high density lipoproteins. *J. Lipid Res.* 1991. **32:** 1929–1940.

Supplementary key words apolipoprotein A-I • apolipoprotein E • corn oil • dyslipoproteinemia • lard • polyunsaturated fat • saturated fat

We have described a number of dyslipoproteinemic families in baboons (1–4). One dyslipoproteinemia is characterized by the presence of high concentrations of large high density lipoproteins (HDL), called HDL_1, in the plasma of baboons fed a high cholesterol and high saturated fat diet (2, 5). The HDL_1 particles that accumulate in the plasma of HDL_1 baboons are heterogeneous in size and composition and are enriched in cholesteryl esters (6, 7). These particles contain mainly apolipoprotein (apo) A-I, but some also contain apoE. Metabolic variables associated with the accumulation of HDL_1 in the plasma include a slower transfer of cholesteryl ester from HDL to VLDL and LDL that is not due to a deficiency of plasma cholesteryl ester transfer protein, but rather to inhibition of its activity (7). These high HDL_1 baboons also accumulate increased amounts of VLDL and LDL in their plasma when fed a high cholesterol and saturated fat diet (5), a response that cannot be explained by the slower plasma cholesteryl ester transfer. Type of dietary fat modulates apoA-I secretion and mRNA levels in the liver

Abbreviations: LC-C, low cholesterol, corn oil; HC-C, high cholesterol, corn oil; LC-L, low cholesterol, lard; HC-L, high cholesterol, lard.

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but not in the intestine (8). Hepatic mRNA levels are associated with plasma apoA-I concentrations (8), and dietary cholesterol affects intestinal apoB mRNA levels (9). Thus, a likely hypothesis is that the expression of the high HDL_1 phenotype on a cholesterol- and saturated fat-enriched diet results from overproduction of apolipoproteins or underproduction of LDL receptor, or both. The present studies were conducted to determine whether overproduction of apolipoproteins A-I, E, and B in response to dietary cholesterol and saturated fat leads to the excessive accumulation of LDL and HDL_1 in the plasma of high HDL_1 baboons (10, 11). We also investigated whether the underproduction of LDL receptor leads to the plasma accumulation of VLDL, LDL, and large HDL with apoE in high HDL_1 baboons.

METHODS

Animals

Eight half-sib pairs of male adult baboons (*Papio* sp.) were selected for these studies. Four half-sib pairs were progeny of sire X102 and the other four the progeny of sire X1672. These sires expressed the HDL_1 phenotype and transmitted this trait to their progeny. The subjects were similar in age and body weight. Because a previous experiment had shown no sex difference in dietary induction of HDL_1 (5), we used only males in this experiment. One-half of the animals from each sire exhibited the HDL_1 phenotype on the HCSF diet and the other animals exhibited very low levels of HDL_1 on the same diet. Phenotypes were determined based on the presence or absence of an HDL_1 peak in lipoproteins separated by high performance liquid chromatography (HPLC) after a 7-week dietary challenge with a high cholesterol, lard diet (2). All baboons were progeny of different dams. The characteristics of baboons used for this study, including their body weights at the beginning and end of the experiment, are described in **Table 1**. The baboons were housed in indoor-outdoor cages in groups of two and were fed once daily ad libitum. They had access to water at all times. 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.

Diets

Prior to the experiment, baboons were maintained on a chow diet, which is low in both cholesterol (0.03 mg/kcal) and fat (10% of total calories). There were four experimental diets: low cholesterol and corn oil (LC-C), high cholesterol and corn oil (HC-C), low cholesterol and lard (LC-L), and high cholesterol and lard (HC-L) (5). Low cholesterol diets had less than 0.01 mg cholesterol/kcal and high cholesterol diets had 1.0 mg cholesterol/kcal. The experimental diets were made by mixing a basic chow with fat, a vitamin mixture, and cholesterol as described in **Table 2**. The ingredients for experimental diets were mixed with water and pelleted, and the feed was stored in a freezer. Lard and corn oil used for all diets were from the same batch. The major fatty acids of lard and corn oil are described in the footnote to Table 2.

Experimental design

High and low HDL_1 baboons were divided into four blocks (two high and two low HDL_1 baboons in each block). Each block had two progeny of each sire: one was of the high HDL_1 phenotype and the other of the low HDL_1 phenotype. Each block was randomly assigned to a diet sequence given in **Table 3**. Each diet was fed for 7 weeks. One of the four diets was repeated in each block to estimate better the carryover effects. Fasting blood samples were obtained from each animal after 6 weeks on each diet. Thus, each animal was bled seven times (twice on the chow diet, four times on the experimental diets,

TABLE 1. Selected characteristics of baboons used in this study

					Body Weight (kg)			Cholesterol (mg/dl)[b]			
			Age (yr)[a]		Period 1[c]		Period 7	Serum	VLDL + LDL	HDL	
Sire	HDL_1	n	Mean	Range	Mean	95% CI[d]	Mean 95% CI	Mean 95% CI	Mean 95% CI	Mean	95% CI
X102	Low	4	8.6	(6.5-11.5)	27.2	(23.1-32.0)	26.8 (23.7-30.2)	197 (162-239)	93 (60-145)	101 (	85-119)
	High	4	7.9	(6.5- 9.3)	30.8	(26.2-36.3)	33.0 (29.3-37.2)	254 (209-308)	110 (71-172)	142	(120-168)
X1672	Low	3	6.8	(6.1- 7.2)	27.3	(22.6-33.0)	29.4 (25.6-33.8)	191 (153-239)	98 (59-163)	78 (	65- 95)
	High	5	7.3	(6.4- 8.5)	26.6	(22.9-30.8)	28.4 (25.5-31.7)	297 (250-354)	182 (123-271)	113 (	98-131)

[a]Age at start of study.
[b]Baboons were surveyed for lipoprotein cholesterol levels following a 7-week HCSF diet at 2-7 years of age.
[c]Period 1 was before the experimental diets were fed and period 7 was after all experimental diets were given.
[d]Confidence intervals.

TABLE 2. Nutritional composition of baboon chow and experimental diets

Nutrients	Chow Diet	Lard Diets[a]	Corn Oil Diets[a]
Carbohydrates (% 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	0.01-1.0	0.01-1.0

[a]Lard and corn oil diets were prepared by mixing 81.4% (dry weight basis) of Purina monkey meal 5-5046-6 (a special mix with dehydrated alfalfa, sodium chloride, and no added fat) with lard 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% for low cholesterol diet and 0.46% for high cholesterol diet). The major fatty acids of the monkey chow were C16:0, 17%, C18:1, 19.7%, and 18:2, 58%; the major fatty acids of the lard diets were C16:0, 22.5%, C18:0, 17%, C18:1, 39% and C18:2, 14%; and the major fatty acids of the corn oil diets were C16:0, 12%, C18:1, 25%, C18:2, 58%.

TABLE 3. Diet sequences

	Diet Sequence			
Time Period	1	2	3	4
1	Chow	Chow	Chow	Chow
2	LC-lard	HC-corn oil	LC-corn oil	HC-lard
3	LC-corn oil	HC-lard	HC-corn oil	LC-lard
4	HC-lard	LC-corn oil	LC-lard	HC-corn oil
5	HC-corn oil	LC-lard	HC-lard	LC-corn oil
6	LC-corn oil	HC-corn oil	LC-lard	HC-lard
7	Chow	Chow	Chow	Chow

LC, low cholesterol; HC, high cholesterol.

and once on the repeated experimental diet). Liver punch biopsies were also obtained at the time of the bleeding. Lipids, lipoproteins, and apolipoproteins in the plasma, and hepatic mRNA levels were measured in individual samples.

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, the animals were weighed and three 25-mg cores of liver were aspirated by standard punch biopsy procedures. The three liver cores were wrapped in aluminum foil, labeled with animal number and date, quick-frozen in liquid nitrogen, and stored at −70°C. Liver samples from two baboons not involved in this experiment were obtained at necropsy, frozen, and stored at −70°C in small pieces to be used as controls.

Cholesterol and triglyceride measurements

Cholesterol and triglycerides in plasma and cholesterol in lipoproteins were measured by enzymatic methods with the ABA 100 Bichromatic Analyzer (Abbott Laboratories, South Pasadena, CA) as described by Allain et al. (12) and Mott and Rogers (13). Serum HDL cholesterol (HDL-C) was measured after precipitation of VLDL and LDL with heparin-manganese according to the Lipid Research Clinics procedure (14). VLDL+LDL cholesterol (VLDL+LDL-C) was calculated as the difference between the total serum cholesterol and HDL-C concentrations. These methods met the criteria of the Centers for Disease Control Lipid Standardization Program.

Detection of HDL_1

To detect the presence of HDL_1 in plasma, lipoproteins (d 1.21 g/ml) were isolated by ultracentrifugation using a 50 Ti rotor and ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 125,000 *g* for 24 h at 6°C. The lipoproteins were harvested by pipetting the upper 2 ml and further separated by HPLC (2). Animals with a peak corresponding to HDL_1 were designated high HDL_1 baboons and those with no peak in the HDL_1 region were designated low HDL_1 baboons. This was a qualitative measurement. The presence of an HDL_1 peak was also confirmed by the separation of plasma lipoproteins by gradient gel electrophoresis using 2% to 16% polyacrylamide slab gels (15) as described earlier (5).

Separation of lipoproteins by density gradient ultracentrifugation

Blood samples collected at the end of each dietary period were centrifuged at low speed to obtain plasma. Lipoproteins were separated by density gradient ultracentrifugation using an SW 41 Ti rotor in a Beckman ultracentrifuge Model L5-50 or L8-70. The density gradient procedure was a modification of the method of Redgrave, Roberts, and West (16) described previously in detail (5). The refractive index was measured and the fractions were pooled on the basis of their densities as described previously (5). The fractions between d 1.042 and 1.068 g/ml were pooled as the HDL_1 fraction. Other fractions pooled were VLDL+IDL (d < 1.019 g/ml), LDL (d 1.020–1.041 g/ml), HDL_{2+3} (d 1.069–1.21 g/ml), and a nonlipoprotein fraction (d > 1.21 g/ml).

Apolipoprotein analyses

ApoB and apoA-I were measured in plasma and in lipoprotein fractions separated by ultracentrifugation by the electroimmunoassay procedure of Laurell (17) as described by Mott et al. (18). The antisera against baboon apoB and apoA-I were prepared in rabbits by injecting purified LDL and apoA-I, respectively. The antisera showed specificity for these apolipoproteins. Baboon serum controls were standardized against purified apoA-I and LDL isolated by ultracentrifugation. The linear range of the assay was between 35 and 470 ng for apoA-I and between 50 and 500 ng for apoB. Samples were diluted to concentrations within these linear ranges. Two baboon serum controls were run in duplicate with each

row of 28 samples. The sample values for each row were calculated from the average peak heights and standardized values of the controls. The coefficient of variation for apoA-I in a control serum pool measured throughout the study was 5.5% and for apoB, 4.8%.

Measurement of hepatic mRNA levels

Liver samples (0.05–0.2 g wet weight) were extracted with guanidine thiocyanate for measurement of total cellular RNA with guanidine thiocyanate (19). The A_{260}:A_{280} ratios were greater than 1.9 and the yield of RNA was 1.0–2.0 mg/g tissue. The integrity of the RNA was determined by Northern blot hybridization (20). We used human cDNA clones for apoE (21) and apoB (22) (corresponding to nucleotides 4649–5289) obtained from Dr. James Scott, MRC, London, U.K.; apoA-I (23), apoC-II (24), and LDL receptor (25) obtained from Dr. David Russell, University of Texas Southwestern Medical School, Dallas, TX; and hamster HMG-CoA synthase cDNA (26) (ATCC) to quantitate mRNA levels in the baboon liver samples on slot blots. The cDNA clones for apolipoproteins E, A-I, and C-II were obtained from Dr. Jan Breslow, Rockefeller University, New York, NY. cDNA inserts were obtained by preparative restriction endonuclease digestion followed by preparative low melt agarose gels. The inserts were labeled by random priming (27) with [α-^{32}P]dCTP (> 3,000 Ci/mmol, Amersham) to a specific activity of 0.5–1.0 $\times$ 10^6 cpm/μg.

Slot blots were prepared as described by Wyne et al. (28). For quantitation of apolipoprotein mRNA levels, at least four aliquots, varying between 0.1 and 1.5 μg of total RNA, were slotted. For quantitation of LDL receptor and HMG-CoA synthase mRNA levels, between 1 and 6 μg of total RNA was slotted. Liver RNA samples from two control baboons were also included on each slot blot. For this purpose, two single baboon liver samples were stored in 100-mg aliquots at −70°C and an aliquot of each was assayed along with each set of samples processed for mRNA determinations. One of these RNAs from control liver samples was used as a reference standard and the second was used as an internal control. Slot blots were hybridized in 50% formamide, 5 $\times$ SSPE, 1 $\times$ Denhardt's solution, 0.1% BSA, 100 μg/ml *E. coli* tRNA, 0.1% SDS, and 5% dextran sulfate at 42°C. The length of time for hybridization and the amount of probe required to reach equilibrium was determined for each probe and varied between 45 and 60 h and 1 $\times$ 10^6 to 5 $\times$ 10^6 cpm/ml, respectively. Blots were washed three times at room temperature in 2 $\times$ SSC with 1 $\times$ Denhardt's solution followed by 3 washes in 0.1 $\times$ SSC with 0.1% SDS at 58°C except for blots probed for apoC-II mRNA which were washed at 55°C.

The autoradiograms of the slot blots were quantitated by densitometric scanning using an LKB scanning densitometer and the slope of the linear portion of the autoradiogram was determined for each sample. The blots were stripped and reprobed a maximum of two times. The correlation coefficients for all of the individual linear regressions of absorbance against RNA mass were better than 0.99. All data are expressed as the ratio of the slope of each individual sample to the slope of the reference standard RNA that was included on each slot blot. This was necessary since multiple slot blots were needed to analyze all the samples. The ratio of the internal standard RNA to the reference standard was also determined on each blot for each probe.

Statistical analyses

The data were analyzed by analysis of variance (29) after logarithmic transformation to satisfy better the assumptions underlying the analysis. The effects of sire, diet sequence, and HDL_1 class were tested against a between-animal error term. For analysis of data obtained during the experimental dietary periods, the effects of time period, diet, sire by diet, HDL_1 class (phenotype) by diet, and carryover were tested against a within-animal error term. The estimated means and confidence intervals were based on robust M-estimates (30). This procedure lessens the effects of outliers in the data. Effects of diet and HDL_1 phenotype are presented as the ratio of the means in the different groups. For example, the multiplicative effect of HDL_1 phenotype is the ratio of the mean, adjusted for diet and sire, for the high HDL_1 phenotype to the adjusted mean for the low HDL_1 phenotype. For each animal, we computed the slope and intercept of the linear regression line of the serum lipoprotein and apolipoprotein concentrations on the mRNA measured in the liver. These slopes and intercepts for the individual animals were then combined in a weighted average. The weights were the inverse of the variance of the coefficients from the linear regression analysis (31). Weighted analysis of variance was used to compare HDL_1 classes. For presentation, we show the slope and the mean serum lipoprotein concentrations after adjusting to the overall mean mRNA level. One baboon was misclassified in the initial measurement of HDL_1, but the data were analyzed according to a later correct classification.

RESULTS

Effects of dietary lipids and HDL_1 phenotype on plasma lipids and apolipoproteins

Fig. 1 shows the mean plasma lipids and apolipoproteins in both high and low HDL_1 phenotypes for each diet. The differences in the levels of these variables between high and low HDL_1 baboons on experimental diets were generally in the same direction as those on the chow, but in several cases were greater in magnitude. Effects of HDL_1 phenotype and diet on plasma variables are

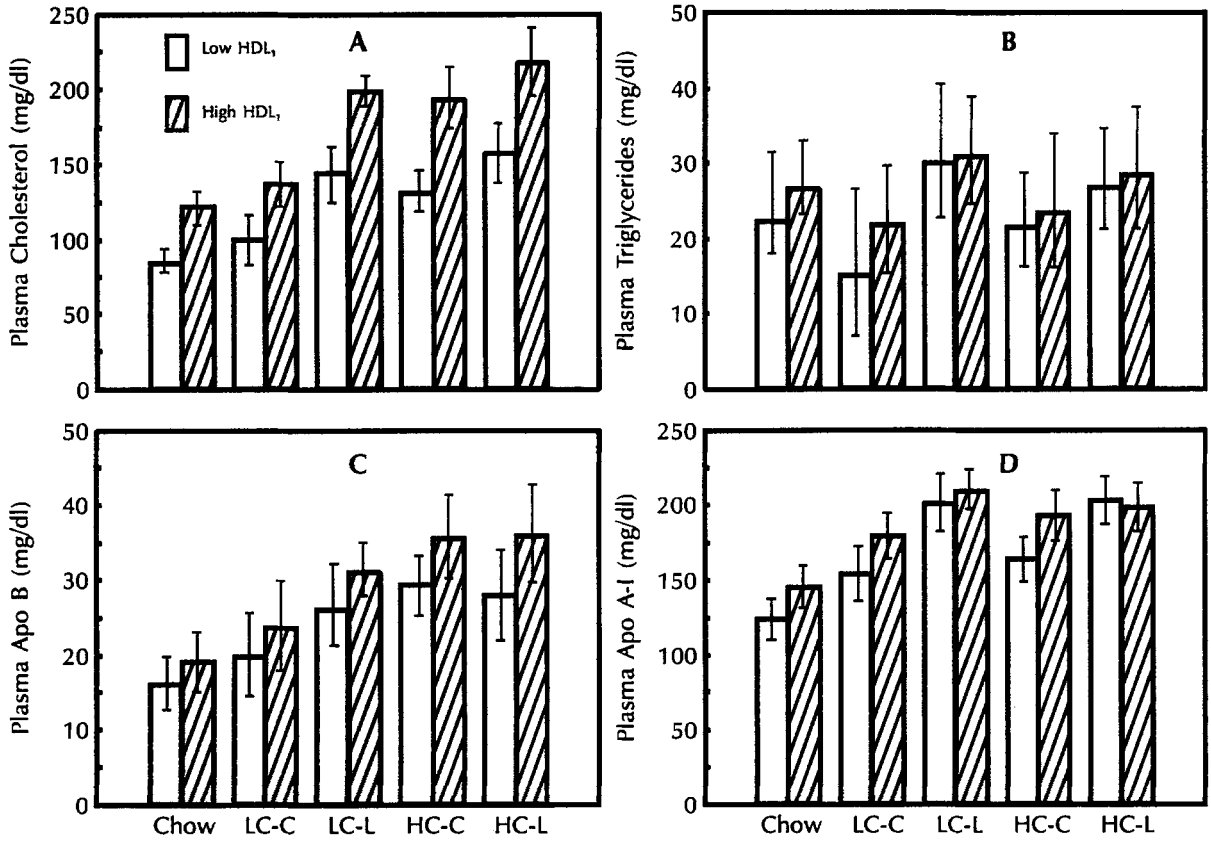

Fig. 1. Means and 95% confidence intervals for plasma cholesterol (A), triglycerides (B), apoB (C), and apoA-I (D) for high and low HDL_1 baboons fed chow, low cholesterol-corn oil (LC-C), low cholesterol-lard (LC-L), high cholesterol-corn oil (HC-C), and high cholesterol-lard (HC-L) diets. Each diet was fed for 7 weeks but blood samples for lipoprotein measurements were obtained after 6 weeks. Significance levels are described in Table 4.

described in **Table 4**. HDL_1 phenotype, dietary cholesterol, and dietary fat significantly increased plasma cholesterol and apoB concentrations. However, only the dietary fat had a significant effect on plasma triglycerides and apoA-I concentrations.

Effects of dietary lipids and HDL_1 phenotype on plasma lipoproteins

Fig. 2 shows mean plasma lipoprotein cholesterol levels for the HDL_1 phenotypes and diets. Table 4 summarizes the effects. As anticipated, the greatest effect of high HDL_1 phenotype was on HDL_1 cholesterol. In contrast, HDL_1 phenotype was not associated with a significant difference in cholesterol in HDL_{2+3}. The changes in cholesterol in lipoproteins due to experimental diets (Fig. 2) paralleled the changes in major apolipoproteins (**Fig. 3**) of these lipoproteins. Lard had a more profound effect on the level of HDL_1 cholesterol in the high HDL_1 phenotype (Fig. 2C). HDL_1 phenotype also had a significant effect on VLDL+IDL and LDL cholesterol and LDL apoB. Dietary cholesterol significantly increased the cholesterol and apoB of the apoB-containing lipoproteins and also significantly increased the cholesterol and apoA-I in HDL_1, but it did not affect apoA-I in the HDL_{2+3} fractions (Table 4).

Interactions among HDL_1 phenotype and dietary lipids

Dietary cholesterol generally had a lesser effect on all plasma and lipoprotein cholesterol and apolipoprotein levels when consumed with lard than with corn oil (multiplicative effects of cholesterol × fat less than 1.00 in Table 4). This attenuation of the effects of dietary cholesterol was greatest for cholesterol and apoB in the lower density lipoproteins, and was less and non-significant for cholesterol and apoA-I in the HDL fractions. A similar but less pronounced attenuation of effects occurred when HDL_1 class and saturated fat were considered together. The effect of lard compared with corn oil on HDL_1 cholesterol was greater in high HDL_1 baboons than in low HDL_1 baboons. The effect of lard compared with corn oil on HDL_{2+3} cholesterol levels was less in high HDL_1 baboons than in low HDL_1 baboons.

Dietary cholesterol generally had similar effects on plasma lipids and lipoproteins in the high and low HDL_1 animals, with the exception that the effect of cholesterol

TABLE 4. Summary of the effects of HDL_1 phenotype, dietary cholesterol, and type of dietary fat on plasma and lipoprotein cholesterol, and plasma and lipoprotein apolipoprotein concentrations

Dependent/Independent Variable	Whole Plasma	VLDL + IDL	LDL	HDL_1	HDL_{2+3}
Cholesterol					
HDL_1 phenotype	1.40^b	1.87^a	1.66^b	2.18^b	1.15
Dietary cholesterol	1.22^b	2.07^b	1.69^b	1.31^b	1.07
Dietary fat	1.30^b	1.25	1.22^a	1.73^b	1.24^b
Dietary cholesterol × dietary fat	0.90^b	0.53^a	0.73^b	0.90	0.96
HDL_1 phenotype × dietary cholesterol	1.02	0.85	1.14	1.07	0.93^a
HDL_1 phenotype × dietary fat	0.99	0.69	1.05	1.24^b	0.92^a
ApoB					
HDL_1 phenotype	1.22^a	1.11	1.30^b		
Dietary cholesterol	1.29^b	1.66	1.40^b		
Dietary fat	1.14^a	1.35	1.13		
Dietary cholesterol × dietary fat	0.86^b	0.34^a	0.77^b		
HDL_1 phenotype × dietary cholesterol	1.02	2.42^a	1.08		
ApoA-I					
HDL_1 phenotype	1.09			3.96^b	1.03
Dietary fat	1.18^b			2.16^b	1.12^b
HDL_1 phenotype × dietary fat	0.93^b			1.16	0.91^b
Triglycerides					
Dietary fat	1.44^b				
Dietary cholesterol × dietary fat	0.85^a				

$^aP < 0.05$.
$^bP < 0.01$.

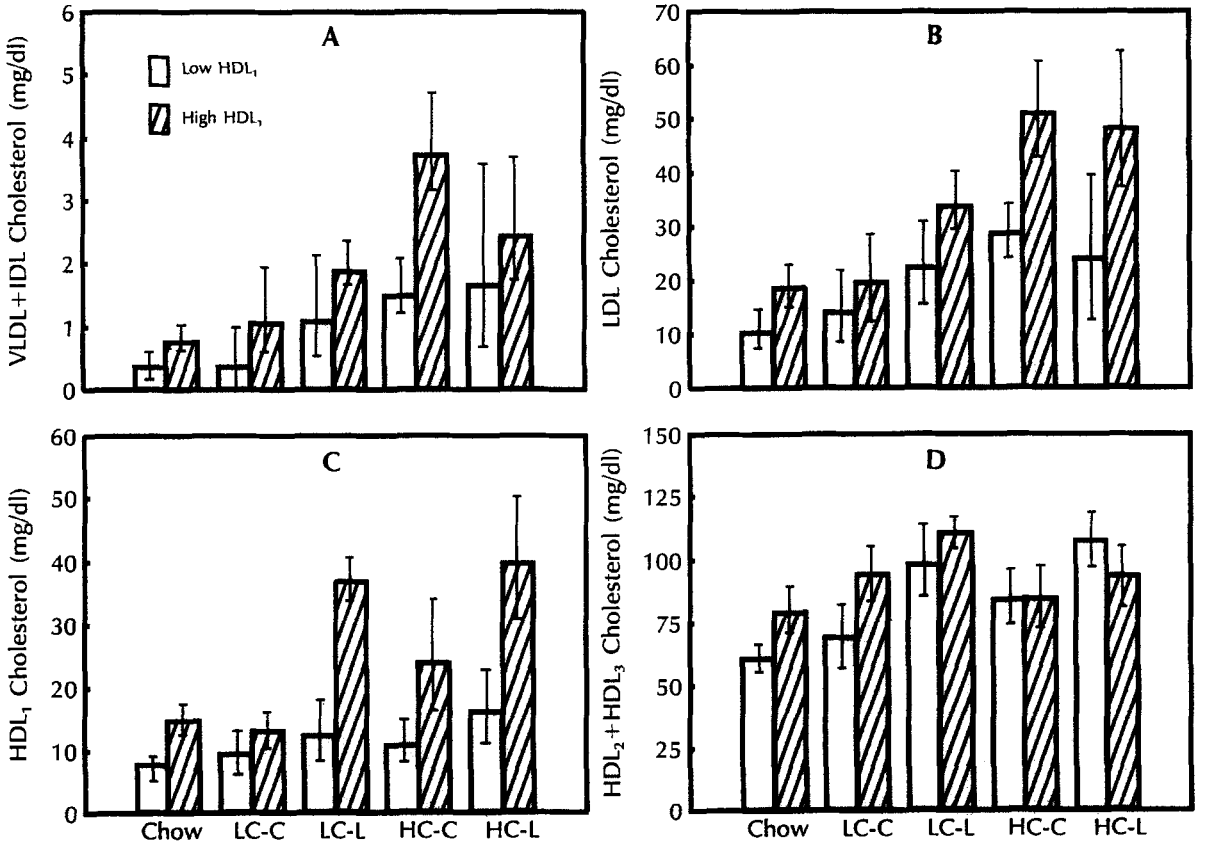

Fig. 2. Means and 95% confidence intervals for cholesterol in VLDL+IDL (A), LDL (B), HDL_1 (C), and HDL_2+HDL_3 for high and low HDL_1 baboons fed chow, low cholesterol-corn oil (LC-C), low cholesterol-lard (LC-L), high cholesterol-corn oil (HC-C), and high cholesterol-lard (HC-L) diets. Each diet was fed for 7 weeks but blood samples for lipoprotein measurements were obtained after 6 weeks. Significance levels are described in Table 4.

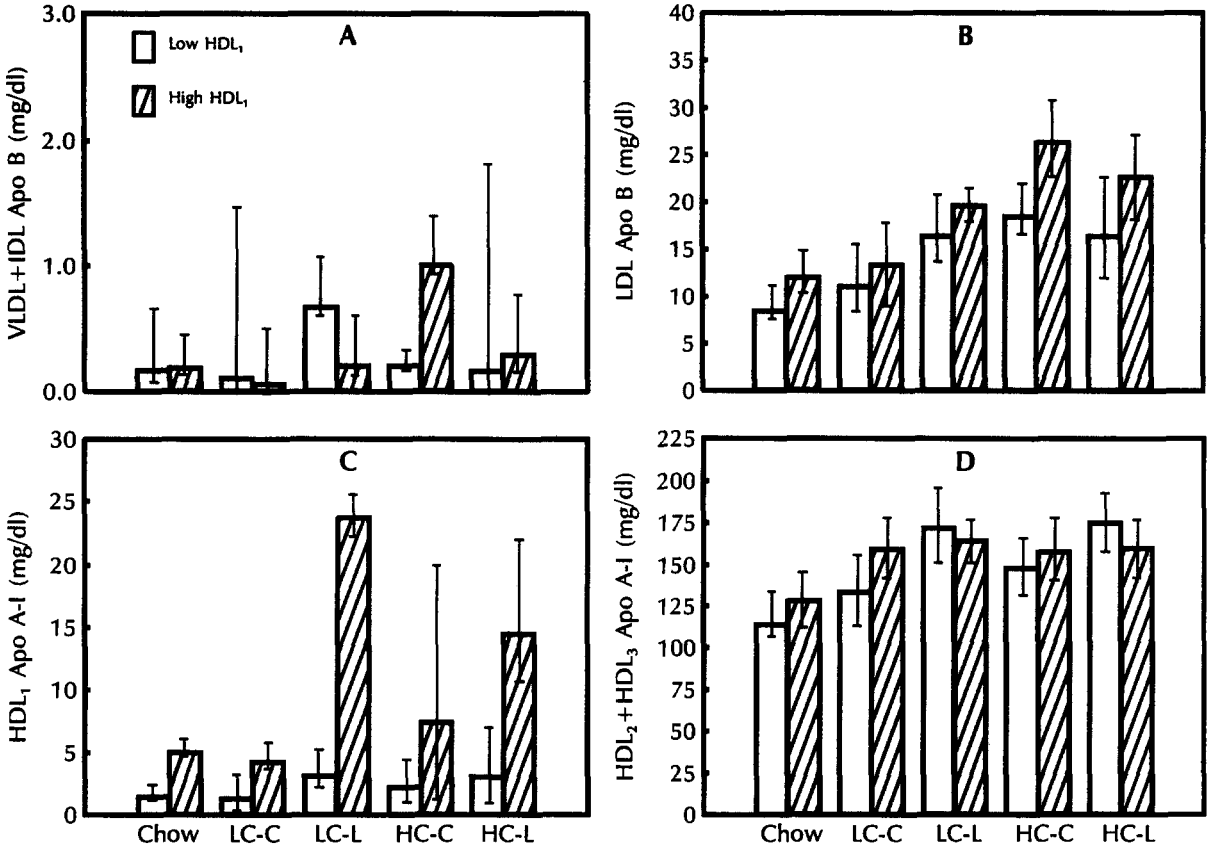

Fig. 3. Means and 95% confidence intervals for VLDL+IDL apoB (A), LDL apoB (B), HDL_1 apoA-I (C), and HDL_2+HDL_3 apoA-I (D) for high and low HDL_1 baboons fed chow, low cholesterol-corn oil (LC-C), low cholesterol-lard (LC-L), high cholesterol-corn oil (HC-C), and high cholesterol-lard (HC-L) diets. Each diet was fed for 7 weeks but blood samples for lipoprotein measurements were obtained after 6 weeks. Significance levels are described in Table 4.

on HDL_{2+3} cholesterol levels was less in high HDL_1 animals than in low HDL_1 animals.

Effects of HDL_1 phenotype and dietary lipids on hepatic mRNA levels

Fig. 4 describes the mean hepatic mRNA levels for apolipoproteins in high and low HDL_1 baboons. **Fig. 5** describes the mean hepatic LDL receptor and HMG-CoA synthase mRNA levels. **Table 5** summarizes the effects of HDL_1 phenotype and diet on hepatic mRNA levels. HDL_1 phenotype and dietary cholesterol had no significant effect on hepatic mRNA levels of apolipoproteins, HMG-CoA synthase, and LDL receptor. Some of the hepatic mRNA levels were significantly affected only by the dietary fat in both high and low HDL_1 baboons. Lard in the diet significantly increased hepatic apoE mRNA levels and decreased hepatic HMG-CoA synthase and LDL receptor mRNA levels. Dietary cholesterol and fat had significant interaction in their effect on hepatic mRNA levels. The effects of dietary cholesterol on message for HMG-CoA synthase were significantly greater when the animals were consuming lard in the diet, as indicated by the significant cholesterol × fat interactions. On the other hand, the effects of dietary cholesterol on message for apoA-I, apoE, and apoC-II were lower when the animals were consuming lard in the diet as compared to corn oil in the diet.

Relationship of plasma lipid and lipoprotein levels to hepatic mRNA levels

Table 6 gives slopes of linear regression of serum lipid concentrations and lipoprotein variables on hepatic mRNA levels. This analysis uses the values derived from whole serum by the precipitation method and those derived from plasma by ultracentrifugation. We included the serum precipitation measurements because the major lipoprotein classes were pooled and the results were not complicated by recovery problems. Total plasma cholesterol and each of its major subfractions were positively correlated with message levels of apolipoproteins. There was significant correlation between apoA-I message level and cholesterol in HDL (precipitation method), HDL_1, and HDL_{2+3}. ApoA-I message was also correlated with apoA-I concentration in whole plasma,

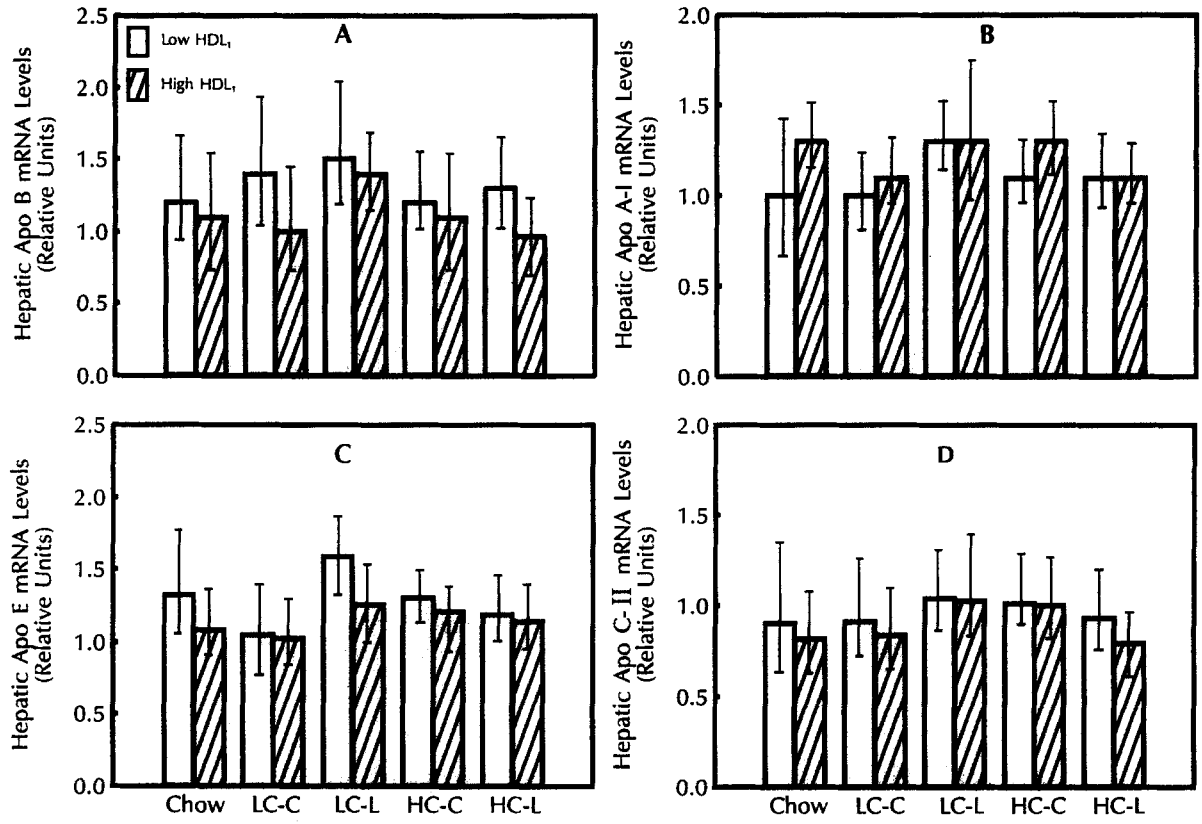

Fig. 4. Means and 95% confidence intervals for hepatic mRNA levels of apoB (A), apoA-I (B), apoE (C), and apoC-II (D) for high and low HDL_1 baboons fed chow, low cholesterol-corn oil (LC-C), low cholesterol-lard (LC-L), high cholesterol-corn oil (HC-C), and high cholesterol-lard (HC-L) diets. Each diet was fed for 7 weeks but liver samples for mRNA measurements were obtained after 6 weeks. Significance levels are described in Table 5.

HDL_1, and HDL_{2+3}. ApoB message was positively correlated with cholesterol in whole plasma and HDL-C. However, hepatic apoB message levels were not correlated with plasma or LDL apoB levels. Hepatic apoE mRNA levels were correlated with cholesterol in whole plasma, VLDL+LDL-C, HDL-C, LDL, and HDL_{2+3}, and apoA-I in whole plasma and HDL_{2+3}. Hepatic apoC-II mRNA levels were not associated with any variable except cholesterol in HDL. There was a consistent significant negative correlation between the concentrations of cholesterol in all lipoprotein fractions, the concentration of apoB and apoA-I levels in the plasma, and the level of hepatic HMG-CoA synthase and LDL receptor messages.

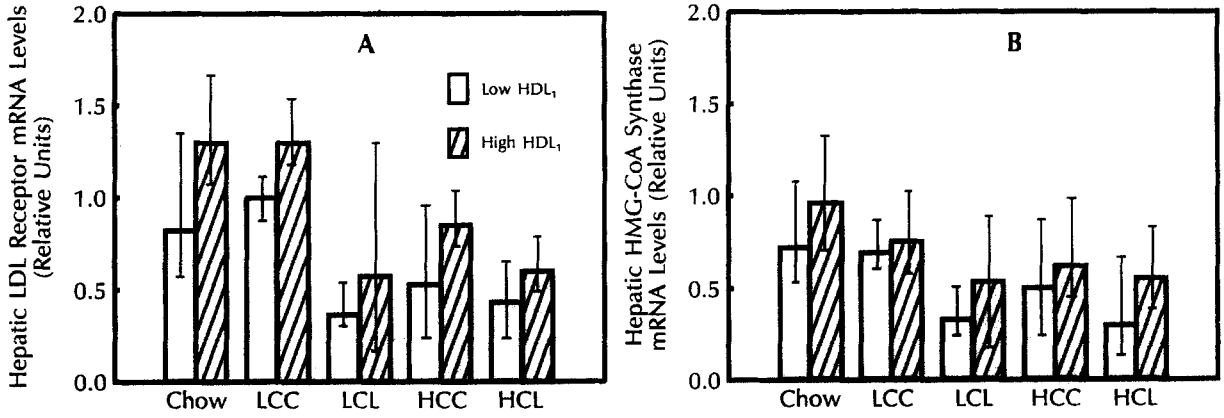

Fig. 5. Means and 95% confidence intervals for hepatic mRNA levels of LDL receptor (A) and HMG-CoA synthase (B) for high and low HDL_1 baboons fed chow, low cholesterol-corn oil (LC-C), low cholesterol-lard (LC-L), high cholesterol-corn oil (HC-C), and high cholesterol-lard (HC-L) diets. Each diet was fed for 7 weeks but liver samples for mRNA measurements were obtained after 6 weeks. Significance levels are described in Table 5.

TABLE 5. Summary of the effects of HDL_1 phenotype, dietary cholesterol, and type of dietary fat on liver mRNA levels

Independent Variable	mRNA					
	ApoA-I	ApoB	ApoE	ApoC-II	HMGS[a]	LDLR[a]
HDL_1 phenotype	1.08	0.83	0.91	0.93	1.47	1.40
Dietary cholesterol	0.99	0.86	1.00	0.98	0.81	0.87
Dietary fat	1.06	1.09	1.12[b]	1.00	0.55[c]	0.66[c]
Dietary cholesterol × dietary fat	0.89[b]	0.89	0.82[c]	0.85[c]	1.37[c]	1.12

[a]HMGS, HMG-CoA synthase; LDLR, LDL receptor.
[b]$P < 0.05$.
[c]$P < 0.01$.

Effect of HDL_1 phenotype on the relationship of hepatic LDL-receptor and HMG-CoA synthase mRNA levels to serum lipid levels

Table 7 shows the slopes of linear regression of serum VLDL+LDL and HDL cholesterol concentrations on hepatic mRNA for HMG-CoA synthase and the LDL receptor, and the mean lipoprotein cholesterol concentrations adjusted to overall mean mRNA levels. The slopes for mRNA for LDL receptor and HMG-CoA synthase in high HDL_1 phenotypes were similar but the mean levels of cholesterol in lipoproteins were different at the same hepatic mRNA levels. High HDL_1 baboons had significantly higher VLDL+LDL-C and HDL-C levels than low HDL_1 baboons at such adjusted message levels. For example, this relationship of cholesterol in HDL-C with hepatic LDL receptor mRNA levels is illustrated in **Fig. 6.** Fig. 6 clearly indicates both the negative relationship between HDL-C and hepatic LDL receptor mRNA levels and the higher HDL-C concentrations in the high HDL_1 phenotype. High HDL_1 baboons had a higher concentration of HDL cholesterol for the same level of hepatic mRNA for LDL receptor. For example, at 1.0 relative unit of hepatic LDL receptor mRNA, high HDL_1 baboons have an HDL cholesterol level of 118 mg/dl, whereas low HDL_1 baboons have an HDL cholesterol level of 95 mg/dl (Fig. 6). Similar relationships exist for whole plasma and LDL cholesterol with hepatic LDL and HMG-CoA synthase mRNA levels when high and low HDL_1 phenotypes are compared.

DISCUSSION

Summary of results

The changes in plasma lipid and lipoprotein levels of different HDL_1 phenotypes in response to modifications of dietary cholesterol and fat confirm similar results reported previously (5). These analyses were done to correlate the measured hepatic transcript levels as a function of diet and HDL_1 phenotype. In spite of significant

TABLE 6. Slope of linear regression of serum lipid, lipoprotein, and apolipoprotein concentrations on mRNA levels

Variable	mRNA					
	ApoA-I	ApoB	ApoE	ApoC-II	HMGS	LDLR
Cholesterol						
Total	26.53	16.28[a]	41.94[b]	25.15	−27.28[b]	−57.11[b]
VLDL + LDL-C	5.81	10.09	24.37[a]	11.42	−14.32[b]	−32.17[b]
HDL-C	20.72[b]	6.19[b]	17.57[a]	13.73[a]	−12.95[b]	−24.94[b]
VLDL + IDL	−0.95	0.31	0.47	0.11	−1.03[b]	−1.91[b]
LDL	6.05	7.73	18.38[a]	9.78	−11.13[b]	−25.47[b]
HDL_1	8.96[a]	3.30[b]	8.62	1.99	−5.12[a]	−11.90[b]
HDL_{2+3}	13.54[a]	5.27	16.37[a]	11.99	−9.80[b]	−17.42[b]
ApoA-I						
Total	28.83[b]	7.44	26.72[b]	23.64	−15.63[b]	−28.71[b]
HDL_1	9.62[b]	3.20	6.31	2.33	−3.12[a]	−8.17[a]
HDL_{2+3}	20.15[a]	4.29	23.23[a]	18.62	−12.23[b]	−18.93[a]
ApoB						
Total	3.78	3.95	7.33	5.86	−5.52[b]	−12.32[b]
VLDL + IDL	0.23	0.18	0.38	0.19	−0.27[a]	−0.59[b]
LDL	3.69	3.62	6.85	5.04	−5.05[b]	−10.71[b]

[a]$P < 0.05$.
[b]$P < 0.01$.

TABLE 7. Slopes and means of linear relationships between lipoprotein cholesterol concentrations and liver mRNA by HDL_1 phenotype

Lipoprotein	HDL_1 Phenotype	mRNA			
		HMGS		LDLR	
		Slope	Mean[a]	Slope	Mean[a]
VLDL + LCL-C	High	−14.6	56.5[b]	−40.5	58.0[c]
	Low	−13.4	30.6	−22.6	31.9
HDL-C	High	−11.7	123.6[b]	−26.6	122.6[c]
	Low	−16.7	100.7	−23.0	96.4
VLDL + IDL	High	−1.0	3.0	−2.4	2.8
	Low	−1.0	1.7	−1.3	1.6
LDL	High	−11.4	42.1	−34.2	42.0[b]
	Low	−10.5	23.9	−15.5	23.8
HDL_1	High	−6.6	28.2[c]	−17.9[b]	30.9[c]
	Low	−0.9	10.4	−5.0	12.3
HDL_{2+3}	High	−7.4[c]	100.1	−12.2	98.1
	Low	−17.0	88.4	−23.4	83.7

[a]Adjusted to HMGS value of 1.05 or LDLR value of 0.72.
[b]High HDL_1 phenotype different from low HDL_1 class ($P < 0.10$).
[c]High HDL_1 phenotype different from low HDL_1 class ($P < 0.05$).

differences in cholesterol and apoB in plasma and lipoproteins, high and low HDL_1 baboons did not have significant differences in mRNA levels for apolipoproteins A-I, B, E, and C-II, regardless of the type of dietary fat or amount of cholesterol consumed. Only the hepatic apoE mRNA levels were significantly affected by the type of fat. ApoE mRNA levels were significantly increased in the livers of baboons fed low cholesterol and lard. In general, the cholesterol level in plasma and lipoproteins was positively associated with hepatic mRNA levels of apolipoproteins. ApoA-I levels in plasma and HDL fractions were significantly positively associated with hepatic apoA-I mRNA levels. In contrast, apoB levels in plasma or apoB-containing lipoproteins were not significantly associated with hepatic apoB mRNA levels. Plasma and lipoprotein cholesterol levels were significantly negatively associated with hepatic mRNA levels of LDL receptor and HMG-CoA synthase. In high HDL_1 animals, the negative relationship of the plasma concentrations of LDL and HDL cholesterol, and the messages of HMG-CoA synthase and LDL receptor were approximately parallel but at a higher level than in low HDL_1 animals (Table 7, Fig. 6).

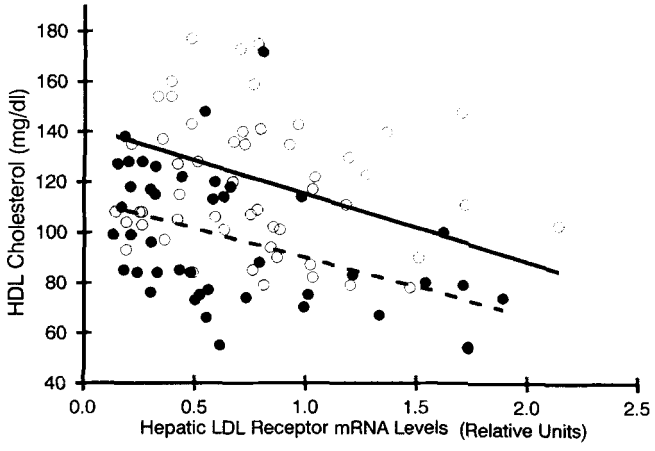

Fig. 6. Relationship of plasma HDL cholesterol and hepatic mRNA levels for LDL receptor in high (○ ——— ○) and low (● - - - - ●) HDL_1 baboons. For slope and significance level see Table 7.

Relationship of dietary cholesterol and fat to mRNA levels of apolipoproteins

Because baboons with the high HDL_1 phenotype accumulate VLDL + LDL and HDL_1 in their plasma, hepatic mRNA levels of apoB, apoE, and apoA-I were measured to determine whether the production of these lipoproteins is increased. ApoC-II mRNA was not expected to respond to the dietary manipulations in this study and therefore can be regarded as an apolipoprotein specificity control. HMG-CoA synthase was selected for study not only because it would monitor the cholesterol synthetic process, but also because it, as well as the LDL receptor gene, is expected to respond to cholesterol homeostasis in the liver.

Dietary cholesterol and fat elevated plasma levels of apoB and dietary fat elevated plasma levels of apoA-I, but the transcripts for these two genes were not increased to a similar degree and the transcript for apoC-II gene was quite stable. Plasma apoC-II was not measured. Dietary fat had an overall influence on hepatic mRNA levels. The increase in apoE levels in baboon plasma caused by

dietary lard, especially on a low cholesterol diet as reported earlier (5), may be due to the increase in hepatic mRNA levels for apoE, perhaps providing the apoE for HDL_1 enlargement. The observations with apoB suggest that the increase of apoB in the plasma upon fat and cholesterol feeding may be due to a decrease in the catabolic processes or to regulation of apoB production at a post-transcriptional level. This observation needs to be explored further by in vivo kinetic studies. Because there was no difference in hepatic mRNA levels of apoA-I in high and low HDL_1 baboons, it is unlikely that increased expression of the apoA-I gene was responsible for production of HDL_1.

Differential down-regulation of LDL receptor and HMG-CoA synthase in high and low HDL_1 baboons

It is well known that hepatic cholesterol synthesis is inhibited by dietary cholesterol. Key enzymes of cholesterol biosynthesis (HMG-CoA reductase and HMG-CoA synthase) and the LDL receptor are down-regulated by increased plasma cholesterol (32–34). Our results are consistent with these reports as shown by negative correlations of hepatic mRNA levels of HMG-CoA synthase and LDL receptor (Table 7) with the serum lipoprotein constituents. This inverse relationship presumably reflects differences in the hepatic cholesterol regulatory pool. There was a negative association between plasma lipoprotein cholesterol levels and hepatic mRNA levels of LDL receptor and HMG-CoA synthase. However, there was a quantitative difference in the level at which the negative association between plasma lipoproteins, and particularly LDL, and hepatic LDL receptor message was set in high HDL_1 animals and in low HDL_1 animals. This relationship is paradoxical. Relative to low HDL_1 animals, high HDL_1 animals have both higher plasma LDL (measured as LDL cholesterol) levels and higher levels of hepatic LDL receptor message. This suggests either that the hepatic LDL receptor message in high HDL_1 animals does not directly reflect the level of LDL receptor function, or that LDL in these animals may not be an efficient ligand for the receptor, or that another substance that inhibits binding is present. Post-transcriptional regulation of LDL receptor expression has recently been reported (35). Sakai et al. (36) have also reported that cholesteryl ester transfer protein-deficient human subjects accumulate in their plasma an unusual species of LDL that is poor in cholesteryl esters. A similar defect in baboon LDL may influence its binding to the receptor. To what extent the inhibition of core lipid exchange among lipoproteins accounts for the differences in LDL composition between high and low HDL_1 animals remains to be clarified.

The observation that the level of two cholesterol-responsive messages, those of the LDL receptor and of HMG-CoA synthase, are both paradoxically higher in high than in low HDL_1 animals in the face of concomitantly higher LDL values, suggests that the control of the cholesterol regulatory pool in the liver may be quantitatively different in these two animal groups. The difference in the control of cholesterol regulatory pool could be due to a lower rate of delivery of cholesterol from LDL, chylomicron remnants, or other lipoproteins in livers of high HDL_1 animals. Alternatively, the cholesterol regulatory pool could be depleted by more active cholesterol esterification, bile acid formation, or lipoprotein secretion. Further work will be needed to differentiate among these possible explanations.

In summary, these studies indicate that the higher level of LDL in the plasma of high HDL_1 baboons is due neither to elevated expression of the apolipoprotein B gene nor of suppressed expression of the LDL receptor gene. Other possibilities, as described above, need consideration.

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