Relationships of Plasma and Hepatic Variables With Rates of Plasma Low-Density Lipoprotein Apolipoprotein B Metabolism in Baboons Fed Low- and High-Fat Diets

Rampratap S. Kushwaha, P. Hugh R. Barrett, Catherine A. Reardon, Douglas S. Lewis, K.D. Carey, Godfrey S. Getz, and Henry C. McGill, Jr

These studies were conducted to determine relationships of plasma low-density lipoprotein (LDL) cholesterol concentrations and hepatic mRNA levels for apolipoprotein (apo) B, LDL receptor, and hepatic hydroxymethyl glutaryl coenzyme A (HMG CoA) synthase with plasma LDL apo B production and catabolic rates in baboons maintained on a low-cholesterol, low-fat chow diet and on a high-cholesterol, high-fat (HCHF) diet. Twelve baboons with LDL cholesterol levels ranging from low to high on the HCHF diet but with similar high-density lipoprotein (HDL) cholesterol levels were selected from a colony of selectively bred pedigreed baboons. LDL apo B turnover and hepatic mRNA concentrations for apo B, LDL receptor, and HMG CoA synthase were measured on a chow diet and again on a HCHF diet fed for 14 weeks. LDL apo B fractional catabolic rates decreased and production rates increased on the HCHF diet. Hepatic mRNA concentrations for apo B were not affected by the HCHF diet. Hepatic LDL receptor and HMG CoA synthase mRNA concentrations decreased on the HCHF diet as compared with the chow diet. LDL apo B fractional catabolic rate was negatively correlated with plasma cholesterol, LDL cholesterol, LDL apo B, and LDL apo B production and positively correlated with hepatic LDL receptor and HMG CoA synthase mRNA concentrations and with plasma LDL triglyceride to cholesterol ratio on the chow diet but not on the HCHF diet. LDL apo B production was positively correlated with plasma cholesterol, LDL cholesterol, and LDL apo B on the HCHF diet and negatively correlated with LDL triglyceride to cholesterol ratio on both chow and HCHF diets. Hepatic apo B mRNA concentrations were not correlated with either the fractional catabolic rate or production rate on either diet. When baboons were grouped by LDL cholesterol response, high responders had lower LDL apo B fractional catabolic rate and lower hepatic LDL receptor mRNA on the chow diet as compared with those having no or moderate response. On the other hand, high responders had high LDL apo B production on the HCHF diet as compared with those having no or moderate response. These results suggest that LDL catabolism regulates LDL cholesterol in plasma on the chow diet, and that hepatic LDL receptor mRNA concentrations are associated with LDL catabolic rate. However, LDL apo B production is the major determinant of LDL cholesterol in the plasma on a HCHF diet, and production of LDL apo B is not associated with hepatic apo B mRNA concentrations.

PLASMA LIPOPROTEIN concentrations, which are major determinants of atherosclerosis,[1-3] differ considerably among individuals and are controlled by both genetic and dietary factors. Dietary cholesterol and fat increase plasma cholesterol levels in humans and experimental animals, but there are wide variations in responsiveness of plasma lipoproteins among mammalian species and among individuals within each species. In many species, including the baboon, saturated fat and cholesterol increase levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL).[4,5] Selective breeding has produced families of baboons that differ mainly in response of plasma LDL cholesterol concentration to dietary cholesterol and saturated fat.[4,5]

Complex segregation analyses of very–low-density lipoprotein (VLDL) and LDL cholesterol concentrations have provided strong evidence that there is a major gene locus that influences VLDL and LDL cholesterol concentrations in baboons fed a chow diet.[6] This major gene locus accounts for approximately 46% of the variability in VLDL and LDL cholesterol in baboons.[6] Thus, baboons with high and low levels of LDL cholesterol are useful for determining the predominant metabolic mechanism that regulates plasma LDL cholesterol concentration, and eventually the major gene that controls this mechanism.

Since the hepatic LDL receptor is responsible for most of the removal of plasma LDL, the liver plays an important role in maintaining plasma LDL cholesterol homeostasis.[7] In addition to the hepatic LDL receptor level, a number of other hepatic metabolic variables, such as cholesterol synthesis, apolipoprotein (apo) B synthesis, and bile acid synthesis, may help regulate plasma LDL cholesterol by modulating hepatic LDL receptor concentrations.[6] Any of these hepatic metabolic variables could account for the differences in LDL cholesterol concentrations among baboons.

Our previous studies have suggested that high-LDL responding baboons have a higher LDL apo B production than low-LDL responding baboons.[8] However, LDL apo B production may be related to a number of variables in the liver that affect LDL receptor expression.[7] The present studies were conducted to determine whether hepatic variables such as mRNA concentrations of LDL receptor, apo B, and hepatic hydroxymethyl glutaryl coenzyme A (HMG CoA) synthase are correlated with LDL apo B metabolic variables in the plasma on a low-cholesterol,

From the Department of Physiology and Medicine, Southwest Foundation for Biomedical Research, San Antonio, TX; Center for Bioengineering, University of Washington, Seattle, WA; Department of Pathology, University of Chicago, Chicago, IL; and Department of Food Science and Human Nutrition, Iowa State University, Ames, IA.

Submitted August 8, 1994; accepted December 7, 1994.

Supported by National Institutes of Health Grants No. HL41256, HL28972, and RR02176 and Contract No. HV53030 from the National Heart, Lung, and Blood Institute.

Address reprint requests to Rampratap S. Kushwaha, PhD, Department of Physiology and Medicine, Southwest Foundation for Biomedical Research, PO Box 28147, San Antonio, TX 78228-0147.

0026-0495/95/4408-0016$03.00/0

low-fat chow diet and on a high-cholesterol, high-fat (HCHF) diet.

MATERIALS AND METHODS

Animals and Diets

Twelve adult baboons (*Papio* sp.) that had LDL cholesterol concentrations ranging from low to high, but similar HDL concentrations on both chow and HCHF diets, were selected from a colony of pedigreed baboons (Table 1). These baboons had been developed by several generations of selective breeding to have no detectable HDL_1 or lipoprotein(a) in their plasma and to differ mainly in LDL cholesterol and LDL apo B concentrations. Therefore, differences in their hepatic variables should be related to LDL apo B metabolism.

The animals were first studied on a chow diet (Monkey chow; Ralston Purina, Indianapolis, IN) and again after consuming an atherogenic diet enriched in cholesterol (1.7 mg/kcal) and fat (lard, 40% of total calories) for 14 weeks.[8] Animals were maintained in individual cages during the metabolic studies and fed once per day with 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 US Department of Agriculture.

Isolation and Labeling of LDL

Animals were studied in three blocks of four animals at one time. LDL for iodination and reinjection was isolated from the blood of the baboons after a 14- to 18-hour fast. The animals were immobilized with ketamine HCl (10 mg/kg), blood was drawn from the femoral vein, and catheters were implanted to allow blood sampling without anesthesia during the turnover procedures. Blood (25 mL from each baboon) was collected into tubes containing EDTA (1 mg/mL), and plasma was obtained by centrifugation in a low-speed centrifuge (TJ6; Beckman Instruments, Palo Alto, CA). LDL was isolated by sequential ultracentrifugation as previously described[8] and dialyzed against saline (0.15 mol/L NaCl) containing EDTA (0.05 mol/L, pH 7.4) to remove potassium bromide.

Protein content of LDL was measured by the Lowry procedure.[9] A known amount of LDL (2 to 4 mg protein) was iodinated by the iodine monochloride procedure[10] with ^{125}I (ICN Chemicals and Radioisotope Division, Irvine, CA) as described previously.[8] Free iodide was removed by passing the labeled lipoproteins through a column of Sephadex G-50 and by further dialysis against saline (0.15 mol/L NaCl) containing EDTA (0.05 mol/L, pH 7.4) at 6°C with four to six changes. After dialysis, iodinated lipoproteins were characterized for radioactivity in trichloroacetic acid precipitate, lipids, and free iodide. Radioactivity in apo B was also measured after isopropanol precipitation as described by Egusa et al.[11] Most of the radioactivity (>95%) was recovered in the trichloroacetic acid–precipitable fraction as described previously,[12] and there was little detectable radioactivity (<0.5%) in the free-iodide fraction. Radioactivity in LDL apo B and lipids was 86.6% ± 1.5% and 5.9% ± 0.7%, respectively, on the chow diet and 91.8% ± 0.4% and 4.8% ± 0.6% on the HCHF diet. The iodinated LDL had β-mobility on agarose gels, and all radioactivity was recovered in the band. Liver punch biopsies were also obtained when the animals had blood withdrawn, and hepatic mRNA levels were measured in individual samples.

Lipoprotein Turnover Procedures

For turnover studies, baboons were housed in individual cages and maintained on a tether system that allowed blood sampling without the use of anesthesia. Fasting baboons were injected with LDL protein 1.0 to 1.5 mg in 1.5 mL saline. Specific activity of LDL varied from 35 to 50 μCi/mg protein. Blood samples (6 mL) were drawn into tubes containing EDTA (1 mg/mL) at 5 and 30 minutes and at 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 168, and 216 hours. The baboons were fed after the 12-hour blood sample was obtained and were allowed to eat for an hour. In subsequent blood withdrawals, the fasting blood sample was obtained in the morning, after which the baboons were fed once per day. Blood samples were centrifuged to obtain plasma. Each plasma sample (2.0 mL) was ultracentrifuged sequentially in L8-70, L8-70M, and L5-50 ultracentrifuges (Beckman Instruments, Fullerton, CA) using 50.3 Ti rotors, and LDL was obtained by slicing the top 2.5-mL layer as described previously.[8]

Urine samples were collected in a pan under the baboon cage and pooled at 6, 12, 24, 48, 72, 96, 120, 168, and 216 hours. Radioactivity in urine at these time points was measured by gamma counting a 1.0-mL sample and multiplying the counts by the urine volume.

Radioactivity in a small aliquot (0.5 mL) of each plasma sample

Table 1. Characteristics of Baboons Used in This Study

			Plasma Cholesterol (mg/dL)		LDL Cholesterol (mg/dL)		LDL Apo B (mg/dL)	
Baboon No.	Sex/Age (yr)	Weight (kg)*	Chow	HCHF	Chow	HCHF	Chow	HCHF
1522	M /12.3	31.2	67	145	12	49	8	26
1864	F /11.8	17.1	74	110	34	44	10	12
4624	M /5.3	19.7	77	77	17	19	10	12
4217	F /7.3	13.8	78	112	26	17	13	14
3566	M /9.4	28.6	81	236	32	161	24	60
920	M /13.7	29.3	84	165	14	49	10	20
6451	M /4.3	18.7	91	154	15	39	10	26
3349	M /9.8	33.4	98	135	45	50	26	27
4736	M /4.6	14.8	101	244	53	164	27	60
2315	F /10.8	18.2	111	245	60	178	36	64
3699	F /9.2	16.0	116	164	58	34	36	29
4726	M /4.7	14.1	117	295	63	157	39	49
Mean ± SE	8.6 ± 1.0	21.2 ± 2.1	91 ± 4.9	174 ± 19.3†	36 ± 1.7	80 ± 5.2	21 ± 0.9	33 ± 1.7

NOTE. HCHF diet was fed for 14 weeks.

*Body weights were measured at the start of the experiment.

†Plasma cholesterol values were significantly increased ($P < .0001$) by the HCHF diet.

and lipoprotein fraction was determined using a gamma spectrometer (Nuclear-Chicago, Des Plains, IL). After radioactivity was determined, the same sample was used to precipitate apo B by adding an equal volume of isopropanol (Sigma Chemical, St Louis, MO) as described by Egusa et al.[11] The precipitate was washed with isopropanol and dissolved in 1N sodium hydroxide.[11] Radioactivity of the suspension was determined before incubating the suspension at 37°C until the pellet was completely dissolved. Protein content of the pellet was then measured by the Lowry method,[9] and radioactivity was determined using a gamma spectrometer.

Separation of Plasma Lipoproteins for Lipid and Apo B Measurements

Blood samples collected at time 0 hours 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, L8-70, or L80-70M. The density gradient procedure was a modification of that reported by Redgrave et al[13] as described in detail previously.[14] The refractive index was measured, and fractions were pooled on the basis of density.[14] The densities of pooled fractions corresponded to VLDL and IDL (d < 1.019 g/mL), LDL (d 1.019 to 1.045), and HDL (d 1.045 to 1.21).

Measurement of Cholesterol, Triglyceride, and Apo B

Cholesterol and triglyceride levels in plasma and lipoproteins were measured by enzymatic methods using kits (Sigma Chemical, and Wako Pure Chemical Industries, Richmond, VA). Apo B level in lipoprotein fractions separated by ultracentrifugation was measured by the electroimmunoassay procedure reported by Laurell[15] as modified by Mott et al.[16] The antisera used for these assays were purchased commercially (Boehringer Mannheim, Indianapolis, IN) and were monospecific. Baboon serum was standardized against LDL (d 1.030 to 1.040 g/mL) isolated by ultracentrifugation. Concentrations of apo B were well within the linear ranges of the assays. Two serum controls were assayed in duplicate in each row of 28 samples to check day-to-day variation in the assay. The sample values were calculated by the average peak heights using a standard curve prepared with standard serum on the same plate. Coefficients of variation for apo B and apo A-I assays were less than 5%.

Measurement of Hepatic mRNA Concentration

Liver samples (0.05 to 0.2 g wet weight) were extracted with guanidine thiocyanate for measurement of total cellular RNA content.[17] mRNA concentrations were measured by a slot-blot procedure as previously described in detail.[18] To confirm the results of slot-blot analysis, some of the same samples were also measured by RNase protection assay, which yielded similar values.

Kinetic Analysis

The CONSAM[19] computer program running on a 486 PC computer (IBM compatible) was used to fit the model to the turnover data. LDL apo B turnover data were analyzed using a two-compartment model. The model is characterized by a plasma compartment and an extravascular-exchange compartment. This model assumes that the plasma LDL apo B pool is kinetically homogeneous. We also assumed that all LDL apo B catabolism occurred via the plasma compartment. In previous studies, we have used a model proposed by Foster et al[20] to account for LDL heterogeneity. However, their model requires that urine radioactivity data be integrated with plasma radioactivity data to support the presence of kinetic heterogeneity within the plasma LDL fraction. In the present study, urine radioactivity data were collected; however, the degree of radioactivity incorporated in smaller peptides and lipids in LDL was too great (9% to 14%) to permit the use of urine radioactivity data. High incorporation of radioactivity in other components in LDL means that radioactivity in the urine is derived from catabolism of those labeled components and from the labeled apo B. Therefore, we have analyzed LDL tracer data using the two-pool model that assumes kinetic homogeneity. In the absence of reliable urine radioactivity data, we cannot assess the impact of LDL heterogeneity on our findings. The fractional catabolic rates presented here therefore represent the average fractional catabolic rate of LDL particles in the LDL density fraction.

LDL apo B production rates were determined by multiplying the LDL apo B fractional catabolic rate by the mass of LDL apo B in plasma. LDL apo B production rates are then expressed on a per-kilogram-weight basis.

Statistical Analysis

Data were log-transformed before analysis to meet the statistical assumptions better. The effect of the HCHF diet on each variable was analyzed using ANOVA with repeated measures. Associations between LDL cholesterol and apo B levels in plasma and lipoproteins and the kinetic parameters and mRNA levels were calculated using univariate and multivariate regression analyses. Although the level of statistical significance was set at P less than .05, we also present differences at a P value less than .10 to obtain a better balance between type I and type II statistical errors.

RESULTS

Effect of HCHF Diet on LDL Lipids and Apo B

The HCHF diet increased plasma LDL cholesterol concentration twofold and LDL apo B concentration 1.5-fold (Table 1), but did not increase either the plasma or LDL triglyceride concentration (Table 2). These observations suggest that LDL became enriched with cholesterol in response to the HCHF diet and that the LDL triglyceride to cholesterol ratio decreased (Table 2). There was no significant change in body weight of the animals during both parts (chow and HCHF diet studies) of the experiment (data not shown).

Effect of HCHF Diet on Metabolic Variables of LDL Apo B

Mean values for catabolic rates and production rates for LDL apo B are listed in Table 3. LDL apo B fractional catabolic rate decreased by 21% on the HCHF diet. LDL apo B production, on the other hand, increased by 25% on the HCHF diet as compared with the chow diet.

Effect of HCHF Diet on Hepatic mRNA Concentrations for Apo B, LDL Receptor, and HMG CoA Synthase

Mean values for hepatic mRNA concentrations for apo B, LDL receptor, and HMG CoA synthase on chow and on the HCHF diet are listed in Table 4. Hepatic apo B mRNA concentrations on the chow diet did not differ from those on the HCHF diet. However, both hepatic LDL receptor and HMG CoA synthase mRNA concentrations decreased on the HCHF diet.

Table 2. Values for Plasma and LDL Triglyceride Concentrations and LDL Triglyceride to Cholesterol Ratio in Baboons on Chow and on a HCHF Diet

	Plasma Triglyceride (mg/dL)		LDL Triglyceride (mg/dL)		LDL Triglyceride to Cholesterol Ratio	
Baboon No.	Chow	HCHF	Chow	HCHF	Chow	HCHF
1522	41	41	11	10	0.92	0.20
1864	43	37	11	9	0.32	0.20
4624	37	24	9	5	0.53	0.26
4217	51	23	13	6	0.50	0.35
3566	21	29	5	8	0.16	0.05
920	29	23	7	6	0.50	0.12
6451	28	28	7	6	0.47	0.15
3349	38	29	10	9	0.22	0.18
4736	20	30	6	12	0.11	0.07
2315	34	23	11	6	0.18	0.03
3699	20	37	5	7	0.09	0.21
4726	30	31	10	9	0.16	0.06
Mean ± SE	32.67 ± 2.83	29.58 ± 1.76	8.75 ± 0.77	7.75 ± 2.09	0.35 ± 0.07*	0.16 ± 0.03

*Different from chow ($P = .001$).

Table 3. Values for Metabolic Variables for LDL Apo B in Baboons on Chow and on a HCHF Diet

	Fractional Catabolic Rate (pools per day)		Production Rate (mg/kg/d)	
Baboon No.	Chow	HCHF	Chow	HCHF
1522	1.23	0.54	4.53	6.46
1864	1.45	0.99	6.67	5.46
4624	1.45	0.76	6.67	4.20
4217	1.40	1.16	8.37	7.47
3566	0.79	0.79	8.72	20.15
920	1.25	0.63	5.75	5.80
6451	1.32	1.28	6.07	15.31
3349	0.60	0.57	7.18	7.08
4736	1.02	0.81	12.67	22.36
2315	0.66	0.97	10.87	38.44
3699	0.60	0.50	9.67	6.68
4726	0.69	0.66	12.38	14.88
Mean ± SE	1.04 ± 0.10	0.80 ± 0.07*	8.30 ± 0.76	12.02 ± 2.32†

*Different from chow ($P = .048$).
†Different from chow ($P = .074$).

Relationship Between Plasma LDL Apo B Metabolic Variables and Plasma and Hepatic Variables

Coefficients for the correlation of plasma LDL apo B catabolic rates and production rates with plasma and hepatic variables affecting LDL metabolism are listed in Table 5. Plasma LDL apo B production rate was positively correlated on both the chow and HCHF diets with plasma cholesterol, LDL cholesterol (illustrated graphically in Fig 1, $P = .000$ for both chow and HCHF diets), and LDL apo B. LDL apo B production on chow and on the HCHF diet was negatively correlated with LDL triglyceride to cholesterol ratio (illustrated graphically in Fig 2, $P = .000$ for both the chow and HCHF diets). Plasma LDL apo B catabolic rate was negatively correlated with plasma cholesterol, LDL cholesterol (illustrated graphically in Fig 1, $P = .000$), and LDL apo B, and positively correlated with LDL triglyceride to cholesterol ratio (Fig 2, $P = .034$) on the chow diet but not on the HCHF diet.

Plasma LDL apo B catabolic rate was positively correlated with hepatic LDL receptor mRNA concentrations

Table 4. Values for Hepatic mRNA Levels for Apo B, LDL Receptor, and HMG CoA Synthase in Baboons on Chow and on a HCHF Diet

	Hepatic Apo B mRNA		Hepatic LDL Receptor mRNA		Hepatic HMG CoA Synthase mRNA	
Baboon No.	Chow	HCHF	Chow	HCHF	Chow	HCHF
1522	0.66	1.27	1.25	1.25	0.76	0.76
1864	1.54	1.10	0.32	0.18	0.74	0.11
4624	0.88	1.50	0.42	0.14	1.11	0.37
4217	0.84	1.90	1.49	0.29	0.76	0.21
3566	1.20	1.22	0.36	0.15	0.59	0.24
920	1.27	1.19	0.65	0.30	0.44	0.21
6451	1.42	1.68	0.45	0.42	0.48	0.61
3349	0.79	1.10	0.19	0.15	0.15	0.15
4736	1.16	1.09	0.23	0.53	0.18	0.20
2315	2.23	1.43	0.38	0.37	0.47	0.22
3699	1.05	1.79	0.23	0.16	0.22	0.27
4726	1.55	1.04	0.21	0.27	0.19	0.26
Mean ± SE	1.22 ± 0.12	1.36 ± 0.09	0.52 ± 0.12	0.35 ± 0.09*	0.51 ± 0.09	0.30 ± 0.06†

NOTE. Expressed in relative units compared with a standard sample from a control baboon.
*Different from chow ($P = .071$).
†Different from chow ($P = .041$).

Table 5. Correlation Coefficients of Variables Between Plasma LDL Apo B Metabolism and Plasma and Liver

Plasma and Hepatic Variables	Plasma LDL Apo B Metabloic Variables			
	Prodution Rate		Catabolic Rate	
	Chow	HCHF	Chow	HCHF
Plasma				
Cholesterol	+.883*	+.782*	−.789*	−.131
LDL cholesterol	+.883*	+.818*	−.759*	−.074
LDL apo B	+.871*	+.875*	−.903*	−.132
LDL triglyceride to cholesterol ratio	−.843*	−.862*	+.764*	+.034
Hepatic				
LDL receptor mRNA	−.526§	+.033	+.640†	+.484
Apo B mRNA	−.472	−.118	−.211	+.322
HMG CoA synthase mRNA	−.562§	−.007	−.730‡	+.065

*P = .000.
†P = .034.
‡P = .007.
§P = .053.

and hepatic HMG CoA synthase mRNA concentrations on the chow diet, but not on the HCHF diet. LDL apo B production and catabolic rates were not correlated with hepatic apo B mRNA concentrations on either the chow or HCHF diet. Plasma LDL apo B production rates were negatively correlated with hepatic LDL receptor and hepatic HMG CoA synthase mRNA concentrations on the chow diet, but not on the HCHF diet.

Relationship Between Plasma Variables and Hepatic mRNA Levels

Coefficients for correlation of plasma variables and hepatic mRNA concentrations are listed in Table 6. There was a negative correlation between hepatic LDL receptor mRNA and plasma cholesterol, LDL cholesterol, and LDL apo B on the chow diet, but not on the HCHF diet. Similarly, there was a significant and negative correlation between hepatic mRNA concentrations for HMG CoA synthase and plasma cholesterol, LDL cholesterol, and LDL apo B on the chow diet, but not on the HCHF diet. Hepatic apo B mRNA concentrations were not significantly correlated with plasma cholesterol, LDL cholesterol, or LDL apo B on either diet.

Separation of Baboons by Plasma LDL Cholesterol Response

The baboons were selected to represent a wide range of LDL cholesterol responses to the HCHF diet in a previous dietary challenge. Their LDL cholesterol responses to the HCHF diet in this study were similar (Table 1) and comprised three groups of four baboons each: no response, with minimal or no increase in LDL cholesterol and a range in challenge levels of 17 to 50 mg/dL; moderate response, with an increase of 10 to 37 mg/dL and a range in challenge levels of 39 to 49; and high response, with an increase of 94 to 139 and a range in challenge levels of 157 to 178. Table 7 lists a comparison of the metabolic variables among these three groups.

The major difference between no-response and moderate-

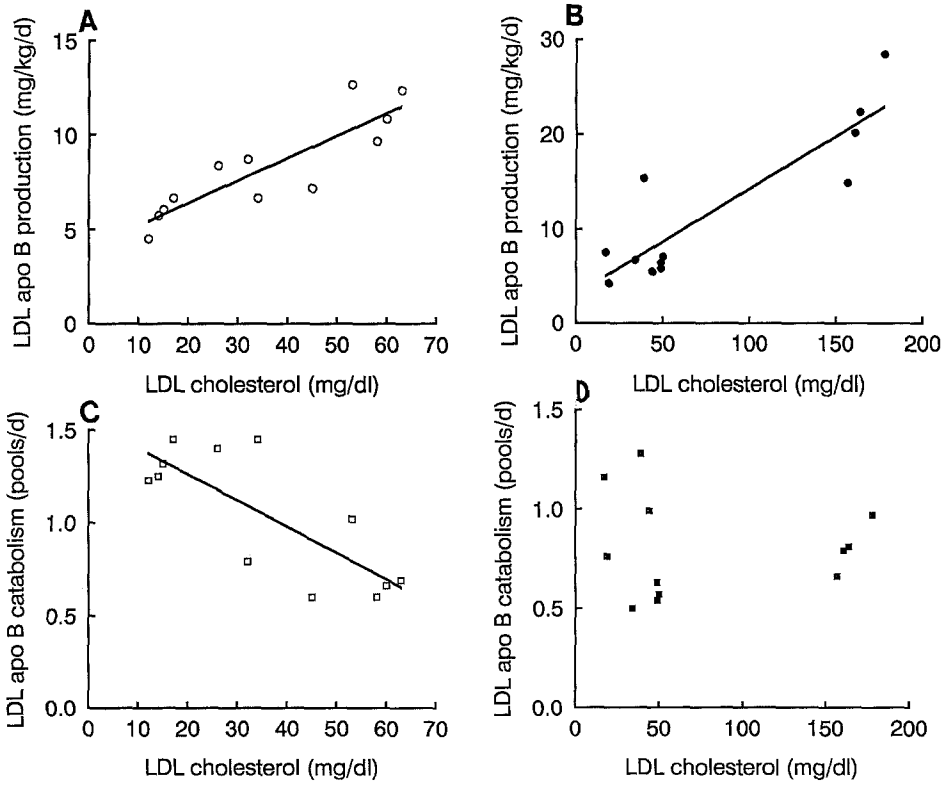

Fig 1. (A and B) Relationship of LDL apo B production with LDL cholesterol concentration on the chow diet (○) and on the HCHF diet (●). (C and D) Relationship of LDL apo B catabolic rate with LDL cholesterol concentration on the chow diet (□) and on the HCHF diet (■). Correlation coefficients and their significance are listed in Table 5.

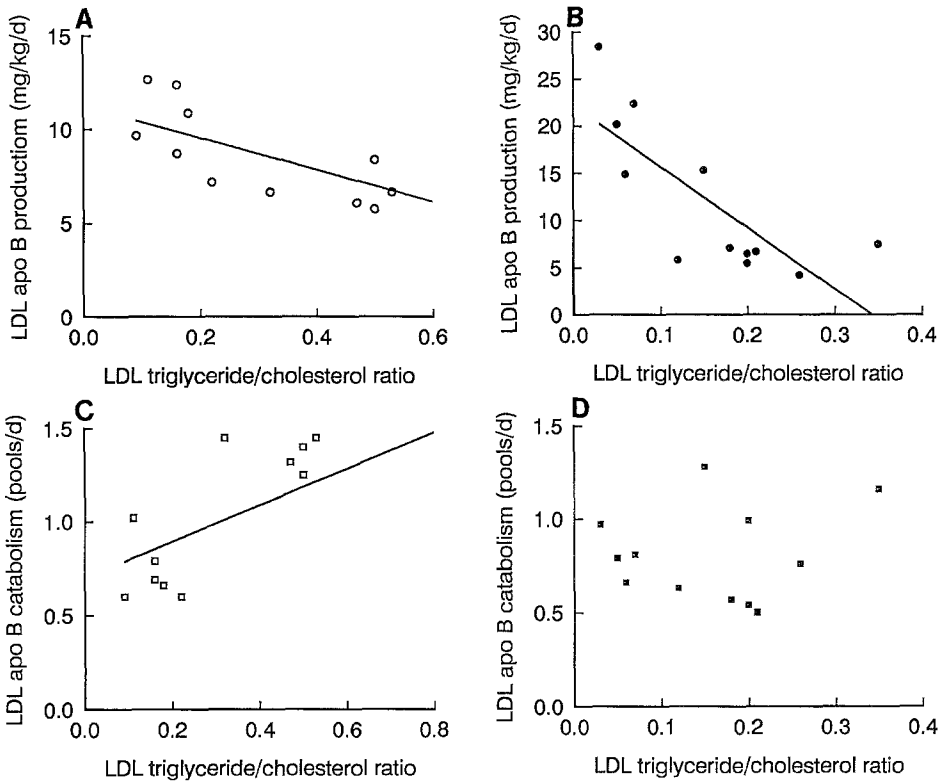

Fig 2. (A and B) Relationship of LDL apo B production with LDL triglyceride to cholesterol ratio on the chow diet (○) and on the HCHF diet (●). (C and D) Relationship of LDL apo B catabolic rate with LDL triglyceride to cholesterol ratio on the chow diet (□) and on the HCHF diet (■). Correlation coefficients and their significance are listed in Table 5.

response groups was in LDL apo B production. On the chow diet, baboons in the no-response group had higher LDL apo B production than those in the moderate-response group. However, on the HCHF diet, baboons in the no-response group had lower LDL apo B production than those in the moderate-response group (Table 7). Baboons in the no-response group had higher plasma LDL cholesterol and apo B on the chow diet than those in the moderate-response group. However, baboons in the no-response group had lower plasma LDL cholesterol and apo B on the HCHF diet than those in the moderate-response group. There was no difference in LDL apo B catabolic rate or hepatic LDL receptor mRNA concentrations between baboons in the no-response group and those in the moderate-response group.

Baboons in the high-response group had higher LDL cholesterol and apo B on both the chow and HCHF diets than those in the no- or moderate-response group. Baboons in the high-response group had a lower triglyceride to cholesterol ratio on both the chow and HCHF diets than those in the no- or moderate-response groups. Baboons in the high-response group had higher LDL apo B production on both the chow and HCHF diets than those in the no- or moderate-response groups. On the other hand, baboons in the high-response group had a lower LDL apo B catabolic rate and lower hepatic LDL receptor mRNA concentration only on the chow diet than those in the no- or moderate-response group.

In baboons having no or moderate response, there was a decrease in hepatic LDL receptor mRNA concentrations with a corresponding decrease in LDL apo B fractional catabolic rate on the HCHF diet as compared with the chow diet. On the other hand, in baboons having high response, there was no change in hepatic LDL receptor message or LDL apo B fractional catabolic rate on the HCHF diet as compared with the chow diet. Hepatic HMG CoA synthase mRNA concentrations were not affected by the degree of response, but were decreased by dietary cholesterol and fat.

Table 6. Correlation Coefficients of Plasma Variables and Hepatic mRNA Levels Affecting LDL Metabolism

	Plasma Variables					
	Cholesterol		LDL Cholesterol		LDL Apo B	
Hepatic mRNA	Chow	HCHF	Chow	HCHF	Chow	HCHF
Apo B	+.471	−.406	+.426	−.490	+.374	−.310
LDL receptor	−.602*	+.002	−.618*	+.344	−.599‡	+.369
HMG CoA synthase	−.785†	−.162	−.669‡	−.171	−.733†	−.035

*$P < .05$.
†$P < .01$.
‡$P < .06$.

Table 7. Metabolic Variables in Baboons Classified by Plasma LDL Cholesterol Response to the HCHF Diet

Variable	No Response		Moderate Response		High Response	
	Chow	HCHF	Chow	HCHF	Chow	HCHF
Plasma C	92.3 ± 9.5	122.0 ± 18.5	79.0 ± 5.3	143.5 ± 11.9	102.5 ± 8.0	225.0 ± 13.5
Plasma TG	36.5 ± 6.5	28.3 ± 3.0	35.3 ± 4.0	32.3 ± 4.1	26.3 ± 3.5	28.3 ± 2.0
LDL C*	36.5 ± 9.0	30.0 ± 7.7	18.8 ± 5.1	45.3 ± 2.2	52.0 ± 7.0	165.0 ± 4.6
LDL TG	9.3 ± 1.5	9.3 ± 1.0	9.0 ± 1.0	7.8 ± 1.0	7.8 ± 1.5	8.8 ± 1.0
LDL TG/C†	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 2.0	0.2 ± 0.0	0.2 ± 0.0	0.05 ± 0.02
LDL apo B‡	21.3 ± 6.0	21.3 ± 4.5	9.5 ± 0.5	21.0 ± 3.5	31.5 ± 3.5	58.3 ± 3.0
LDL C/apo B§	1.8 ± 0.1	1.4 ± 0.2	2.0 ± 0.5	2.4 ± 0.5	1.6 ± 0.2	2.8 ± 0.1
LDL apo B FCR‖	1.0 ± 0.3	0.7 ± 0.2	1.3 ± 0.1	0.8 ± 0.3	0.8 ± 0.1	0.8 ± 0.0
LDL apo B production¶	7.97 ± 0.6	6.4 ± 0.8	5.8 ± 0.5	8.3 ± 4.7	11.1 ± 0.9	21.5 ± 5.1
LDL-R mRNA	0.6 ± 0.3	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
Apo B mRNA	0.9 ± 0.1	1.6 ± 0.2	1.2 ± 0.2	1.3 ± 0.3	1.5 ± 0.3	1.2 ± 0.1
HMG CoA synthase mRNA#	0.6 ± 0.3	0.3 ± 0.0	0.6 ± 0.1	0.42 ± 0.1	0.4 ± 0.1	0.2 ± 0.0

NOTE. Mean ± SE (n = 4).

Abbreviations: C, cholesterol; TG, triglycerides; FCR, fractional catabolic rate; LDL-R, LDL receptor.

*LDL is significantly different among groups on chow diet ($P = .025$), and plasma cholesterol is significantly different among groups on HCHF diet ($P = .002$).

†Significantly different among groups on chow diet ($P = .039$) and on HCHF diet ($P = .000$).

‡Significantly different among groups on chow diet ($P = .005$) and on HCHF diet ($P = .002$).

§Significantly different on HCHF diet ($P = .013$).

‖LDL apo B FCR and hepatic LDL receptor mRNA concentrations are significantly different among groups on chow diet ($P = .1$).

¶Significantly different among groups on chow diet ($P = .001$) and on HCHF diet ($P = .002$).

#Levels in liver are significantly decreased by the HCHF diet in all groups ($P = .014$).

DISCUSSION

Summary of Results

These studies were conducted to determine whether LDL apo B catabolism or production plays a major role in regulating plasma LDL cholesterol in baboons on chow and HCHF diets, and whether hepatic LDL receptor and apo B mRNA concentrations are related to variables of plasma LDL apo B metabolism. Pedigreed baboons differing mainly in LDL cholesterol concentration were used for these studies, and therefore, differences in hepatic and plasma variables should be related to LDL cholesterol concentrations. The results indicate that the relationships differ with the dietary cholesterol and fat intake. On the chow diet, plasma LDL cholesterol concentrations were correlated with LDL apo B catabolic rates and with hepatic LDL receptor mRNA concentrations. These observations suggest that with minimal cholesterol and fat intake, hepatic LDL receptor mRNA concentration controls LDL cholesterol concentration. In contrast, on the HCHF diet, plasma LDL cholesterol concentrations were correlated with LDL apo B production rates but not with hepatic apo B mRNA concentrations. These observations suggest that with a cholesterol- and fat-enriched diet, LDL apo B production controls LDL cholesterol concentration. However, hepatic apo B transcription does not control apo B production. These correlations were also evaluated by analysis of data for baboon groups separated by plasma LDL cholesterol response to the HCHF diet.

Role of Hepatic LDL Receptor in LDL Metabolism on Chow Diet

The hepatic LDL receptor is the major mechanism for regulating plasma LDL cholesterol homeostasis.[7] The hepatic LDL receptor not only removes LDL cholesterol from plasma but also removes VLDL remnants. Since VLDL-remnant removal is inversely related to LDL production, the LDL receptor also regulates LDL production.[7] In the present studies, hepatic LDL receptor mRNA concentrations were negatively correlated with plasma LDL cholesterol concentrations and positively correlated with LDL apo B catabolic rates on the chow diet. LDL apo B catabolic rates were also negatively correlated with LDL apo B production rates ($r = -.581$, $P < .05$; Fig 3) in baboons maintained on the chow diet. Thus, in baboons fed a low-cholesterol, low-fat chow diet, LDL receptor transcription explains a substantial portion of the variability in LDL cholesterol concentrations, and also a portion of the variability in LDL apo B catabolic and production rates.

Role of Hepatic LDL Receptor in LDL Metabolism on HCHF Diet

On the HCHF diet, both LDL apo B fractional catabolic rates and hepatic LDL receptor mRNA concentrations decreased as compared with levels on the chow diet. Therefore, the decrease in LDL apo B catabolic rate on the HCHF diet seems to be mediated by the decrease in hepatic LDL receptor mRNA concentration. Since LDL apo B production rate was also increased on the HCHF diet, production of LDL apo B might have been influenced by the LDL receptor. However, unlike results observed on the chow diet, hepatic mRNA concentrations of LDL receptor did not correlate with either the production or catabolic rates of LDL apo B on the HCHF diet. Thus, changes in LDL apo B production, which was the major determinant of plasma LDL cholesterol on the HCHF diet, were not

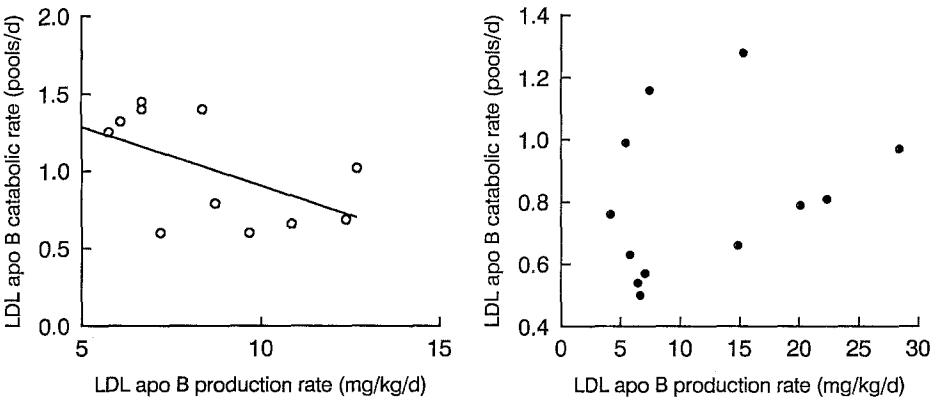

Fig 3. Relationship of LDL apo B production rate with LDL apo B catabolic rate in baboons maintained on the chow diet (○) and on the HCHF diet (●). Correlation coefficients and their significance are listed in Table 5.

explained by variability in hepatic LDL receptor concentrations, in contrast to the situation on the chow diet.

Comparison of Results With Human Studies

Kesaniemi and Grundy[21] measured LDL apo B turnover rates in human subjects fed a diet of mixed solid foods and liquid formula high in fat (40% of calories), whose LDL cholesterol levels varied from low to moderately high, similar to those of baboons used for this study. LDL cholesterol levels in the human subjects were regulated by LDL apo B production and not by LDL apo B catabolism. Thus, the results of these studies of baboons fed a HCHF (40% of total calories) diet are consistent with those of human subjects.[21] Baboons fed the HCHF diet are therefore similar to humans in regulating plasma LDL cholesterol mainly by LDL apo B production. Glatz et al[22] conducted LDL apo B turnover studies in high- and low-responding humans and concluded that variability in the response of serum cholesterol to dietary lipids was related to variability in LDL production, as we found in baboons on the HCHF diet. However, in these human studies it was not possible to relate the production and catabolic rates to measures of hepatic mRNA concentrations of LDL receptor.

Role of Hepatic Apo B mRNA in LDL Production

Hepatic mRNA concentrations for apo B did not increase on the HCHF diet, despite increased LDL apo B production. These observations indicate that increased LDL apo B production on the HCHF diet was not caused by increased concentrations of mRNA for apo B. These results are consistent with a number of in vitro and in vivo studies of animals and humans reviewed by Dixon and Ginsberg.[23] In most of the in vivo studies, hepatic apo B mRNA concentrations were correlated with steady-state concentrations of plasma apo B,[18,23,24] and it was not determined whether increased plasma and LDL apo B concentrations were related to an increase in LDL apo B production. The present studies therefore offer new apo B results, in that LDL apo B production rates were not correlated with hepatic mRNA levels.

Parallel Responses of LDL Receptor and HMG CoA Synthase Transcription

The key and rate-limiting enzyme of cholesterol biosynthesis is HMG CoA reductase. HMG CoA synthase is regulated in coordination with HMG CoA reductase in response to cholesterol delivery to hepatocytes, although the mechanism of regulation of these two enzymes differs in detail. Hepatic mRNA concentrations of HMG CoA synthase decreased on the HCHF diet. Like hepatic LDL receptor mRNA concentrations, hepatic HMG CoA synthase mRNA concentrations were positively correlated with LDL apo B catabolic rate and negatively correlated with plasma cholesterol, LDL cholesterol, and LDL apo B on the chow diet, but not on the HCHF diet. Thus, in response to dietary cholesterol and fat, HMG CoA synthase mRNA concentrations in the liver are regulated in the same direction as hepatic LDL receptor mRNA concentrations.

Possible Role of Bile Acid Metabolism in the Regulation of LDL Cholesterol Levels

In these studies, increased LDL apo B production rates on the HCHF diet were not correlated with either the hepatic LDL receptor or apo B mRNA concentrations. Because increased apo B production was not associated with hepatic apo B mRNA concentrations, apo B output may be regulated posttranscriptionally as suggested by Dixon and Ginsberg.[23] Intracellular degradation of newly synthesized apo B in rat hepatocytes[25] and HepG2 cells[26] represents the most likely translational locus at which apo B output may be regulated. Dixon et al[26] reported that oleate stimulates the secretion of apo B by protecting the degradation of nascent apo B in HepG2 cells. Similarly, availability of cholesteryl esters in the hepatocyte may also increase apo B secretion.[27] The present studies suggest that an increase in the cholesterol to triglyceride ratio in LDL was associated with an increase in apo B production on both the chow and HCHF diets (Fig 2). Decreased cholesterol absorption or increased bile acid synthesis in baboons may affect availability of cholesteryl esters in the liver and affect the posttranslational modification and secretion of apo B. Our previous studies suggest that low-responding baboons have lower cholesterol absorption than high-responding

baboons[28] and also have a greater accumulation of 27-hydroxycholesterol in the plasma and liver than high-responding baboons.[29] Further studies are required to determine if decreased cholesterol absorption and increased production of 27-hydroxycholesterol in livers of low-responding versus high-responding baboons are associated with increased bile acid production, decreased availability of cholesteryl esters, and decreased apo B secretion.

ACKNOWLEDGMENT

We thank William Ehler, Karen Couch, Diane Martinez, and Kathleen Born for technical help.

REFERENCES

1. Kukita H, Hiwada K, Kokubu T: Serum apolipoprotein A-I, A-II and B levels and their discriminative values in relatives of patients with coronary artery disease. Atherosclerosis 51:261-267, 1984

2. Kwiterovich PO Jr, Sniderman AD: Atherosclerosis and apoproteins B and A-I. Prev Med 12:815-834, 1983

3. McGill HC Jr, McMahan CA, Kruski AW, et al: Relationship of lipoprotein cholesterol concentrations to experimental atherosclerosis in the baboon. Arteriosclerosis 1:3-12, 1981

4. McGill HC Jr, McMahan CA, Mott GE, et al: Effects of selective breeding on the cholesterolemic responses to dietary saturated fat and cholesterol in baboons. Arteriosclerosis 8:33-39, 1988

5. Williams MC, Kushwaha RS, McGill HC Jr: Quantitation of baboon lipoproteins by high performance gel exclusion chromatography. Lipids 22:366-374, 1987

6. Konigsberg LW, Blangero J, Kammerer CM, et al: Mixed model segregation analysis of LDL-C concentration with genotype-covariate interaction. Genet Epidemiol 8:69-80, 1991

7. Dietschy JM, Turley SD, Spady DK: Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 34:1637-1659, 1993

8. Kushwaha RS, Barnwell GM, Carey KD, et al: Metabolism of apoprotein B in selectively bred baboons with low and high levels of low density lipoproteins. J Lipid Res 27:497-507, 1986

9. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951

10. McFarlane AS: Efficient trace-labeling of proteins with iodine. Nature 182:53-54, 1958

11. Egusa GD, Brady W, Grundy SM, et al: Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. J Lipid Res 24:1261-1267, 1983

12. Kushwaha RS, Foster DM, Barrett PHR, et al: Effect of estrogen and progesterone on metabolism of apoprotein B in baboons. Am J Physiol 258:E172-E183, 1990

13. Redgrave TG, Roberts DCK, West CE: Separation of plasma lipoproteins by density-gradient ultracentrifugation. Anal Biochem 65:42-49, 1975

14. McGill HC Jr, McMahan CA, Kushwaha RS, et al: Dietary effects on serum lipoproteins of dyslipoproteinemic baboons with high HDL_1. Arteriosclerosis 6:651-663, 1986

15. Laurell CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 15:45-52, 1966

16. Mott GE, Jackson EM, Prihoda TJ, et al: Effects of dietary cholesterol and fat, sex and sire on lecithin:cholesterol acyltransferase activity in baboons. Biochim Biophys Acta 919:190-198, 1986

17. Chirgwin JM, Przybyla AE, MacDonald RJ, et al: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294-5299, 1979

18. Kushwaha RS, McMahan CA, Mott GE, et al: 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 32:1929-1940, 1991

19. Foster DM, Boston RC: The use of computers in compartmental analysis: The SAAM and CONSAM programs, in Robertson JS (ed): Compartmental Distribution of Radiotracers. Boca Raton, FL, CRC, 1983, pp 73-142

20. Foster DM, Chait A, Albers JJ, et al: Evidence for kinetic heterogeneity among human low density lipoproteins. Metabolism 35:685-696, 1986

21. Kesaniemi YA, Grundy SM: Significance of low density lipoprotein production in the regulation of plasma cholesterol level in man. J Clin Invest 70:13-22, 1982

22. Glatz JFC, Turner PR, Katan MB, et al: Hypo- and hyperresponse of serum cholesterol level and low density lipoprotein production and degradation to dietary cholesterol in man. Ann NY Acad Sci 676:163-179, 1993

23. Dixon JL, Ginsberg MN: Regulation of hepatic secretion of apolipoprotein B–containing lipoproteins: Information obtained from cultured liver cells. J Lipid Res 34:167-179, 1993

24. Kroon PA, DeMartino JA, Thompson GM, et al: Molecular cloning of partial cDNAs for rabbit liver apolipoprotein B and the regulation of its mRNA levels by dietary cholesterol. Proc Natl Acad Sci USA 83:5071-5075, 1986

25. Borchardt RA, Davis RA: Intrahepatic assembly of very low density lipoproteins. Rate of transport out of the endoplasmic reticulum determines rate of secretion. J Biol Chem 262:16394-16402, 1987

26. Dixon JL, Furukawa S, Ginsberg HN: Oleate stimulates secretion of apolipoprotein B–containing lipoproteins from HepG2 cells by inhibiting early intracellular degradation of apolipoprotein B. J Biol Chem 266:5080-5086, 1991

27. Fungwe TV, Cagen L, Wilcox HG, et al: Regulation of hepatic secretion of very low density lipoprotein by dietary cholesterol. J Lipid Res 33:179-191, 1992

28. Kushwaha RS, Rice KS, Lewis DS, et al: The role of cholesterol absorption and hepatic cholesterol content in explaining high and low responses to dietary cholesterol and fat in pedigreed baboons (*Papio* sp.). Metabolism 42:714-722, 1993

29. Hasan SQ, Kushwaha RS: Differences in 27-hydroxycholesterol concentrations in plasma and liver of baboons with high and low responses to dietary cholesterol and fat. Biochim Biophys Acta 1182:299-302, 1993