Relationship of high density lipoprotein cholesterol to cholesterol metabolism in the baboon (*Papio* sp.)

Bryan L. Flow* and Glen E. Mott*,**

Department of Pathology, University of Texas Health Science Center at San Antonio* and Southwest Foundation for Research and Education,** San Antonio, TX 78284

Abstract Genetic effects on serum high density lipoprotein (HDL) cholesterol concentration and several parameters of a two-pool model of cholesterol metabolism were investigated in 79 baboons, the progeny of 6 sires. Significant differences ($P < 0.05$) were observed among the sire progeny groups for HDL cholesterol (HDL-C), cholesterol production rate, cholesterol mass of pool A, and the rate constants K_A and K_{AB}. Rank correlations (r_s) revealed that the sire progeny group means for HDL-C are closely correlated with those for the cholesterol mass of pool A ($r_s = 0.89$), K_A ($r_s = -0.78$), and K_{AB} ($r_s = -0.94$). These strong correlations suggest that pool A, K_A, and K_{AB} are influenced to a large degree by the same genes that regulate HDL-C concentration. The strong inverse relationship ($r_s = -0.78$) between HDL-C and K_A suggests that the differences among these sire progeny groups for HDL-C are due chiefly to those metabolic processes which regulate cholesterol excretion from pool A. This conclusion is consistent with reports that HDL-C is a preferred precursor for bile acid synthesis.—**Flow, B. L., and G. E. Mott.** Relationship of high density lipoprotein cholesterol to cholesterol metabolism in the baboon (*Papio* sp.). *J. Lipid Res.* 1984. **25:** 469–473.

Supplementary key words genetics • two-pool model • cholesterol turnover

Several studies (1–8) support Glomset's hypothesis (9) that high density lipoproteins (HDL) transport cholesterol from peripheral tissues to the liver for catabolism and excretion. Nevertheless, the relationship between serum HDL cholesterol concentrations (HDL-C) and most in vivo measures of cholesterol metabolism are not clear (10–13).

We previously reported genetically mediated differences among baboon sire progeny groups for serum cholesterol concentrations (14) and several parameters of cholesterol metabolism (15). Further study (16) indicated that there were also differences among these sire groups for HDL-C. The present report describes the relationship between HDL-C and parameters of a two-pool model of cholesterol metabolism in these baboon sire families.

MATERIALS AND METHODS

Experimental design

The present study is part of a long-term study of the influence of infant diet, adult diet, sex, and heredity on cholesterol metabolism and experimental atherosclerosis in the baboon. Detailed descriptions of the experimental design have been published in previous reports (14–16). This design included sire progeny groups produced by random assignment of adult female baboons to seven sires. Sires and dams were selected only for their breeding potential without consideration of their serum cholesterol concentration or other characteristics. One sire group was excluded from this study since it contained only three progeny. Each of the remaining six sires contributed from 7 to 17 progeny to the 79 progeny available for this report. Each dam contributed only one progeny to the study.

We assigned each infant at birth to one of three infant formulas or to breast feeding and, upon weaning, to one of four adult diets by a restricted random procedure (14). The adult diets consisted of two levels of dietary cholesterol and two types of fat. The effects of the infant diets during infancy have been reported (14). The long term effects of the infant and adult diets on serum lipoprotein concentrations also have been described (17). The effects of infant and adult diets on cholesterol metabolism will be described in a separate report.

Isotope kinetic procedure for analysis of cholesterol metabolism

We estimated parameters of cholesterol metabolism in the progeny at 3.5 years of age by the isotopic kinetic method of Goodman and Noble (18). A dose of

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Abbreviations: HDL, high density lipoprotein; C, cholesterol.

[4-^{14}C]cholesterol was injected intravenously and the serum cholesterol specific radioactivity was measured 25 times over a 4-month interval (15). Parameters of cholesterol metabolism were estimated for a two-pool model (**Fig. 1**) by fitting two exponential functions to the serum specific radioactivity data as previously described (15, 18). We also fitted the specific radioactivity data to a three-pool model (19) by a similar procedure, but in some animals the three-pool model did not provide a better fit of the data than the two-pool model. Therefore, we present only the results of the two-pool model.

In this report, the cholesterol mass of pool B is a minimum estimate based on the assumption that no cholesterol synthesis occurs in pool B. We have also assumed that irreversible cholesterol loss occurs only from the rapidly exchanging compartment (pool A) as shown in Fig. 1. The cholesterol output or excretion from pool A (Q_A) is defined to be equivalent to the production rate of cholesterol in pool A. Q_A is equal to the product of the rate constant K_A and the mass of pool A. The flux of cholesterol from pool A to pool B (Q_{AB}) is defined as the product of K_{AB} and the mass of pool A.

Determination of serum HDL cholesterol concentrations

Measurements of HDL-C were obtained twice 3 weeks apart at 4–6 years of age by heparin-Mn^{2+} precipitation (16, 20, 21) and an enzymatic cholesterol procedure (22). The HDL-C value of each progeny represents the mean of the two samples.

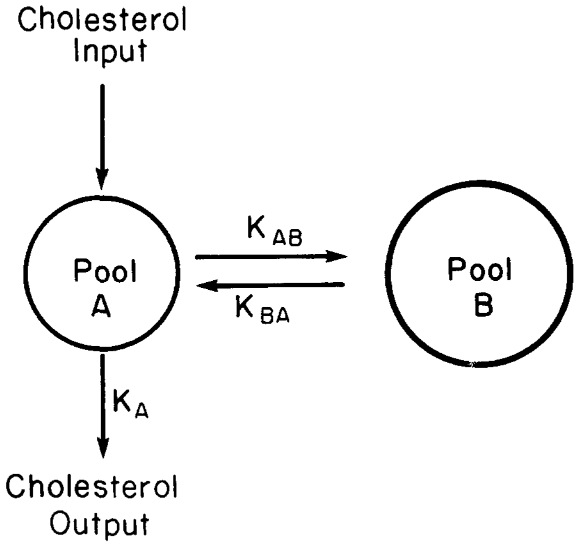

Fig. 1. Parameters of a two-pool model of cholesterol metabolism. Pool A is the rapidly exchanging cholesterol pool; pool B, the slowly exchanging cholesterol pool; K_{AB}, the rate constant for transfer of cholesterol from pool A to pool B; K_{BA}, the rate constant for transfer of cholesterol from pool B to pool A; and K_A, the rate constant for excretion of cholesterol from pool A.

We previously reported that there were no significant age effects for these HDL-C measurements from 4–6 years of age (16). The absence of an age effect for these HDL-C values indicates that by 4 years of age HDL-C had reached a plateau which was maintained through 6 years. Since there was no age effect for HDL-C from 4–6 years, these HDL-C values were compared with the cholesterol metabolism data which were obtained during a 4-month period from 3.5–4 years of age.

Statistical methods

Since the number of males and females and number of progeny in each diet group were not balanced within the sire progeny groups, the data were analyzed by least squares procedures as described for the analysis of data with unequal subclass numbers (23, 24). The linear model included the effects of infant diet, dietary cholesterol, dietary fat, sex, sire, and the two factor interactions of infant diet by dietary cholesterol, infant diet by dietary fat, and dietary cholesterol by dietary fat. The effect of age also was included in the linear model for the analysis of HDL-C values. Preliminary analysis indicated that the sex by diet, sire by sex, and sire by diet interactions were not significant for any of the dependent variables; therefore, these effects were not included in the final model. Data were log-transformed to better meet the assumption of homogeneity of variance.

The absence of sire by sex and sire by diet interactions indicated that the genetic effects due to sire could be determined from sire family means averaged across sex and diet effects. In this report, the sire progeny group means are presented as least squares means. These least squares means are the sire family means that would be expected if the effects of sex and diets had been balanced within sire groups (24).

Correlations among sire progeny group means were determined as Spearman rank correlations (r_s) (25). The rank correlation has the advantage that no assumptions are made about the distribution of the two traits being correlated.

RESULTS

Effect of sire on HDL-C and parameters of cholesterol metabolism

We previously reported (16) that there were no differences ($P > 0.05$) among these sire progeny groups for the cholesterol concentrations of serum very low density plus low density lipoproteins (VLDL + LDL). However, the differences in HDL-C among sire progeny groups were statistically significant ($P < 0.01$). Sire progeny group means for HDL-C are reproduced in **Table 1** to permit comparison with variables of the two-pool model.

TABLE 1. Serum HDL cholesterol concentration and parameters for a two-pool model of cholesterol metabolism for 79 baboon progeny by sire group[a]

Sire Progeny Group	Number of Progeny	HDL Cholesterol Concentration	Cholesterol Mass, mg/kg		Rate Constants, days^{-1}			Flux, mg/kg per day	
			Pool A	Pool B[b]	K_A	K_{AB}	K_{BA}	Q_A[c]	Q_{AB}
A772[d]	15	83.2 (75.9–91.1)	344 (318–374)	402 (375–430)	0.095 (0.087–0.104)	0.070 (0.059–0.083)	0.061 (0.055–0.067)	32.8 (31.0–34.9)	24.2 (21.1–27.8)
A947	16	75.8 (69.7–82.4)	352 (327–379)	406 (380–432)	0.107 (0.098–0.116)	0.073 (0.062–0.085)	0.063 (0.058–0.069)	37.6 (35.5–39.7)	25.6 (22.4–29.1)
A956	8	75.6 (66.9–85.3)	324 (291–362)	384 (350–421)	0.115 (0.102–0.130)	0.077 (0.061–0.096)	0.065 (0.056–0.075)	37.3 (34.4–40.5)	24.8 (20.5–30.0)
A776	16	69.4 (63.7–75.5)	330 (306–356)	424 (398–452)	0.102 (0.094–0.111)	0.082 (0.070–0.095)	0.064 (0.058–0.070)	33.7 (31.9–35.7)	26.9 (23.6–30.7)
A982	7	67.8 (59.5–77.3)	302 (269–340)	428 (388–473)	0.115 (0.101–0.131)	0.079 (0.062–0.101)	0.056 (0.048–0.065)	34.8 (31.9–38.0)	23.9 (19.5–29.3)
A943	17	65.2 (60.2–70.7)	300 (279–322)	427 (402–454)	0.119 (0.110–0.129)	0.100 (0.086–0.117)	0.070 (0.064–0.077)	35.7 (33.8–37.7)	30.1 (26.5–34.1)
Tests of significance among sire groups		$P < 0.01$	$P < 0.05$	P = NS	$P < 0.01$	$P < 0.05$	P = NS	$P < 0.05$	P = NS

[a] Sire progeny group means are least squares means. Values in parentheses represent 95% confidence intervals for the mean.
[b] The cholesterol mass of pool B is a minimum estimate based on the assumption that no cholesterol synthesis occurs in pool B.
[c] Q_A is equivalent to production in pool A.
[d] Sire progeny groups are ranked in decreasing order of their serum HDL cholesterol concentrations.

We also observed significant differences among the sire progeny groups for the cholesterol mass of pool A, the rate constants K_A and K_{AB}, and Q_A (Table 1). Differences among the sire progeny group means were not statistically significant ($P > 0.05$) for the mass of pool B, the rate constant for cholesterol transfer from pool B (K_{BA}), or Q_{AB}.

Relationships between HDL-C and measures of cholesterol metabolism

We observed a strong positive relationship (**Table 2, Fig. 2**) among sire progeny group means for HDL-C and those for the cholesterol mass of pool A (r_s = 0.89, $P < 0.01$). In contrast, means of sire progeny groups for HDL-C are inversely correlated with those for K_A (r_s = −0.78, $P < 0.05$) and K_{AB} (r_s = −0.94, $P < 0.01$). The sire family means for pool A also were inversely related (Fig. 2) to those for K_A (r_s = −0.81, $P < 0.05$) and K_{AB} (r_s = −0.77, $P < 0.05$), but we observed a positive correlation between K_A and K_{AB} (r_s = 0.64, P = 0.08). Although the differences among sire progeny means for mass of pool B were not statistically significant, we observed an inverse correlation (Table 2) between mass of pool B and HDL-C (r_s = −0.77, $P < 0.05$).

TABLE 2. Rank correlations among sire progeny group means for serum HDL cholesterol concentration with measures of cholesterol metabolism in baboons

Metabolic Variables	Correlations with Serum HDL Cholesterol Concentrations
Mass of pool A	0.89[a]
Mass of pool B	−0.77[b]
K_A	−0.78[b]
K_{AB}	−0.94[a]
K_{BA}	−0.37
Q_A	−0.09
Q_{AB}	−0.37

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

DISCUSSION

We previously reported significant differences among these baboon sire groups for HDL-C (16) and several parameters of cholesterol metabolism (15). These sire effects indicated that genetic factors are important in the control of HDL-C and cholesterol metabolism in these baboons. In the present study, the rank correlations revealed that the sire family means for HDL-C are closely correlated with those for pool A (r_s = 0.89), K_A (r_s = −0.78), and K_{AB} (r_s = −0.94). These strong correlations suggest that pool A, K_A, and K_{AB} are influenced to a large degree by the same genes that regulate HDL-C.

The strong inverse relationships between sire progeny group means for K_A and those for HDL-C (r_s = −0.78)

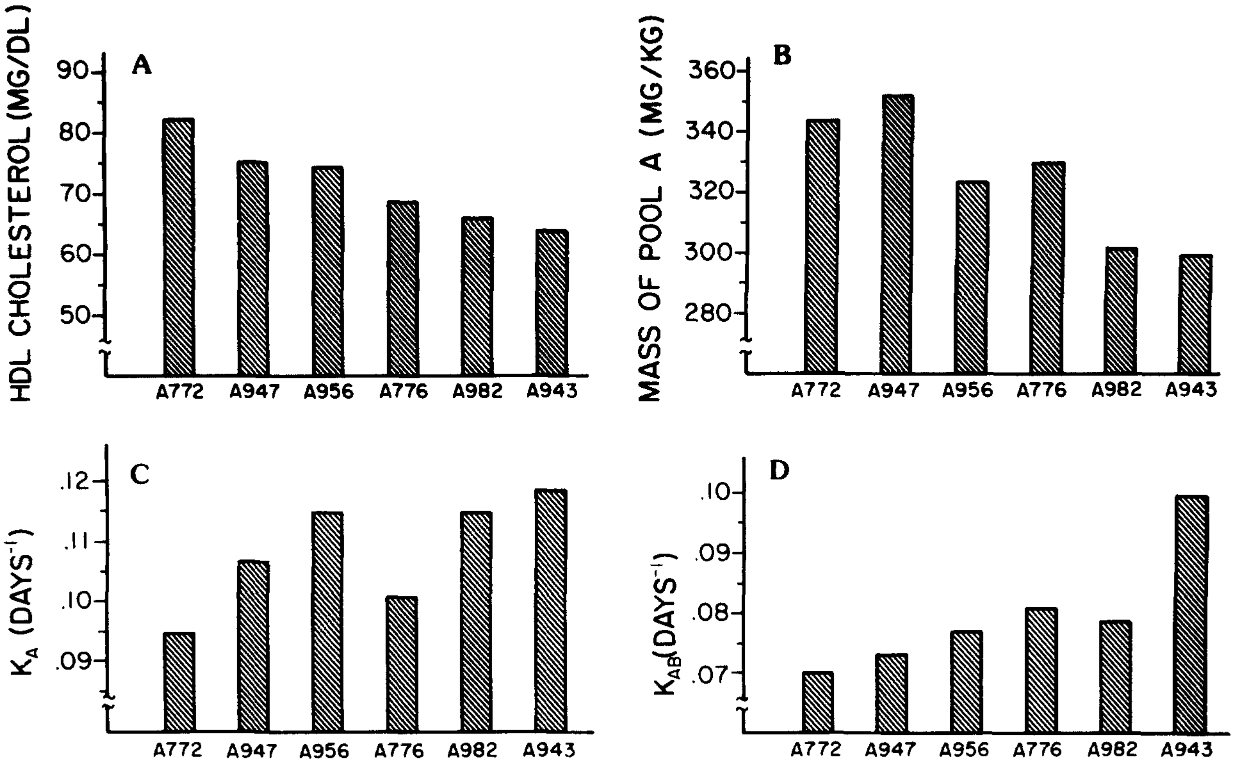

Fig. 2. Sire progeny group means: A) HDL-C; B) mass of pool A; C) K_A; D) K_{AB}. The order of sire groups in figure A is maintained in figures B–D. Groups are ordered by decreasing serum HDL cholesterol concentration. Each sire group value is the mean of 7–17 progeny. Sire progeny group means and 95% confidence intervals are presented in Table 1.

or pool A (r_s = −0.81) indicate that in these baboons cholesterol excretion is likely the primary mechanism for the genetic control of HDL-C and the cholesterol mass of pool A. This conclusion is consistent with our previous reports (15, 16) of strong negative genetic correlations for HDL-C and pool A with cholesterol turnover rate.

Several studies (4–6) have shown that HDL-C is a preferred precursor for bile acid synthesis. That observation is consistent with the strong correlation (−0.78) of HDL-C with K_A. If production of HDL-C were the primary process controlling HDL-C concentration and were also the principal regulator of cholesterol excretion, we would have expected a positive correlation between HDL-C and K_A. However, the strong negative correlation of HDL-C with K_A (r_s = −0.78) suggests that the differences among sire progeny groups for HDL-C and K_A are likely due to genetically mediated differences in the hepatic receptors or enzymes which regulate HDL-C catabolism. However, this speculation can be confirmed only by characterization of these sire progeny groups for metabolic processes which can simultaneously influence HDL-C and cholesterol turnover rate. These metabolic processes may include, among others, genetically mediated differences in activities of lecithin cholesterol acyl transferase (LCAT), hepatic lipase, or cholesterol 7α-hydroxylase or in processes controlling steroid reabsorption from the intestine.

We previously reported (16) significant differences among these sire progeny groups for cholesterol turnover rate measured by an isotopic balance procedure. The sire progeny group means for cholesterol turnover rate were inversely correlated (r_s = −0.55) with those for HDL-C,[1] a finding consistent with the negative correlation (Table 2) between sire family means for HDL-C and K_A (r_s = −0.78). In contrast, the correlation between sire family means for HDL-C and Q_A is low (r_s = −0.09). Cholesterol turnover rate and Q_A measure fecal excretion of bile salts and neutral steroids. However, Q_A also includes steroid losses due to skin sloughing (26) and steroid hormone production. These additional sources of variation which contribute to Q_A may explain the lower correlation between HDL-C and Q_A (r_s = −0.09) as compared to that for HDL-C with turnover rate (r_s = −0.55).

The differences among sire progeny group means for K_{AB} (Table 1) are likely due to genetically mediated differences for those metabolic processes that regulate cellular cholesterol concentrations. Recent in vitro studies have shown that the HDL_2 and HDL_3 subfractions have opposite effects on cellular cholesterol metabolism (7, 27). Further study of these HDL subfractions could clarify

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[1] Flow, B. L., and G. E. Mott. Unpublished results.

the metabolic basis for the strong inverse relationship (Table 2) of HDL-C with K_{AB} ($r_s = -0.94$). This additional information should also greatly enhance our understanding of the relationships between HDL-C and other parameters of cholesterol metabolism.

We thank Cynthia M. Farley and Evelyn M. Jackson for excellent technical assistance. Dr. Douglas Eggen, Louisiana State University Medical Center, New Orleans, provided advice and the computer program for fitting data to a two-pool model of cholesterol metabolism. Drs. C. Alex McMahan and Steve Ingram adapted the two-pool model program to our computer system. Dr. T. C. Cartwright, Texas A&M University, provided the experimental design and assisted during the analysis of data. We also thank Dr. H. C. McGill, Jr., University of Texas Health Science Center, at San Antonio for his support of this project and for reviewing the manuscript. Dr. Herman Wigodsky stimulated many discussions and offered valuable comments during preparation of the manuscript. Drs. DeWitt Goodman, George Barnwell, and Jim Kelley also reviewed the manuscript. This research was supported by grants HL-15914, HL-19362, and HL-28972 from the National Heart, Lung, and Blood Institute.

Manuscript received 22 July 1983.

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