Lack of relationship in humans of the parameters of body cholesterol metabolism with plasma levels of subfractions of HDL or LDL, or with apoE isoform phenotype

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Abstract The factors involved in regulating parameters of whole body cholesterol metabolism in humans have been explored in a series of investigations. Several physiological variables have been identified (weight, excess weight, plasma cholesterol, and age) that can predict 53–76% of the variation in production rate (PR) and in the sizes of the rapidly exchanging pool of body cholesterol (M_1) and of the minimum estimates of the slowly exchanging pool of body cholesterol (M_3min) and of total body cholesterol (M_{tot}min). Surprisingly, measurements of the plasma levels of HDL cholesterol and of the major HDL apolipoproteins (apoA-I, A-II, and E) did not provide additional information useful in predicting parameters of whole body cholesterol metabolism. A study was therefore conducted to investigate possible relationships of the plasma levels of subfractions of lipoproteins, determined by analytic ultracentrifugation, and of apoprotein E phenotype, with the parameters of whole body cholesterol metabolism. Ultracentrifugal analysis of plasma lipoprotein subfractions was performed at the Donner Laboratory in 49 subjects; all of these subjects were currently undergoing whole body cholesterol turnover studies or had previously had such studies and were in a similar metabolic state as judged by plasma lipid and lipoprotein values. Apoprotein E phenotyping was carried out in 71 subjects. Differences in model parameters were sought among subjects with various apoprotein E phenotypes. Ultracentrifugal LDL subfractions S_f° 0–2 (the region of LPa), S_f° 0–7 (smaller LDL), S_f° 7–12 (larger LDL), S_f° 12–20 (IDL), and ultracentrifugal HDL subfractions $F_{1.20}^\circ$ 0–1.5 (smaller HDL_3), $F_{1.20}^\circ$ 2–9 (larger HDL_3 plus HDL_2), and $F_{1.20}^\circ$ 5–9 (larger HDL_2 or HDL_{2b}) were examined for correlations with each other and with parameters of whole body cholesterol metabolism. Although an interesting negative correlation between the smaller LDL subfraction (S_f° 0–7) and the HDL_2-enriched subfraction ($F_{1.20}^\circ$ 2–9) was confirmed, neither the apoprotein E phenotype nor any of the analytic ultracentrifugal measurements (or their ratios) that were examined provided additional predictive information about parameters of whole body cholesterol metabolism. We conclude that these variables are not important determinants of parameters of whole body cholesterol metabolism in humans, and that other explanations must be sought for the remaining variability in these parameters. —**Palmer, R. H., A. V. Nichols, R. B. Dell, R. Ramakrishnan, F. T. Lindgren, E. L. Gong, C. B. Blum, and D. S. Goodman.** Lack of relationship in humans of the parameters of body cholesterol metabolism with plasma levels of subfractions of HDL or LDL, or with apoE isoform phenotype. *J. Lipid Res.* 1986. **27:** 637–644.

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It is generally accepted that there is a relationship between levels of circulating lipoproteins, particularly LDL and HDL, and the amount of cholesterol in tissues, but the precise relationship and the factors that govern it are poorly understood. In previous work from this laboratory, the turnover of [^{14}C]cholesterol in plasma has been used in conjunction with a three-pool model to study various parameters of whole body cholesterol metabolism in man (1–4). This method provides estimates of the masses of exchangeable cholesterol and of the rates of flux of cholesterol in the pools and in the whole body.

In a previous study (4), several physiological variables were identified that predicted 53–76% of the variation among humans in four major model parameters, namely production rate (PR), the size of the rapidly exchanging compartment (M_1), and the minimum values of the size of the slowly exchanging pool 3 (M_3min) and of total body exchangeable cholesterol (M_{tot}min). These model parameters could be predicted by simple equations involving various combinations of body weight, plasma lipid levels, and age (4). More recently, we conducted an extensive

Abbreviations: PR, production rate; M_1, M_2, M_3, M_{tot}, pool sizes; k, rate constant; tot, total; min, minimum estimate; HDL, high density lipoprotein; apo, apolipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein.

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study to determine whether or not relationships exist between the parameters of body cholesterol metabolism and the plasma levels of HDL cholesterol and of apoproteins A-I, A-II, and E. This study (5), carried out in 55 subjects who underwent long-term cholesterol turnover studies, focused particularly on possible relationships of HDL cholesterol and apoprotein levels with the mass of slowly exchangeable (and total) body cholesterol. Neither the levels of HDL cholesterol nor of any of these apoproteins provided additional information (beyond that provided by the previously identified (4) physiological variables) about the pool sizes or other parameters of whole body cholesterol metabolism.

The present investigation was undertaken to determine whether additional information about the parameters of whole body cholesterol metabolism can be obtained from ultracentrifugal measurements of the plasma levels of various lipoprotein subfractions or from the determination of apoprotein E isoform phenotype. These analyses were carried out in a large number of patients who were undergoing or had previously undergone long-term cholesterol turnover studies. Neither the apoprotein E isoform status nor any of the lipoprotein subfraction measurements provided additional information for predicting the parameters of whole body cholesterol metabolism beyond that provided by the previously confirmed determinants.

METHODS

Subjects studied

A total of 103 hyperlipidemic patients or normal volunteers have had long-term cholesterol turnover studies conducted in our laboratory. The clinical characteristics of the 21 patients not reported previously are shown in **Table 1**, identified by their study number. (Study numbers are assigned sequentially. Since several of the 103 subjects have had more than one study, the total number of studies is greater than 103.)

Apoprotein E isoform phenotyping was possible in 71 of the 103 subjects: subjects E. M., G. W., D. F., J. F., and C. G. (n = 5) from reference 3; subjects 26, 27, 31–35, 38, 39, 41–51, 53, 54 (n = 22) from reference 4; subjects 55, 57, 58, 60, 63–65, 67–70, 74, 76–88 (n = 25) from reference 5; and subjects 89–101, 103–106, 108, 109 (n = 19) from Table 1, this study. Four patients (subjects 55 and 72 (E3,3 phenotype) and subjects 73 and 82 (E4,4 phenotype)) from reference 5 had undergone repeat cholesterol turnover studies, and the results of all 75 studies were used to search for an effect of apoE phenotype on parameters of whole body cholesterol metabolism.

Plasma samples were obtained from 59 subjects for ultracentrifugal analyses. Aliquots of plasma were taken for lipid and lipoprotein analyses, and the remainder of each sample was sent to the Donner Laboratory for analytic ultracentrifugation. Plasma from 10 subjects was rejected for the following reasons: *a*) the total cholesterol level of the plasma sample sent to the Donner Laboratory differed by more than 2 SD from the subject's mean plasma cholesterol level during the cholesterol turnover study 1–7 years previously (n = 5); *b*) the total triglyceride level of the sample sent to the Donner Laboratory differed by more than 3 SD from the subject's mean plasma triglyceride level during the turnover study (n = 2); or *c*) there were uncertainties about the representative nature of the sample sent to the Donner Laboratory (n = 3). Note that the SD values used in these evaluations were the values observed for the cholesterol or triglyceride levels for each individual subject during the turnover study. The remaining 49 subjects either had Donner Laboratory samples collected during the cholesterol turnover studies (n = 17) or were considered to be in a metabolic state at the time of blood sampling that was reasonably similar to that of the cholesterol turnover study, as manifested by lipid and lipoprotein values in the Donner Laboratory sample that were similar to those characterizing the cholesterol turnover study period. All patients who had received lipid-lowering drug therapy had discontinued the medication for at least a month prior to donating blood for the Donner Laboratory sample.

For the 32 subjects whose Donner Laboratory samples were collected after the completion of the cholesterol turnover study, the time intervals between turnover study completion and sample collection were, as follows: < 1 year, 7 subjects; 1–3 years, 10 subjects; 3–5 years, 10 subjects; 5–7 years, 5 subjects. The fact that half of the subjects in this study had Donner Laboratory samples that were collected 1 or more years after the completion of the turnover study does, of course, somewhat weaken the potential power of the study. We felt, however, that more analytical power was gained by inclusion of a larger subject population (n = 49) in the overall study than by restricting the final study to subjects whose Donner Laboratory samples had been collected contemporaneously with (n = 17) or within a year of the end of (n = 7) the turnover study.

The clinical characteristics of the 49 subjects used for the ultracentrifugal studies have been presented in detail as subjects #26, 31–34, 39, 41–44, 49, 51–53 in reference 4, subjects #55, 57, 60, 67–70, 74–88 in reference 5, and subjects #89, 90, 92–101, 103 in the present study. In two subjects, two plasma samples were analyzed by analytic ultracentrifugation, and in three subjects three samples were analyzed. In addition, two patients had had two cholesterol turnover studies. For all patients with repeat studies, the average values of the repeat studies were used. An overall summary of the lipid status of the 49 study subjects is as follows (using the classification criteria pro-

TABLE 1. Characteristics of new subjects studied[a]

Subject #	Sex	Age	Height	Weight	Ideal Body Weight[b]	Plasma Cholesterol[c]	Plasma Triglyceride[c]	Familial Disorder[d]
		yr	*cm*	*kg*	*%*	*mg/dl*		
89	F	60	159	65	125	318 ± 18	211 ± 107	IND
90	M	37	189	85	101	307 ± 28	96 ± 14	FH
91	F	70	149	57	122	297 ± 16	191 ± 64	IND
92	M	65	182	80	110	342 ± 18	73 ± 10	FH
93	F	18	159	60	115	218 ± 15	89 ± 15	FH
94	F	42	157	70	137	261 ± 17	176 ± 55	IND
95	F	51	157	50	97	277 ± 20	233 ± 88	FH
96	F	25	164	48	93	315 ± 17	67 ± 15	FH
97	F	59	163	55	101	290 ± 21	115 ± 25	IND
98	F	62	173	70	114	326 ± 21	196 ± 31	IND
99	F	58	175	90	133	329 ± 17	229 ± 46	IND
100	M	27	183	88	111	259 ± 27	210 ± 41	FH
101	F	55	160	52	105	360 ± 26	140 ± 22	IND
103	M	52	173	85	129	302 ± 17	293 ± 84	FH
104	F	40	171	64	107	290 ± 21	37 ± 6	FH
105	M	46	183	100	137	279 ± 14	207 ± 54	FH
106	M	58	177	80	116	390 ± 22	87 ± 16	FH
107	F	41	159	61	117	258 ± 24	143 ± 65	FH
108	F	50	166	59	104	369 ± 44	107 ± 24	FH
109	F	50	154	55	111	369 ± 19	117 ± 34	FH
111	M	64	188	74	101	346 ± 28	154 ± 23	IND

[a] See text for complete list of subjects used in each study.
[b] Percent of ideal body weight = actual weight divided by mean desirable weight for the patient's frame as determined from actuarial data times 100 (21).
[c] Mean ± SD during the period of study.
[d] Familial disorder: IND, indeterminate; FH, familial hypercholesterolemia, as determined by the patient and at least one first degree relative having an initial plasma cholesterol level >95th percentile (22).

vided in Table 1 of reference (5)): 12 subjects were normal; 17 subjects had hypercholesterolemia (10 with FH); 11 subjects had hypertriglyceridemia; and 9 subjects had mixed hyperlipidemia (3 with FH).

Cholesterol turnover studies and their analyses

[4-^{14}C]Cholesterol (New England Nuclear, Boston, MA), complexed with the subject's own serum lipoproteins, was injected intravenously and the plasma total cholesterol specific activity decay curve was determined as described previously (1–3). In some subjects, a simplified sampling strategy was utilized (6). The specific activity decay data were analyzed by a weighted, least squares technique to determine the parameters of a three-pool mammillary model that gave the best fit (7). Six unique parameters were determined: PR (cholesterol production rate in g/day), M_1 (size of pool 1 in g), and the constants k_{12}, k_{21}, k_{13}, and k_{31} (rate constants for transfer between pool 2 or 3 and pool 1 in days^{-1}). Minimum values for the size of pool 2 (M_2), of pool 3 (M_3), and for the amount of total exchangeable body cholesterol (M_{tot}) were computed by assuming that all new cholesterol enters pool 1. Different (and physiologically more reasonable (8)) assumptions about the amount of cholesterol synthesis in the side pools would not be expected to alter the relative relationships of the computed minimum pool sizes to the independent variables.

Sample treatment—analytic ultracentrifugation studies

Venous blood samples were obtained after an overnight fast, with EDTA (1 mg/ml) as an anticoagulant, and immediately placed in ice-cold water. Plasma was separated within 4 hr of blood collection and shipped on ice to the Donner Laboratory by overnight express. Prior to shipment, aliquots were taken for cholesterol, triglyceride, and lipoprotein cholesterol determinations. At the Donner Laboratory, the d ≤ 1.063 g/ml lipoproteins were separated by ultracentrifugation at 114,000 *g* for 24 hr and then subjected to analytic ultracentrifugation. A second aliquot of plasma was adjusted to d 1.2168 g/ml and subjected to ultracentrifugation, followed by analytic ultracentrifugation of the d ≤ 1.20 g/ml lipoproteins. The mass of d ≤ 1.063 g/ml lipoproteins was determined in 29 flotation intervals between S_f^o 0–400 and the mass of HDL lipoproteins was determined in 15 flotation intervals between $F^o_{1.20}$ 0–9. The details of the ultracentrifugal procedures have been described previously (9).

Analytical techniques

Cholesterol and triglyceride concentrations were measured using Technicon AAI methodology (Technicon In-

struments Corp., Tarrytown, NY) (10). Lipoprotein cholesterol was determined using the Lipid Research Clinics methodology (11). ApoE isoforms were determined by isoelectric focusing with and without cysteamine treatment, as described by Rall et al. (12).

Data analysis—apolipoprotein E study

The statistical significance of differences in model parameters among different apoE isoform phenotypes was tested by an analysis of covariance using weight as a covariate for PR; weight, plasma cholesterol concentration, and triglyceride group as covariates for M_1; weight, plasma cholesterol concentration, and age as covariates for M_3min; and weight and plasma cholesterol concentration as covariates for M_{tot}min (4). Since no equations have been found that significantly relate M_2min or the various rate constants (k values) to physiological variables, possible differences in these latter model parameters between subjects with different apoE isoform phenotypes were examined by analysis of variance.

Data analysis—analytic ultracentrifugation study

Lipoprotein mass data from the ultracentrifugal analyses were combined in flotation intervals that were considered to be of physiological interest and that might reveal new associations between plasma lipoprotein subfractions and parameters of whole body cholesterol metabolism. The d $\leq$ 1.063 g/ml lipoproteins were divided into those with flotation rates between S_f^o 0–2 (the region of LPa), S_f^o 0–7 (smaller LDL), S_f^o 7–12 (larger LDL), S_f^o 12–20 (IDL), and S_f^o 10–14 (13). The HDL (d 1.063–1.20 g/ml) lipoproteins were divided into those with flotation rates between $F_{1.20}^o$ 0–1.5 (smaller HDL_3), $F_{1.20}^o$ 2–9 (larger HDL_3 plus HDL_2), and $F_{1.20}^o$ 5–9 (larger HDL_2 or HDL_{2b}) (14, 15). In addition, in order to use some measures of the relative proportions of various lipoprotein species (i.e., to reflect patterns of distribution rather than absolute amounts), several ratios were constructed: S_f^o (12–20)/(0–12), (or the ratio of IDL to LDL); S_f^o (7–12)/(0–7) (or the ratio of larger LDL to smaller LDL); and $F_{1.20}^o$ (0–1.5)/(2–9).

Possible relationships between the levels (and ratios) of the selected lipoprotein subfractions and the model parameters of body cholesterol metabolism were tested for statistical significance by multiple regression analysis (16). Each of the previously identified variables (4) and all of the lipoprotein measurements were included as independent variables. All possible regressions were examined to test whether any of the subfractions substituted for or improved the predictive accuracy of each model parameter. Since 11 new variables (see Table 4) were being tested for significance, tests were declared significant at the probability levels of 0.05/11 or 0.004 and 0.01/11 or 0.0009 for the 5% and 1% level, respectively. Correlation coefficients (r) of 0.384 and 0.443 were considered significant at the 5% and 1% levels, respectively. If a correlation coefficient was found that exceeded 0.384, then the significance of the correlation was assessed by the "jackknife" procedure (17). The jackknife procedure is a method for assessing whether the correlation just found is due to one or two outliers and hence may be a chance observation. In this procedure, data for each subject are deleted one at a time and the correlation coefficient is recomputed using data from the remaining subjects. (With our population of 49 subjects, the correlation was recomputed on the remaining 48 subjects when 1 subject was dropped, and on the remaining 47 when 2 subjects were dropped.) If there is a large fall in the correlation coefficient when 1 or 2 subjects are dropped, then the correlation may be due to the chance inclusion of the outlier(s).

Finally, because Krauss, Lindgren, and Ray (15) had described a relationship between LDL S_f^o 0–7 and HDL $F_{1.20}^o$ 2–9, as part of this study we also looked for correlations between these two lipoprotein subfractions.

RESULTS

Apolipoprotein E study

The top portion of **Table 2** gives the characteristics of the subjects in the apoE study and the distribution of the characteristics among the apoE isoform phenotype groups. The bottom portion of Table 2 gives the major model parameters for the entire group, as well as the model parameters (adjusted as indicated) in the apoE isoform groups. The values for the different apoE phenotype groups are generally similar, and no effect of apoE isoform phenotype on any of the model parameters was found on statistical analysis.

Analytic ultracentrifugation studies

Table 3 summarizes the characteristics of the subjects in the analytic ultracentrifugation study. **Table 4** summarizes the lipoprotein mass levels found within the various flotation intervals in this study population. In each interval there was a broad distribution of values (indicated by the high SD values) in the population, providing an opportunity to demonstrate relationships if such existed.

Relation of lipoprotein subfractions to model parameters

Table 5 shows the effects of the various HDL and LDL subfractions on the model parameters of whole body cholesterol metabolism. On the basis of the previously established relationships (4) between certain independent variables (weight, age, total plasma cholesterol, total plasma triglyceride group, excess weight, and plasma cholesterol concentration times weight) and some of the dependent variables (PR, M_1, M_3min, and M_{tot}min), a multiple R

TABLE 2. Summary of characteristics of subjects used in the apoE isoform study[a]

	Total	ApoE Isoform Phenotype					
		3,3	3,4	4,4	2,3	2,2	2,4
Number	75	44	15	7	6	2	1
Age (yr)	48.9	47.7	53.8	48.3	47.7	48.0	40
Weight (kg)	76.1	73.8	80.6	73.4	81.4	91.0	64
% I.B.W.	115	115	120	108	118	122	106
Plasma Chol (mg/dl)	275	284	234	251	321	324	287
Plasma TG (mg/dl)	239	221	274	150	362	401	37
PR (g/day)	1.20	1.19	1.30	1.12	1.27	1.43	0.95
M_1 (g)	23.3	22.7	24.7	26.2	22.5	24.8	21.4
M_3min (g)	38.6	38.3	40.9	31.7	43.3	28.2	59.5
M_{tot}min (g)	79.1	77.1	85.5	81.8	77.9	75.6	97.7
$k_{12}(\times 10^3)$ (day^{-1})	89.1	89.4	96.5	68.0	97.5	88.5	67.0
$k_{13}(\times 10^3)$ (day^{-1})	16.2	16.4	16.8	13.1	17.8	18.0	11.0

[a]Mean values (single values for the E2,4 subject) are presented for the physiological variables of age, weight, % I.B.W., and plasma lipid levels. For the model parameters of PR and pool size (M), the isoform group values were adjusted by analysis of covariance for weight (PR); weight, cholesterol and triglyceride group (M_1); weight, cholesterol and age (M_3min); or weight and cholesterol (M_{tot}min).

(third column) was calculated using the independent variables shown in the second column. As in our previous studies, these R values were highly significant, and accounted (as R^2) for 32% to 71% of the variation in the dependent variables. (The LDL subfractions S_f^o 7–12 and S_f^o 12–20 (IDL) correlated highly with total cholesterol, and could be substituted for total cholesterol in the predictive equations, but in each case the significance of the relationship decreased.) We then determined the partial R for each lipoprotein subfraction or ratio of subfractions as an additional independent variable, or as a single independent variable in the case of those dependent variables for which no previous correlates had been found (M_2min, k_{12}, k_{21}, k_{13}, and k_{31}).

As shown in Table 5, the partial R values for the added lipoprotein subfraction variables were small and statistically different from zero only for k_{21} (see below). For the major model parameters PR, M_1, M_3min, and M_{tot}min, for which predictive equations had been found previously (4), the |partial R| values varied from 0.00 to 0.29, and more than three-fourths of these 66 |partial R| values were less than 0.20. Hence, less than 9% of the remaining variations in any of these major model parameters could be explained by any of the lipoprotein variables. For the other model parameters, the largest |partial R| values among the lipoprotein subfractions were found with k_{21}. Using values of 0.384 and 0.443 (see Methods) as representing the 5% and 1% levels of significance, the correlations of k_{21} with lipoprotein subfractions $F_{1.20}^o$ 2–9, $F_{1.20}^o$ 5–9, S_f^o 7–12, and S_f^o 10–14 were all judged initially to be significant (see Table 5). In all four cases, however, when one subject (#83) was dropped (jackknife procedure, see Methods), the partial correlation coefficients fell to insignificant levels (0.04 to 0.14); thus the entire apparent significance observed originally was due to an unusual k_{21} for this one subject. (There was nothing unusual about the fit of the three-pool model to the data from this subject.) Thus, the apparent correlation probably does not reflect a physiological association between this model parameter and these lipoprotein subclasses.

As indicated in the Methods section, as part of this study we also examined the relationship between the LDL S_f^o 0–7 and HDL $F_{1.20}^o$ 2–9 lipoprotein subfractions (**Table 6**). LDL S_f^o 0–7 did correlate negatively with HDL $F_{1.20}^o$ 2–9 ($r = -0.474$), as described by Krauss et al. (15). The negative correlation was slightly greater for HDL $F_{1.20}^o$ 5–9, but the difference was not statistically significant. This LDL subfraction also correlated positively with HDL $F_{1.20}^o$ 0–1.5, although the correlation ($r = 0.309$) was not strong. Finally, we looked at the remaining possible relationships between lipoprotein subfractions, but none were significant.

DISCUSSION

The study reported here was designed to explore possible relationships of the plasma levels of subfractions of HDL and LDL, and of apoE phenotype, with the parameters of whole body cholesterol metabolism. The present study aimed to extend the recent report from this laboratory (5), which indicated that the plasma levels of total HDL cholesterol and of apoproteins A-I, A-II, and E are

TABLE 3. Summary of characteristics of subjects used in the analytic ultracentrifugation study

Variables	Mean ± SD
Age (yr)	49 ± 13
Weight (kg)	74 ± 14
% Ideal weight	114 ± 12
Height (cm)	172 ± 10
Plasma cholesterol (mg/dl)	284 ± 79
Plasma triglycerides (mg/dl)	213 ± 160

TABLE 4. Lipoprotein mass in selected flotation intervals

Lipoprotein Flotation Interval	Mean ± SD
	mg/dl
$F^{o}_{1.20}$ 0–1.5	73 ± 17
$F^{o}_{1.20}$ 2–9	161 ± 85
$F^{o}_{1.20}$ 5–9	22 ± 37
$F^{o}_{1.20}$ (0–1.5)/(2–9) (%)	59 ± 32
S^{o}_{f} 0–2	7.7 ± 7.0
S^{o}_{f} 0–7 (smaller LDL)	289 ± 113
S^{o}_{f} 7–12 (larger LDL)	216 ± 143
S^{o}_{f} 10–14	65 ± 34
S^{o}_{f} 12–20 (IDL)	70 ± 40
S^{o}_{f} (12–20)/(0–12) (IDL/LDL) (%)	15 ± 8.2
S^{o}_{f} (7–12)/(0–7) (%)	120 ± 272

not quantitatively important determinants of any of the parameters of whole body cholesterol metabolism in humans. This previous work (5) had its impetus in hypotheses on the mechanism responsible for the known inverse relationship between HDL levels in plasma and coronary heart disease rates (18, 19). A leading proposed mechanism for this inverse relationship is that HDL may reduce tissue stores of cholesterol by transporting cholesterol from peripheral tissues, including the arterial wall, to the liver (20). In support of this hypothesis, Miller, Nestel, and Clifton-Bligh (23) reported in 1976, in a study of 8 subjects using a two-pool model, that the plasma level of HDL cholesterol was inversely correlated with the mass of slowly exchanging body cholesterol. However, this 1976 study had several major shortcomings, as discussed in detail recently (5). Moreover, an extensive study of 55 subjects recently reported from this laboratory (5) showed that the plasma levels of HDL cholesterol or HDL apoproteins are not independent determinants of the kinetically defined mass of exchangeable pools of body cholesterol. This study (5) hence provided no support for the concept that the inverse relationship between levels of HDL cholesterol and coronary heart disease rate is mediated by an HDL-induced reduction of the amount of cholesterol that accumulates in tissues.

We next considered the possibility that significant relationships might exist between the plasma levels of certain lipoprotein subfractions and parameters of whole body cholesterol metabolism that might not be demonstrable by an analysis that used gross measurements of an entire lipoprotein fraction. Accordingly, the present study was undertaken to examine this possibility, using analytic ultracentrifugal analysis to define lipoprotein subfractions in terms of their flotation characteristics within the LDL/IDL and HDL density ranges. It should be noted, moreover, that the ultracentrifugal analysis measured total lipoprotein mass in the various flotation fractions, whereas the previous analysis (5) had used the cholesterol content of the lipoprotein fraction. In the data analysis used here, we searched both for possible relationships between plasma levels of the various lipoprotein subfractions and parameters of the three-pool model, and also examined the possibility that the distribution (pattern) of lipoprotein subfractions within a fraction might have importance beyond that of the mass in any particular fraction or subfraction. For the latter purpose we used several ratios (to reflect differences in distribution) in the analysis: $F^{o}_{1.20}$ (0–1.5)/(2–9) (ratio of smaller HDL_3 to larger HDL_3 plus HDL_2), S^{o}_{f} (12–20)/(0–12) (the ratio of IDL to LDL), and S^{o}_{f} (7–12)/(0–7) (the ratio of larger to smaller LDL).

Analyses were also carried out to investigate possible relationships between the apoE isoform phenotype and the parameters of body cholesterol metabolism. The rationale for these analyses was the fact that apoE is known to play an important role in lipoprotein binding to, and cell uptake via, specific cell surface receptors. Moreover, the apoE2 isoform is known to bind very poorly to

TABLE 5. Independent effects of lipoprotein subfractions in determining model parameters of total body cholesterol metabolism

Dependent Variable	Independent Variables[a]	R[b]	Partial R for HDL Flotation Intervals as Additional Independent Variables[c]				Partial R for LDL and IDL Flotation Intervals as Additional Independent Variables[c]						
			F 0–1.5	F 2–9	F 5–9	F 0–1.5 / F 2–9	S 0–2	S 0–7	S 7–12	S 10–14	S 12–20	S 12–20 / S 0–12	S 7–12 / S 0–7
PR	Wt	0.73	0.11	0.05	0.02	0.01	0.23	−0.12	−0.25	−0.22	−0.10	0.16	−0.03
M_1	Wt, Chol	0.80	−0.02	−0.04	0.02	−0.03	0.12	−0.06	0.11	0.02	−0.10	0.01	0.12
M_3min	Wt, Age	0.57	0.13	−0.26	−0.29	0.26	0.00	0.29	0.12	0.19	0.20	−0.06	−0.07
M_3min	EWt, Age	0.71	0.18	−0.17	−0.27	0.24	−0.04	0.06	0.05	0.24	0.00	−0.09	−0.10
M_{tot}min	Wt, Chol	0.83	−0.04	−0.13	−0.12	0.18	−0.02	0.00	−0.21	−0.18	−0.09	−0.10	−0.06
M_{tot}min	EWt, Chol-Wt	0.84	0.11	−0.10	−0.17	0.17	−0.13	−0.24	−0.18	−0.23	−0.21	−0.03	−0.05
M_2min			0.02	−0.03	−0.03	0.10	−0.07	−0.10	−0.28	−0.37	−0.32	−0.13	−0.25
k_{12}			0.20	−0.02	0.05	0.11	0.06	0.00	0.10	0.15	0.18	0.10	0.09
k_{21}			−0.06	0.40	0.46	−0.05	−0.05	−0.30	−0.39	−0.45	−0.32	−0.14	−0.18
k_{13}			0.06	0.00	0.05	−0.04	−0.01	−0.04	0.17	0.28	0.25	0.20	0.32
k_{31}			0.04	−0.07	−0.04	0.11	−0.03	−0.02	−0.01	0.17	0.21	0.17	0.23

[a] Abbreviations used (not already defined in text): Chol, serum cholesterol concentration (mg/dl); Wt, observed body weight (kg); EWt, excess weight (observed minus ideal weight); Chol-Wt, total plasma cholesterol concentration times weight.

[b] R is the multiple correlation coefficient for the listed independent variables.

[c] F refers to the flotation rate ($F^{o}_{1.20}$) of the HDL (d 1.063–1.20 g/ml) lipoproteins; S refers to the flotation rate (S^{o}_{f}) of the d ≤ 1.063 g/ml (LDL, IDL, and VLDL) lipoproteins.

TABLE 6. Correlations between lipoprotein subfractions[a]

LDL Subfractions	HDL Subfractions		
	F 0-1.5	F 2-9	F 5-9
S 0-2	0.212	-0.264	-0.267
S 0-7	0.309[b]	-0.474[c]	-0.516[c]
S 7-12	-0.063	0.242	0.103
S 10-14	-0.069	0.112	0.038
S 12-20	0.075	-0.100	-0.157

[a] Definitions of F and S as in Table 5. The values listed are the univariate correlation coefficients (r) for the indicated LDL and HDL subfractions.
[b] $P < 0.05$.
[c] $P < 0.01$.

the apoB,E (LDL) receptor (12, 24), and several laboratories have reported that the apoE isoform phenotype influences plasma lipoprotein (and lipid) concentrations (25, 26). Hence, it seemed reasonable to investigate whether the apoE phenotype might influence the sizes of kinetically defined pools of body cholesterol or other parameters of whole body cholesterol metabolism.

The results reported here show that neither lipoprotein subfraction levels and ratios nor apoE phenotype provided additional predictive information beyond that provided by the previously identified (4) physiological variables. The data thus suggest that HDL and LDL subfractions and apoE phenotype are not important determinants of the major parameters of whole body cholesterol metabolism in humans. This conclusion, however, does have certain limitations. As discussed previously (5), cholesterol turnover studies such as these provide a "low power" view of whole body cholesterol metabolism. Thus, arterial wall cholesterol content is a small part of pool 3 but is the locus of the atherosclerotic process. Lipoproteins certainly have major effects on cholesterol metabolism in cells, and these effects include influences on the transport of cholesterol into and out of cells. Nevertheless, our results suggest that the variables explored here may not be rate-limiting in these processes. It is also worth noting that a large amount of the variability in the dependent variables has already been accounted for by the known independent variables (4); further definition of the factors accounting for the remaining variability may be quite difficult.

The failure to demonstrate any independent variable with any significant relationship to any of the rate constants is an interesting and provocative finding of our studies to date. It is tempting to speculate that these processes may be unpredictable from the variables studied because they are closely involved in the hour to hour and day to day regulation of cholesterol homeostasis.

We have confirmed the inverse relationship noted previously by Krauss et al. (15) between the smaller LDL fraction (S_f^o 0-7) and the $F_{1.20}^o$ 2-9 HDL subfraction. The negative correlation was slightly greater for the HDL_{2b} subfraction, compared with the whole $F_{1.20}^o$ 2-9 (larger HDL_3 plus HDL_2) fraction, and there was, as also noted by Krauss et al. (15), a significant *positive* correlation between this LDL fraction and the smaller HDL_3 subfraction ($F_{1.20}^o$ 0-1.5). If, as suggested by Krauss et al., part of the inverse relationship noted epidemiologically between HDL_2 and coronary heart disease is related to a second inverse relationship between HDL_2 and smaller LDL, this latter fraction may indeed represent a particularly atherogenic class of LDL. It is of interest that the correlation of LDL S_f^o 0-2 with $F_{1.20}^o$ 2-9 or the HDL_{2b} subfraction was less strong than the correlation of LDL S_f^o 0-7, suggesting that it is not Lp(a), which is found in the S_f^o 0-2 fraction, that is accounting for the relationship.

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