Relationship of the parameters of body cholesterol metabolism with plasma levels of HDL cholesterol and the major HDL apoproteins

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Abstract The inverse relationship between plasma levels of high density lipoprotein (HDL) and coronary heart disease rates has suggested that HDL might influence body stores of cholesterol. Therefore, we have investigated potential relationships between the parameters of body cholesterol metabolism and the plasma levels of HDL cholesterol and the major HDL apoproteins. The study involved 55 human subjects who underwent long-term cholesterol turnover studies, as well as plasma lipoprotein and apolipoprotein assays. In order to maximize the likelihood of detecting existing relationships, the subjects were selected to span a wide range of plasma levels of lipids, lipoproteins, and apolipoproteins. Single univariate correlation analyses suggested weak but statistically significant inverse relationships of HDL cholesterol and apoA-I levels with the following model parameters: production rate (PR), the mass of rapidly exchanging body cholesterol (M_1), the minimum estimate of the mass of slowly exchanging body cholesterol (M_3min), and of the mass of total exchangeable body cholesterol (M_{tot}min). These correlations, however, were quantitatively quite small ($|r| = 0.28–0.42$) in comparison to the strength of the univariate relationships between body weight and PR ($r = 0.76$), M_1 ($r = 0.61$), M_3min ($r = 0.58$), and M_{tot}min ($r = 0.78$). Correlations for apoA-II and apoE levels were even smaller than those for apoA-I and HDL cholesterol. In additional analyses using multivariate approaches, HDL cholesterol, apoA-I, apoA-II, and apoE levels were all found not to be independent determinants of the parameters of body cholesterol metabolism ($|\text{partial } r| < 0.17$, $P > 0.3$ in all cases). Thus the weak univariate correlations reflect relationships of HDL cholesterol and apoA-I levels with physiological variables, such as body size, which are primarily related to the model parameters. We conclude that plasma levels of HDL cholesterol and apoproteins A-I, A-II, and E are not quantitatively important independent determinants of the mass of slowly exchanging body cholesterol or of other parameters of long-term cholesterol turnover in humans. These studies give no support to the hypothesis that the inverse relationship between HDL cholesterol levels and coronary heart disease rates is mediated via an influence of HDL on body stores of cholesterol. — **Blum, C. B., R. B. Dell, R. H. Palmer, R. Ramakrishnan, A. H. Seplowitz, and D. S. Goodman.** Relationship of the parameters of body cholesterol metabolism with plasma levels of HDL cholesterol and the major HDL apoproteins. *J. Lipid Res.* 1985. **26:** 1079–1088.

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The inverse relationship between plasma levels of high density lipoproteins (HDL) and coronary risk is well established (1–3), and has sparked considerable interest in the mechanism responsible for this relationship. A considerable amount of indirect biochemical evidence has suggested that the mechanism may involve HDL-mediated removal of cholesterol from tissues with its transport to the liver (4–6), a process that has been termed reverse cholesterol transport. The ability of HDL and its apoproteins to remove cholesterol from cultured cells (7–9) indicates that HDL may play such a role in reverse cholesterol transport. If this process is an important regulator of tissue stores of cholesterol in general, it may limit net accumulation of cholesterol in peripheral tissues including arteries. Such effects on arteries might, in turn, be responsible for the diminished coronary risk associated with high levels of HDL.

Detailed reports of the relationships between plasma levels of HDL and tissue stores of cholesterol are limited. In 1976, Miller, Nestel, and Clifton-Bligh (10) reported a strongly inverse relationship between plasma levels of HDL cholesterol and the mass of slowly exchanging body cholesterol ($r = -0.88$). However, that result was based on only eight subjects who underwent short-term cholesterol turnover studies of 10–14 weeks duration. Such studies do not give accurate estimates of the mass of slowly exchanging pools of body cholesterol (11, 12). In a subsequent investigation that also used short-term choles-

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; VLDL, very low density lipoprotein.

terol turnover studies, it was found that five subjects with high plasma levels of HDL had significantly smaller pools of slowly exchanging body cholesterol than did a group of normal volunteers (13).

Because of these limitations in the presently available studies, we have conducted an extensive investigation 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, carried out in 55 subjects who underwent long-term cholesterol turnover studies, focused particularly upon possible relationships of HDL cholesterol and apoprotein levels with the mass of slowly (and of total) exchangeable body cholesterol. Some of this work has previously been reported in abstract form (14).

METHODS

Subjects studied

A total of 55 volunteer subjects participated in these studies; written informed consent was obtained from each. Every one of these 55 subjects underwent long-term cholesterol turnover study and had analyses for HDL cholesterol, apoA-I, apoA-II, and apoE. The characteristics and results obtained with the first 25 of these volunteers have been reported previously (12); these first 25 are listed as subjects #25, 26, 31–35, and 37–54 in Table 1 of that report. The clinical characteristics of the remaining 30 subjects in the present study population are listed in **Table 1.**

All subjects were studied as outpatients. All hyperlipidemic subjects had been instructed previously in a diet containing less than 300 mg/day of cholesterol and approximately 35% of total calories as fat, with less than 10% of calories as saturated fat. Subjects were asked not to change their diet, and no subject exhibited significant changes in weight during the study. Serum cholesterol levels were reasonably stable during the study as indicated by the small standard deviation values given in Table 1. Serum triglyceride concentration fluctuated more widely, particularly in hypertriglyceridemic subjects, but there were no time trends seen in any subject. These observations support the validity of the use of the model and the kinetic analysis employed here, since the model assumes the existence of a physiological steady state during the period of study.

Turnover studies and their analysis

[4-^{14}C]Cholesterol (New England Nuclear, Boston, MA), complexed with the subject's own serum lipoproteins was injected intravenously, and the specific radioactivity of serum total cholesterol was determined in samples collected serially thereafter, as described in detail previously (11, 12, 15). The amounts of radioactivity injected (approximately 25 μCi per subject) were measured precisely. In 39 subjects, samples were collected at 35 to 46 different times during the 38- to 46-week duration of the study. In the remaining subjects, as described in more detail below, a simplified sampling strategy involving six time points was used. The specific radioactivity of the cholesterol in each sample, and the serum concentrations of cholesterol were determined as described previously (11, 12, 15).

The specific radioactivity data were analyzed by a weighted, least squares technique described previously (16) to determine the parameters of a three-pool mammillary model that would provide the best fit. The model used is illustrated in **Fig. 1.** The fitting process yields six unique model parameters: 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}). As discussed previously (11), assumptions regarding the relative rates of synthesis of cholesterol in pools 2 and 3 lead to various estimates of pool size. Minimum values for M_2 and M_3 were computed by assuming that no synthesis occurs in the side pools, i.e., that all of cholesterol production enters pool 1. The sum of these minimum pool size estimates plus the size of pool 1 provided a minimum estimate for total exchangeable body cholesterol. Intermediate and maximum values for M_2 and M_3 were calculated as well, as described previously (12).

In 39 of the 55 subjects, a blood sampling strategy involving collection of 35–46 different samples in the course of the study was used. Because the need for such a large number of samplings limited the number of persons willing to participate in these studies, we developed and validated a simplified blood sampling strategy involving collection of samples at only six times to determine the six unique parameters of the three-pool model (17). This simplified, six-point sampling strategy was used in 16 of the 55 subjects (subjects numbered 73 to 88 in Table 1).

The approach used for this six-point sampling strategy has been described elsewhere (17). In brief, for the three-pool model, only six accurate points, at optimal times, are required to estimate the model parameters. The optimal sampling times were selected as follows. First, on the basis of previous experience with long-term cholesterol turnover studies, we selected days 1, 7, and 24 after injection of the isotope (for nonhypercholesterolemic subjects), or days 1, 8, and 28 (for hypercholesterolemic subjects) as the first three sampling times. Then, the remaining three optimal sampling times for each subject were chosen individually. This was done by using the results of the specific activity determinations in the first three samples together with experience from previously studied subjects to identify the best fourth sampling time. The process was repeated to further refine parameter estimates in order to

TABLE 1. Characteristics of new subjects studied[a]

Subject #	Sex	Age	Height	Weight	Ideal Body Weight[b]	Plasma Cholesterol[c]	Plasma Triglyceride[c]	Classification[d]	Familial Disorder[e]
		yr	*cm*	*kg*	*%*	*mg/dl*			
58	M	54	188	107	128	159 ± 11	147 ± 37	NL	
65	F	56	167	87	152	237 ± 16	114 ± 30	NL	
74	M	51	188	84	108	183 ± 14	94 ± 19	NL	
78	M	55	162	70	119	251 ± 28	136 ± 31	NL	
79	M	31	185	82	109	258 ± 15	101 ± 20	NL	
82	F	41	174	62	100	173 ± 11	87 ± 19	NL	
87	M	58	188	93	120	247 ± 17	119 ± 30	NL	
88	M	37	183	87	119	271 ± 11	170 ± 50	NL	
73	M	53	183	69	87	233 ± 11	59 ± 5	NL-α	
76	F	44	169	57	97	185 ± 14	82 ± 21	NL-α	
77	M	49	168	63	101	267 ± 14	106 ± 25	NL-α	
83	F	48	164	55	107	216 ± 11	61 ± 10	NL-α	
86	M	68	174	67	106	233 ± 7	80 ± 10	NL-α	
55	M	46	183	87	119	276 ± 16	118 ± 35	H-Chol	IND
57	M	23	185	74	99	365 ± 18	92 ± 16	H-Chol	FH
66	F	80	156	51	102	297 ± 17	104 ± 21	H-Chol	IND
69	F	66	160	59	112	364 ± 26	164 ± 40	H-Chol	IND
70	M	30	173	67	101	393 ± 30	105 ± 24	H-Chol	FH
75	M	47	178	83	119	439 ± 21	98 ± 14	H-Chol	FH
80	M	22	182	76	105	288 ± 17	94 ± 26	H-Chol	FH?
60	F	58	159	59	113	320 ± 20	274 ± 120	Mixed	IND
61	F	53	147	47	103	295 ± 17	304 ± 73	Mixed	IND
68	M	28	175	88	130	337 ± 24	265 ± 101	Mixed	FH
81	M	56	174	73	109	279 ± 30	209 ± 41	Mixed	Comb
85	F	59	163	69	127	322 ± 19	290 ± 73	Mixed	IND
62	M	55	184	94	127	271 ± 16	269 ± 50	H-TG	IND
63	M	32	173	76	115	170 ± 11	834 ± 470	H-TG	Comb?
64	M	49	183	98	124	224 ± 26	338 ± 85	H-TG	III
67	M	46	173	80	122	274 ± 19	217 ± 71	H-TG	IND
84	M	55	175	101	139	236 ± 17	277 ± 83	H-TG	IND

[a]The first 25 subjects studied were numbered 25, 26, 31–35, and 37–54 in reference 12; they are not included in this table.

[b]Percent of ideal body weight = actual weight divided by mean desirable weight for the patient's frame as determined from actuarial data (41).

[c]Mean ± SD during the period of study.

[d]Classifications: NL, cholesterol < 275 mg/dl, triglycerides < 200 mg/dl; α, HDL cholesterol > 95th percentile for age and sex; H-Chol, cholesterol > 275 mg/dl, triglycerides < 200 mg/dl; Mixed, cholesterol > 275 mg/dl, triglycerides > 200 mg/dl; H-TG, cholesterol < 275 mg/dl, triglycerides > 200 mg/dl.

[e]Familial disorder: IND, indeterminate; FH, familial hypercholesterolemia; Comb, familial combined hyperlipidemia; III, dysbetalipoproteinemia with apoE 2,2 phenotype; ?, classification based on history.

obtain the fifth and finally the sixth sampling times. To achieve a high level of accuracy in the specific activity determinations, each sample was divided into six portions and specific activity was measured separately in each portion. A mean of the six specific activity measurements so obtained was used as the value for that sample. Thus, the simplified, six-point sampling strategy involves 36 different specific activity measurements, as many as had been used in studies with 36 separately collected blood samples. In 26 subjects studied both with the convenient six-point sampling strategy and with the 36-point schedule, the convenient schedule has been found to result in no parameter estimate bias and only small deviations from the results of the 36-point schedule for any subject (17).

Analytical techniques

Cholesterol and triglyceride concentrations were measured using Technicon AAI methodology (Technicon Instruments Corp., Tarrytown, NY) (18). Cholesterol in HDL and LDL was measured by the procedure specified for the Lipid Research Clinics (19).

Radioimmunoassay of apoproteins (A-I, A-II, and E was performed as described in detail for apoE (20, 21). ApoE was purified from the apoproteins of VLDL by chromatography on heparin-agarose and then on DEAE-cellulose, followed by preparative SDS polyacrylamide gel electrophoresis. ApoA-I and apoA-II were purified by DEAE cellulose chromatography of the apoproteins of

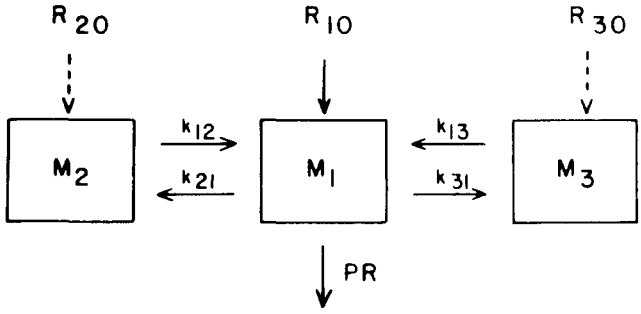

Fig. 1. Three-pool model of cholesterol turnover in humans (see text for definition and discussion of symbols).

HDL. The purified apoproteins (0.5 mg) were emulsified in complete Freund's adjuvant and injected into rabbits to generate specific antisera. The purified apoproteins were iodinated by the iodine monochloride procedure as described (22).

Plasma samples were incubated overnight in a solution of 50 mM Na decyl sulfate prior to assay. The assays were performed with final concentrations of 5 mM Na decyl sulfate, 50 mM Na phosphate, 100 mM NaCl, 0.02% Na azide, 0.04% non-immune rabbit serum, and 0.014% antiserum to apoA-I, 0.006% antiserum to apoA-II, or 0.011% antiserum to apoE, pH 7.4. Each assay tube contained 30,000 cpm of iodinated apoprotein. Goat anti-rabbit serum was added after a 48-hr incubation period, and the following day the assay was harvested by centrifugation. An identical content of apoA-I, apoA-II, and apoE was found with or without prior delipidation of serum or lipoprotein fractions with organic solvents, probably reflecting the fact that samples were routinely incubated in decyl sulfate prior to assay. Standard curves were prepared from a calibrated plasma pool that was stored in ampoules at −80°C. Each assay contained three separate standard curves, each pipetted in duplicate, and each containing 15 different concentrations of apoprotein. The plasma was calibrated using primary standards of purified apoproteins quantitated by the method of Lowry et al. (23).

The within-assay coefficient of variation was 10.6% for apoA-I, 5.0% for apoA-II, and 9% for apoE. The coefficient of variation for assay-to-assay sources of error was 9.0% for apoA-I, 4.4% for apoA-II, and 3% for apoE. A minimum of three different plasma samples from each subject's turnover study underwent radioimmunoassay, and the mean for each subject was used in analysis. For serum concentrations of cholesterol and triglycerides, a mean of all samples collected in the study (at least six for every subject) was used. For HDL cholesterol and LDL cholesterol, a mean of at least three different samples was used.

Treatment of samples

Blood samples were obtained after an overnight fast. Venous blood was collected with EDTA (1 mg/ml) as anticoagulant and was stored at 4°C. Plasma was separated within 4 hr of the time of blood collection. Plasma was stored at 4°C for up to 1 week prior to lipoprotein fractionation. Aliquots of plasma were then stored frozen at −20°C. For apoprotein radioimmunoassay, selected frozen samples were thawed and subjected to assay. The samples assayed had been stored at −20°C for periods of 0 to 5 years. Samples collected between 1975 and 1979 were analyzed in 1979 and 1980. Samples collected later were analyzed within 1 year of the time of sample collection. In order to be certain that the frozen samples had not undergone changes in concentration due to evaporation during storage, those stored for more than 1 year were assayed for total plasma cholesterol concentration immediately prior to sample selection for apoprotein radioimmunoassay. Only those samples with plasma cholesterol concentrations within 4% of the value obtained when fresh were considered for radioimmunoassay. This criterion excluded 26% of the samples that had undergone long-term storage. For each subject at least one sample was obtained from the early, middle, and late portions of the study for apoprotein radioimmunoassay. Furthermore, we examined the measured apoprotein concentrations for a trend with year of sample collection. If the older samples had deteriorated with storage, the older values would be expected to differ from more recent values. We found, however, that samples undergoing long-term storage gave results similar to those assayed when fresh. Furthermore, no trend of apoprotein concentrations with year of sample collection was found. In addition, within the 9-month duration of the individual studies, there was no tendency of the measured apoprotein concentrations to increase or decrease. We have reported previously for serial assays of apoE that immunoreactivity in plasma was stable for at least 18 months (20), and for all three apoproteins (apoA-I, apoA-II, and apoE) repeated freezing and thawing of plasma did not alter radioimmunoassay results. Thus, we are confident that values obtained on samples stored for 5 years at −20°C are reliable. Finally, it should be noted that for 25 of the subjects, radioimmunoassays were performed within 1 year of sample collection. The within-subject coefficients of variation for the apolipoproteins were as follows: apoA-I 9.3%; apoA-II 8.1%, and apoE 19.4%.

Data analysis

Simple univariate correlation analyses were first carried out to look for relationships between the HDL and apoprotein levels and the model parameters. This represents the simplest statistical method of exploring the influence, if any, of HDL cholesterol, apoA-I, apoA-II, or apoE levels on the parameters of whole body cholesterol turnover. However, this method could give misleading results

when other physiological variables directly influence model parameters. The simple correlation coefficient may be misleadingly large or small in these situations.

For these reasons, it was necessary also to examine the correlations of model parameters with HDL cholesterol or the apoproteins by multivariate methods that accounted for the influence of other physiological variables on the model parameters. Three approaches were taken for these multivariate analyses.

In our earlier work (12), we have shown (in a population of 54 subjects with a wide range of lipid levels) that a substantial part (50–70%) of the variation of four important model parameters (PR, M_1, minimum estimate of M_3, minimum estimate of M_{tot}) may be explained by the variation in body weight, serum cholesterol, age, and adiposity. Therefore, we performed regression analyses that included HDL cholesterol and the apoprotein levels as independent variables in addition to the variables previously found (12) to be predictive of model parameters. A partial F test (24) was used to determine whether the inclusion of each apoprotein reduced the residual variation of the model parameter significantly. Regression coefficients for the new variables as well as the previously chosen variables were determined in the 55 subjects.

A second set of multivariate analyses considered the possibility that one of the new variables (HDL cholesterol or apoprotein levels) might replace another physiological variable in some regression equation, rather than be an additional variable. If this were the case, we would find that the residual variation of the model parameter remained the same or decreased when the new variable was added to the regression equation. Furthermore, the regression coefficient of the new variable would be statistically significant while that of the previously chosen variable would cease to be significant. In order to explore the possible existence of these kinds of relationships, a multivariate regression analysis was carried out for each model parameter to search for relationships between the model parameter and the levels of the apoproteins and HDL cholesterol, along with any variables previously found to be statistically significant for that model parameter.

The third and final set of multivariate analyses was designed to consider the possibility that the new variables enter the regression equations not separately but in combination with another physiological variable, e.g., multiplied by weight, or as discrete variables rather than as continuous ones, e.g., low or high levels of apoE instead of the actual level of apoE. To this end, a very large number of possible linear and nonlinear relationships and those involving interactions between variables were examined. The variables included the present lipoprotein, cholesterol, and apoprotein values, the previously considered (12) physiological variables, and their cross-products as independent variables. More than 100,000 regression equations were considered for each model parameter. Since, at random, a large number of these relationships would appear statistically significant at the $P = 0.05$ level, the following strategy, which we have used previously (12), was employed to guard against selection of relationships that are unlikely to be generally valid. The entire population of 55 subjects was randomly divided into a hypothesis-generating subset of 36 subjects and a hypothesis-testing subset of 19 subjects exactly as described in detail previously (12). The two groups were closely matched with respect to the following key variables: age, sex, weight, percent ideal body weight, and plasma concentration of LDL cholesterol, HDL cholesterol, and the apoproteins. To assess the similarity of the groups, the mean of each of these variables in the hypothesis-testing subset was compared to the mean in the entire group of 55 subjects. The mean of the absolute values of the percent difference [(parameter mean in subset of 19 subjects minus parameter mean in all 55 subjects) divided by the parameter mean in all 55 subjects] was 3.5%. The potential relationships were first examined in the hypothesis-generating group of 36 subjects. The following criteria were applied to select regression equations for testing in the hypothesis-testing group of 19 subjects: *a*) high predictivity for a model parameter ($R^2 > 0.50$), *b*) all coefficients significantly different from zero, *c*) HDL cholesterol, apoA-I, apoA-II, or apoE included as a term, and *d*) the coefficient had to remain significant even if one or two subjects were excluded from the analysis as determined by a statistical "jackknife" procedure (25). This procedure guards against the possibility of a coefficient owing its significance to just one or two outlying subjects.

RESULTS

Lipid, lipoprotein, and apolipoprotein concentrations in the study population

Table 2 summarizes the characteristics of the 55 subjects studied. They included 11 women and 44 men. The

TABLE 2. Summary of characteristics of subjects studied

	Mean ± SD	10th Percentile	90th Percentile
Age (yr)	50 ± 11	32	65
Weight (kg)	79 ± 16	57	103
Height (cm)	174 ± 9	161	185
Surface area (m^2)	1.9 ± 0.2	1.6	2.2
% Ideal weight	116 ± 14	100	137
Excess weight (kg)	11 ± 10	−0.3	27
Plasma cholesterol (mg/dl)	260 ± 80	176	364
Plasma triglyceride (mg/dl)	271 ± 201	90	565
HDL cholesterol (mg/dl)	48 ± 17	29	73
ApoA-I (μg/ml)	1049 ± 301	731	1459
ApoA-II (μg/ml)	323 ± 67	254	401
ApoE (μg/ml)	65 ± 29	36	109

subjects in the study population manifested a wide range of values of serum concentrations of cholesterol, triglycerides, HDL cholesterol, apoA-I, apoA-II, and apoE. These wide ranges were selected in order to maximize the likelihood of detecting relationships between these variables and the parameters of body cholesterol metabolism.

Univariate correlation analyses

Table 3 gives the correlation coefficients observed between the various lipid, lipoprotein, and apoprotein values in the study subjects. As has been previously reported in other populations, there was a very strong relationship between the concentration of LDL cholesterol and the concentration of total plasma cholesterol (r = 0.95). Furthermore, the concentrations of apoA-I, apoA-II, and HDL cholesterol were all related to one another, as has been reported previously (26–28). Since the magnitude of these relationships approximated those previously reported by others, this provides further evidence for the validity of our measurements of these variables. Furthermore, as we had reported previously, there were positive relationships between the plasma concentration of apoE and plasma concentrations of cholesterol and triglyceride. Inverse relationships between apoE concentration and the concentrations of HDL cholesterol and apoA-I were noted.

The simple correlation coefficients of the model parameters production rate (PR), M_1, M_3min, and M_{tot}min, and k_{21} with the lipoprotein and apoprotein variables and body weight are shown in **Table 4.** Other rate constants not listed (k_{12}, k_{23}, k_{32}) did not correlate significantly with any of the lipoprotein or apoprotein variables. The levels of HDL cholesterol and apoA-I were negatively correlated with PR and with the pool size (mass) parameters (M_1, M_3min, and M_{tot}min). For apoA-II, a weakly significant ($P < 0.05$) negative association was found with M_1, while for apoE, none of the correlation coefficients was statistically significant. Furthermore, in no case was a lipoprotein or apolipoprotein variable a powerful predictor of a model parameter. Thus, the square of the correlation coefficient, indicating the fraction of the variance of a model parameter described by an independent variable, was never greater than 0.19. In contrast to these relatively weak relationships, body weight described 58% of the variance in production rate, 37% of the variance in M_1, 33% of the variance in M_3min, and 61% of the variance in M_{tot}min. Thus, univariate analysis suggested quantitatively weak, but possibly significant, relationships of lipoprotein and apoprotein variables with the parameters of body cholesterol metabolism. However, it was possible that this result might reflect known relationships of the lipoprotein or apoprotein variables with other physiological variables such as body size, which more directly influence production rate and the pool sizes. This possibility was investigated with multiple regression techniques.

Multivariate analyses

In previous work, we had validated five highly predictive equations for production rate, M_3min, and M_{tot}min (12). The independent variables in these equations were indices of body size, serum cholesterol concentration, and age. The relationships involving those variables, with multiple correlation coefficients calculated from the present population (partly overlapping our previously reported (12) population), are summarized in **Table 5.** The multiple correlation coefficients obtained with these equations range from 0.58 to 0.83. To determine whether the serum levels of HDL cholesterol or of apoproteins A-I, A-II, or E were important independent determinants of these model parameters, the remaining variance that they could describe was calculated when they were inserted as additional independent variables. The partial correlation coefficients (partial r) resulting from these calculations are listed in Table 5. In every case, the partial r for the added variables was very small and statistically was never sig-

TABLE 3. Correlation of independent variables with one another[a]

	Chol	TG	LDL Chol	HDL Chol	ApoA-I	ApoA-II	ApoE	% IBW	Weight	Age
Chol[b]	1.00									
TG	−0.25	1.00								
LDL Chol	0.95	−0.38	1.00							
HDL Chol	0.20	−0.62	0.19	1.00						
AI	0.08	−0.47	0.08	0.83	1.00					
AII	0.23	−0.17	0.14	0.59	0.61	1.00				
E	0.19	0.42	0.06	−0.29	−0.28	0.04	1.00			
% IBW	−0.14	0.34	−0.15	−0.46	−0.38	−0.14	0.08	1.00		
Weight	−0.33	0.33	−0.32	−0.53	−0.45	−0.25	−0.04	0.73	1.00	
Age	−0.04	−0.01	−0.11	−0.19	−0.09	−0.05	−0.29	0.18	0.08	1.00

[a]The values listed are the linear correlation coefficients (r) between each pair of variables. $P = 0.05$ for $|r| = 0.27$; $P = 0.01$ for $|r| = 0.31$; $P = 0.005$ for $|r| = 0.35$.
[b]Chol, cholesterol; TG, triglycerides; % IBW, % ideal body weight.

TABLE 4. Simple correlations (r) of model parameters of cholesterol metabolism with HDL-cholesterol and apoproteins A-I, A-II, and E in 55 subjects[a]

	HDL Cholesterol	ApoA-I	ApoA-II	ApoE	Weight
PR	−0.42	−0.39	−0.09	0.07	0.76
M_1	−0.38	−0.445	−0.28	0.08	0.61
M_3min	−0.31	−0.36	−0.09	0.02	0.58
M_{tot}min	−0.41	−0.42	−0.19	−0.02	0.78
k_{21}	0.40	0.43	0.36	−0.22	−0.04

[a]Correlation coefficients were not significantly different from zero for k_{12}, k_{13}, and k_{31}. $P = 0.05$ for $|r| = 0.27$; $P = 0.01$ for $|r| = 0.31$; $P = 0.005$ for $|r| = 0.35$.

nificantly different from zero. The partial r never exceeded 0.10 for HDL cholesterol, 0.17 for apoA-I, 0.19 for apoA-II, and 0.23 for apoE.

The lack of an independent relationship of M_3min with HDL-cholesterol and the apoproteins is shown graphically in **Fig. 2.** Here, M_3min has been corrected for the subjects' weight, serum cholesterol concentration, and age, and then plotted against plasma levels of HDL cholesterol, apoA-I, apoA-II, and apoE. It is quite evident that there is no independent relationship of M_3min with HDL-cholesterol or these apoproteins.

A final series of analyses was performed to be certain that no relationships had been overlooked in which the levels of HDL cholesterol or of apoproteins A-I, A-II, or E were independent determinants of any of the model parameters of body cholesterol metabolism. The goal of this effort was to conduct an extensive search for significant relationships between model parameters and physiological variables that would involve a lipoprotein or apoprotein variable as a significant term. This search was conducted using the procedure outlined in Methods. On the basis of the criteria listed in the Methods section, 18 relationships were selected for validation in the hypothesis-testing subset of 19 subjects. When tested in the 19 patients who comprised the hypothesis-testing group, none of the 18 relationships was found to be statistically significant. Thus, using this technique to be certain that important relationships of HDL and the apoproteins with the model parameters were not overlooked in the simpler analysis, no significant relationships became apparent.

DISCUSSION

The experiments described in this report had their impetus in hypotheses on the mechanism responsible for the inverse relationship between HDL levels in plasma and coronary disease rates. The inverse relationship itself has been documented in many prospective (2, 3, 29) and retrospective (1, 30) population studies. It has also been demonstrated in observations on patients with familial elevation of HDL (31), and in studies of certain genetic diseases causing depression of HDL levels (32–34). The mechanism responsible for this relationship, however, remains uncertain. A leading proposed mechanism is that HDL may reduce tissue stores of cholesterol by transporting cholesterol from peripheral tissues, including the arterial wall, to the liver (4). Two sorts of evidence support this hypothesis. First, HDL and the apoproteins of HDL remove cholesterol from cells in culture. Second, two reports (by the same group of investigators) indicated that HDL levels were found to be strongly and inversely related to the mass of slowly exchanging body cholesterol (10, 13). Those reports, however, were based on small numbers of subjects, (eight normal volunteers for one, five subjects with hyperalphalipoproteinemia for the other) and on short-term cholesterol turnover studies, which do not give valid estimate of the mass of slowly exchanging body cholesterol (11). The present experiments, involving long-term cholesterol turnover studies in 55 volunteers, were carried out in order to evaluate this question in a more extensive way.

The results of the present study appear to contradict the conclusions of Miller et al. (10). They concluded that

TABLE 5. Independent effects of HDL cholesterol and apoprotein levels in determining model parameters of body cholesterol metabolism[a]

Dependent Variable	Independent Variables[b]	R[c]	Partial R for Additional Independent Variable			
			HDL Cholesterol	ApoA-I	Apo-II	ApoE
PR	Wt	0.76	−0.02	−0.09	0.15	0.07
M_3min	Wt, Chol, Age	0.58	−0.10	−0.16	0.01	0.08
M_3min	EWt, Age	0.71	0.04	−0.17	0.00	0.03
M_{tot}min	Wt, Chol	0.83	0.01	−0.10	−0.08	−0.19
M_{tot}min	EWt, Chol-Wt	0.81	−0.07	−0.17	−0.19	−0.23

[a]Data are from the 55 subjects who are the subjects of the present study.
[b]Abbreviations used (not already defined in text): Chol, serum cholesterol concentration (mg/dl); Chol-Wt, serum cholesterol concentration times weight; EWt, excess weight (observed weight minus ideal weight); Wt, observed body weight.
[c]R is the multiple correlation coefficient for the listed independent variables.

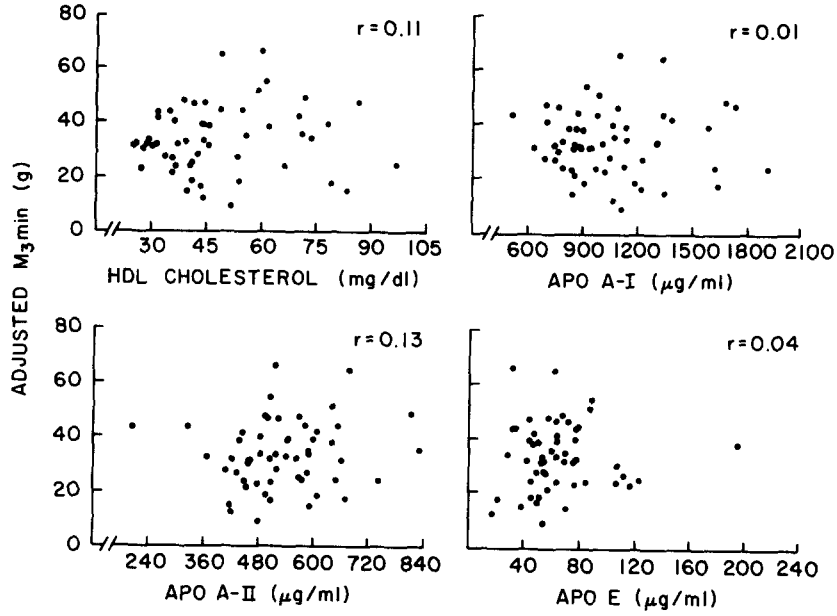

Fig. 2. Relationships of M_3min (adjusted for differences in weight, in age, and in plasma cholesterol concentration) with plasma concentration of HDL cholesterol (upper left), apoA-I (upper right), apoA-II (lower left), and apoE (lower right). Body weight was adjusted to 70 kg, age to 50 years, and plasma cholesterol to 275 mg/dl.

there was a strong negative correlation of HDL cholesterol concentration with both rapidly and slowly exchanging pools of tissue cholesterol. Furthermore, the correlation remained significant when pool size was adjusted for body weight. We have reanalyzed the data presented in reference (10) and conclude that their data are in fact consistent with our conclusions and not with theirs.

First, it should be noted that the studies of Miller et al. (10) were carried out in only eight subjects. Such a small number would make any conclusions tentative even if statistically significant. A particular characteristic of this population of eight subjects was a high negative correlation of −0.75 between HDL cholesterol and weight. The presence of such a correlation in the small study population makes it difficult to choose between the two variables as determinants of turnover parameters. In particular, this HDL-weight correlation complicates interpretation of the findings when the primary model parameters, namely M_A and M_B, the sizes of the two pools A and B, are adjusted by functions of body weight. The size of the rapidly exchanging pool A was reduced by the amount of cholesterol estimated to be in plasma (a function of body weight) while the size of pool B was adjusted for excess body weight using a regression equation developed in a different study population (35). Since body weight and HDL cholesterol are strongly correlated, adjustments using one of the two can lead to spurious (large or small) correlations with the other.

In our reanalysis of the data in reference (10), we took a more direct approach by regressing unadjusted model parameters on weight and HDL cholesterol. We found that the pool sizes were correlated positively with weight and negatively with HDL cholesterol. However, when M_A was adjusted for weight, the relationship with HDL was no longer significant statistically, though it remained negative. As for M_B, when it was divided by body weight, the correlation with HDL went from −0.72 to −0.027, an insignificant value.

Thus we conclude that the data of Miller et al. (10) are entirely consistent with our findings. The pool sizes (and production rate) are positively correlated with body weight, as we would expect. The negative correlation of pool sizes with HDL cholesterol found by Miller et al. (10) is attributable to the strong negative correlation between HDL cholesterol and body weight in this population.

In the present study of a large group of subjects, we found no independent relationship between any of the model parameters of body cholesterol metabolism and the plasma levels of HDL cholesterol or apoproteins A-I, A-II, or E. Weak but statistically significant univariate correlations did exist for HDL cholesterol and apoA-I with production rate, M_1, M_3min, and M_{tot}min. However, the much stronger correlation of these model parameters with body size and the results of multiple regression analyses indicated that the univariate correlations reflected relationships of HDL cholesterol and apoA-I with physiological variables, such as body size, which were previously shown to be related to the model parameters. A final series of analyses, examining a very large number of

potential relationships of the model parameters with HDL cholesterol, the apoproteins, and other physiological variables corroborated this conclusion.

Thus, the present work does not support the hypothesis that higher concentrations of plasma HDL cholesterol or the major HDL apoproteins are associated with reduced cholesterol stores in peripheral tissues, as a result of an increased rate of reverse cholesterol transport from peripheral tissues to the liver. The studies reported here clearly demonstrate that the plasma levels of HDL or its apoproteins are not independent determinants of the kinetically defined mass of exchangeable pools of body cholesterol. Hence, these experiments provide 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.

However, it must be noted that the studies reported here cannot absolutely disprove the hypothesis that increased levels of HDL in plasma are associated with an increased flux of cholesterol from, and a decreased amount of cholesterol in, certain selected tissues (such as arteries). Cholesterol turnover studies such as these provide a "low power" view of whole body cholesterol metabolism. Although the present studies show that there is no independent relationship between the levels of HDL cholesterol or the apoproteins and the mass of kinetically defined pools of body cholesterol, lipoproteins certainly have major effects on cholesterol metabolism in cells. These effects include influences on the transport of cholesterol into and out of cells. Long-term cholesterol turnover studies are uniquely able to provide information about the size (mass) of exchangeable pools of body cholesterol in intact human beings. Nevertheless, such studies do not give information on cholesterol kinetics or masses in individual cells, or in specific individual tissues.

Cholesterol in atheromata represents a very tiny fraction of the mass of exchangeable body cholesterol, M_{tot}min usually being approximately 80–90 g (12, 36). Therefore, if there were effects of HDL level that mainly influenced the mass of cholesterol in atherosclerotic plaque but had very little influence on the mass of other pools of body cholesterol, such effects would probably be undetected in the present studies. Furthermore, cholesterol in atherosclerotic plaque exchanges very slowly with plasma cholesterol, with turnover times exceeding 400 days (37), and plaque cholesterol is physically and kinetically heterogenous (38, 39). The extremely long turnover time (years) of cholesterol in the cholesterol monohydrate crystal phase (39) precludes cholesterol in this phase from being investigated in the standard 9-month "long-term" cholesterol turnover studies we have performed. Cholesterol in other physical states in plaque would enter into pool 3 in our long-term studies (39), but would be minimally detected in kinetic analysis of short-term cholesterol turnover studies as performed by Miller et al. (10) and Nestel and Miller (13).

Accordingly, these long-term cholesterol turnover studies might not detect effects of HDL level restricted specifically to modifying cholesterol deposition in atherosclerotic plaque but not elsewhere. However, such extreme specificity seems unlikely. The existence of more general effects of HDL on the mass of exchangeable pools of body cholesterol is inconsistent with the results of the present studies. The present results thus suggest that the inverse relationship of HDL and coronary rates is not mediated via an influence of HDL on the mass of pools of exchangeable body cholesterol. Other mechanisms may well be involved and warrant investigation. Such mechanisms, e.g., possible effects of HDL on prostacyclin production by endothelial cells (40), may have little or nothing to do with cholesterol metabolism or homeostasis or may reflect antiatherogenic metabolic activities primarily involving other lipoproteins.

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