Prediction of the parameters of whole body cholesterol metabolism in humans

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Abstract Total body turnover of cholesterol was studied in 54 subjects by fitting a three-pool mathematical model to plasma decay curves of 32–49 weeks duration following [^{14}C]cholesterol injection. Fifteen subjects were normal, 10 hypercholesterolemic, 21 hypertriglyceridemic, and 8 had both hypercholesterolemia and hypertriglyceridemia; 21 had a familial form of hyperlipidemia. In every subject in this heterogeneous population, the three-pool model gave the best fit for the data. An extensive search was conducted for relationships between model parameters and physiological variables (body size, serum lipid levels, age, and sex). Both linear and nonlinear relationships, and those involving interactions between pairs of variables, were explored. Fifty different forms of the model parameters and 53 forms of the physiological variables were examined. To guard against declaring statistical significance when none was present, subjects were first randomly divided into two matched groups. In the first (hypothesis-generating) group of 36 subjects, more than 100,000 regression equations were considered for each form of the model parameters. Twenty-one highly significant equations were found that were then tested in the second group (hypothesis-testing, 18 subjects). Eighteen of the 21 equations were found to be significant; of these, 6 were selected that accounted for a large part of the observed variation in the four model parameters for which equations were found (production rate (PR), and the sizes of pool 1, pool 3, and total exchangeable body cholesterol). The major determinant of cholesterol PR was body weight alone (r = 0.80). No function of serum lipid levels significantly influenced PR. Both body weight and serum cholesterol level entered into the equations for cholesterol mass. Age influenced the size of pool 3. Serum triglyceride level only had an effect on the size of pool 1. Since these equations were generated in one group of subjects and tested in another, they can be considered a confirmed set of predictive equations.—**Goodman, D. S., F. R. Smith, A. H. Seplowitz, R. Ramakrishnan, and R. B. Dell.** Prediction of the parameters of whole body cholesterol metabolism in humans. *J. Lipid Res.* 1980. **21:** 699–713.

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During the past few years, kinetic studies have provided a considerable amount of information about cholesterol turnover and metabolism in intact humans (1–13). These studies have generally involved analysis of the turnover of plasma cholesterol following injection of radioactively labeled cholesterol, in experiments of about 10–12 weeks duration (1, 2, 7) or of much longer duration (5, 6, 8, 10). In 1976, we reported the results of long-term studies (32–49 weeks) of the turnover of plasma cholesterol in 8 normal and 16 hyperlipidemic subjects (10). In all subjects (except one for whom complete data were not available), a simple three-pool model previously described (6) gave the best fit for the data. The parameters of the three-pool model observed in the normal subjects were compared with the model parameters found in the patients with hyperlipidemia. In addition, relationships between the model parameters of cholesterol turnover and a number of physiological variables were explored, using simple linear correlations and multiple linear regression analyses.

Although the previous report (10) provided information about cholesterol turnover and metabolism in normal and hyperlipidemic humans, the studies were limited in a number of ways. First of all, only 2 of the 16 hyperlipidemic subjects had hypertriglyceridemia alone (without hypercholesterolemia), and only two subjects (both with hypercholesterolemia plus hypertriglyceridemia, and a type IIb lipoprotein phenotype (14)) had plasma triglyceride levels above 300 mg/dl. Thus, relationships between model parameters and plasma triglyceride concentrations could not be explored effectively. Secondly, because of the small size of the total study population, the data analysis that was conducted was quite limited, and only a relatively small number of linear relationships were explored.

In order to overcome these significant limitations, long-term studies of the turnover of plasma cholesterol were carried out in an additional 30 subjects, including 19 subjects with hypertriglyceridemia alone.

Abbreviations: PR, production rate; M_1, M_2, M_3, pool sizes; k, rate constant; R, rate of transfer of cholesterol mass.

We now report the results of these studies and of the data analysis conducted with the entire series of 54 long-term studies. This analysis involved an extensive search for correlations between many different forms of the model parameters and of physiological variables relating to body size, serum lipid levels, age and sex. The goal of this work was to define the relationships that may exist between the model parameters of whole body cholesterol turnover and the various physiological variables that were measured in each subject. A confirmed set of highly predictive equations has been obtained which describes some of the major body cholesterol kinetic parameters in humans.

METHODS

Subjects studied

A total of 54 volunteer subjects participated in these studies; written informed consent was obtained from each. The characteristics of and results obtained with the first 24 subjects studied have been reported previously (10).[1] The characteristics of the 30 subjects subsequently studied are shown in **Table 1.** Nineteen of these 30 subjects had hypertriglyceridemia, 2 had hypercholesterolemia, 2 had elevations of both cholesterol and triglycerides (mixed hyperlipidemia), and 7 were normal. The diagnosis of hyperlipidemia was based on an arbitrary working definition of levels of cholesterol (at the time of the study) in excess of 275 mg/dl, and/or levels of triglyceride in excess of 200 mg/dl, in serum obtained after a 12-hr fast. Patients were classified as having hypercholesterolemia, hypertriglyceridemia, or both (mixed hyperlipidemia). Of the total study population, 15 subjects were normolipidemic, 10 had hypercholesterolemia alone, 21 had hypertriglyceridemia alone, and 8 had mixed hyperlipidemia.

A genetic classification of each hyperlipidemic patient was attempted, following the approach of Goldstein et al. (15) as previously described (10). A familial disorder was considered to be present, absent, or indeterminate based upon the screening of first-degree relatives. For relatives older than 14 years of age, we again used the 95th and 99th percentile values established in the Seattle study (16). For younger children, 95th percentile values were estimated from the results of the Bogalusa Heart Study (17). Since mildly hypercholesterolemic children frequently have elevated high density lipoprotein and normal low density lipoprotein concentrations (17–19), we did not base a diagnosis of familial hyperlipidemia upon pediatric values without either high density lipoprotein quantification or the presence of at least one child with cholesterol greater than 250 mg/dl (see Table 1). With these criteria, of the 23 hyperlipidemic subjects listed in Table I, 11 were considered to have a familial disorder, 4 were considered not to have a familial disorder, and in 8 the presence or absence of a familial disorder was indeterminate. For 8 of the 11 subjects with familial hyperlipidemia, 3 or more first-degree relatives were tested; only 2 first-degree relatives were available for the ninth subject, and only one each for the remaining two. For the total study population of 54 subjects, 21 of the 39 hyperlipidemic subjects had a familial disorder, and the presence or absence of a familial disorder was indeterminate in 11 hyperlipidemic subjects. The subjects with familial hyperlipidemias included five with familial hypercholesterolemia, two with familial hypertriglyceridemia, and five with familial combined hyperlipidemia.

Table 1 also shows the lipoprotein phenotypes (14) of the study subjects (see column headed "LP Pattern"). Only two of the subjects (Nos. 32 and 33, identical twin sisters with familial hypercholesterolemia), had xanthomas. Five of the patients listed in Table 1 had clinical evidence of cardiovascular disease. The twin sisters with hypercholesterolemia (Subjects Nos. 32 and 33) had angina pectoris, and on exercise testing demonstrated ischemic electrocardiographic changes and chest pain. Subject No. 31 was the normolipidemic sister of these two subjects. A third subject (No. 37) had experienced occasional episodes of supraventricular tachycardia, and was taking propranalol during the study. Two patients with peripheral artery disease had studies which were truncated by surgery. One subject (No. 26) underwent resection of a right femoral artery aneurysm; the second subject (No. 25) required a leg amputation.

Of the other subjects listed in Table 1, subject No. 46 had an episode of mild, acute pancreatitis during the turnover study, but recovered and completed the study. Subject No. 38 had an abbreviated study because of cholecystectomy for symptomatic gallstones. For the three subjects who underwent surgery, data analysis was carried out only on blood samples collected prior to surgery. The other subjects listed in Table 1 were clinically well and without cardiovascular disease.

Table 2 summarizes the characteristics of the 54 subjects studied. In this table, the means and standard deviations are presented for each physiological vari-

[1] For the data analyses reported here, values slightly different from those previously reported (10) were used for percent of ideal body weight for a number of the previously reported subjects. The final corrected values for the previously reported subjects can be obtained, if desired, from the authors. In addition, in the previous report (10), the height of subject J.B. should have been given as 171 instead of 145 cm.

TABLE 1. Characteristics of subjects studied

Subject #	Study Duration	Sex	Age	Height	Weight	Ideal Body Wt. (%)	Serum Cholesterol[a]	Serum Triglyceride[a]	Classification[b]	LP Pattern	Familial Disorder[c]	Genetic Classification[d]
	wk		*yr*	*cm*	*kg*		*mg/dl*					
25	22	M	60	174	75.5	113	134 ± 8	73 ± 11	NL	NL		
26	18	M	65	170	75.5	118	163 ± 10	105 ± 20	NL	NL		
27	43	F	35	173	62.4	101	181 ± 11	67 ± 24	NL	NL		
28	43	M	35	182	75.7	97	186 ± 8	86 ± 37	NL	NL		
29	43	F	43	166	60.1	106	204 ± 12	64 ± 14	NL	NL		
30	40	M	38	187	88.8	107	216 ± 16	154 ± 37	NL	NL		
31	40	F	67	165	65.5	108	272 ± 14	192 ± 35	NL	NL		
32	40	F	56	161	60.5	114	511 ± 33	122 ± 42	H-Chol	IIa	+	FH
33	40	F	56	158	57.0	111	560 ± 29	126 ± 33	H-Chol	IIa	+	FH
34	46	M	57	182	91.8	117	290 ± 25	259 ± 48	Mixed	IIb	−	
35	39	M	54	175	102.7	141	305 ± 20	513 ± 138	Mixed	IIb, IV	IND	
36	38	M	38	184	92.8	116	196 ± 14	205 ± 81	H-TG	IV	IND	
37	40	M	51	183	104.2	132	202 ± 30	211 ± 77	H-TG	IV	+	ND[e]
38	32	M	44	174	80.0	120	222 ± 22	218 ± 54	H-TG	IV	+	Comb.[f]
39	40	M	47	178	92.1	132	228 ± 9	250 ± 58	H-TG	IV	+	ND[g]
40	40	M	35	185	77.7	104	250 ± 28	253 ± 73	H-TG	IV, IIb	+	Comb.
41	39	M	51	195	105.0	117	246 ± 11	269 ± 66	H-TG	IV	IND	
42	39	M	57	178	77.7	112	225 ± 15	279 ± 96	H-TG	IV	−	
43	40	M	51	168	78.6	126	248 ± 25	362 ± 281	H-TG	IV	+	Comb.
44	38	M	43	178	87.3	116	264 ± 13	443 ± 143	H-TG	IV	IND	
45	40	M	58	173	105.9	148	178 ± 14	467 ± 155	H-TG	IV	+	ND[h]
46	40	M	51	185	86.1	106	179 ± 11	498 ± 144	H-TG	IV	+	ND[i]
47	40	M	52	173	103.6	145	206 ± 10	510 ± 83	H-TG	IV	IND	
48	38	M	53	175	91.8	136	234 ± 12	510 ± 185	H-TG	IV	+	F-H-TG
49	40	M	43	161	68.2	117	201 ± 18	526 ± 367	H-TG	IV, V	IND	
50	39	M	53	178	74.5	107	239 ± 12	535 ± 374	H-TG	IV, V	IND	
51	40	M	47	175	67.0	99	207 ± 10	611 ± 237	H-TG	IV	+	F-H-TG
52	40	M	68	173	85.9	130	254 ± 17	690 ± 257	H-TG	IV, V	IND	
53	38	M	51	175	99.1	136	179 ± 41	737 ± 773	H-TG	IV, V	−	
54	46	M	42	169	69.1	109	253 ± 12	751 ± 211	H-TG	IV	−	

[a] Mean ± SD during the period of the study (32–46 samples per subject).

[b] Classifications: NL, normolipidemia; H-Chol, hypercholesterolemia; H-TG, hypertriglyceridemia; Mixed, mixed hyperlipidemia.

[c] (+), present; (−), absent; IND, indeterminate.

[d] Classifications: FH, familial hypercholesterolemia; F-H-TG, familial hypertriglyceridemia; Comb., familial combined hyperlipidemia; ND, not definite. No notation is made in this column for subjects where a familial disorder was absent or was "indeterminate".

[e] Only one first-degree relative was available for testing. The subject's 52-year old sister had a serum cholesterol of 242 and triglycerides of 253 mg/dl.

[f] Two first-degree relatives were available for testing. An 11-year old daughter had a serum cholesterol of 234 and triglycerides of 79 mg/dl. A 10-year old daughter had a serum cholesterol of 286 and triglycerides of 44 mg/dl. The subject's wife had normal total, LDL, and HDL cholesterol. The subject's HDL cholesterol was 34 mg/dl.

[g] A diagnosis of familial hyperlipidemia was based upon the finding of a serum cholesterol of 283 and triglycerides of 228 mg/dl in the subject's 38-year old brother. The subject's 12-year old son had a serum cholesterol of 224 and triglycerides of 66 mg/dl, but HDL was not quantitated; five other children are normolipidemic.

[h] Only two siblings were available for testing. A 60-year old sister had a serum cholesterol of 200 and triglycerides of 297 mg/dl. A 52-year old brother was normolipidemic. A 47-year old brother was said to have high cholesterol and triglyceride levels by history, but this was not documented.

[i] Seven children, ages 14 to 26 were normolipidemic. An eighth child, a 10-year old girl, had hyperbetalipoproteinemia; for six determinations, mean plasma total cholesterol was 216 mg/dl (range 186–233) with mean LDL cholesterol of 136 mg/dl (range 103 to 161) and mean HDL cholesterol of 52 mg/dl (range 40 to 73). The subject's wife was normolipidemic.

able for each subgroup according to plasma lipid classification, and for the total study population.

All subjects were studied as outpatients. All hyperlipidemic subjects had previously been instructed in a diet containing less than 300 mg/day of cholesterol, 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 (see SD values in Table 1), but there

TABLE 2. Subjects by groups: physiological variables (mean ± SD)

Physiological Variable	Group (number of subjects)				
	Normal (15)	Hypercholesterolemia (10)	Hypertriglyceridemia (21)	Mixed Hyperlipidemia (8)	All Subjects (54)
Age (yrs)	43.9 ± 14.1	54.4 ± 10.5	50.0 ± 7.7	51.5 ± 8.8	49.3 ± 10.8
Weight (kg)	74.1 ± 7.5	66.5 ± 15.9	85.0 ± 13.5	79.4 ± 15.5	77.7 ± 14.4
Height (cm)	177.6 ± 7.6	165.6 ± 9.5	176.7 ± 7.2	174.7 ± 6.3	174.6 ± 8.7
Surface area (m^2)	1.91 ± 0.14	1.73 ± 0.24	2.02 ± 0.17	1.94 ± 0.20	1.92 ± 0.20
Ideal body weight (%)	106.5 ± 5.3	111.1 ± 13.0	119.5 ± 14.8	114.5 ± 13.7	113.6 ± 13.1
Excess weight (kg)	4.4 ± 3.5	6.9 ± 9.1	13.9 ± 10.5	10.4 ± 9.9	9.5 ± 9.4
Serum cholesterol (mg/dl)	199. ± 33.	406. ± 105.	223. ± 27.	368. ± 91.	272. ± 104.
Serum triglyceride (mg/dl)	103. ± 35.	131. ± 28.	421. ± 182.	361. ± 175.	270. ± 197.

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 for the 30 studies shown in Table 1, was injected intravenously, and the specific radioactivity of serum total cholesterol was determined in samples collected serially thereafter, as described in detail previously (1, 6). The amounts of radioactivity injected (22 to 28 μCi per subject) were measured precisely. Samples of venous blood were collected daily for 5 days starting 1 day after injection, and then with decreasing frequency so that by the end of the study sampling frequency was every 2 to 3 weeks. For most of the subjects (27 of the 30 listed in Table 1) 36–46 samples were collected during study periods of from 38 to 46 weeks. The specific radioactivity of the cholesterol in each sample, and the serum concentrations of cholesterol and triglyceride, were determined as described previously (1, 6, 10).

The specific radioactivity data were analyzed by a weighted, least-squares technique described before (6, 20), to determine the parameters of a three-pool mammillary model which would provide the best fit. The model used is illustrated in **Fig. 1;** the notations used are identical with those described and used previously (6). 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 (6), 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). Maximum values for M_2 and M_3 were obtained by assuming that all of cholesterol production (except for absorbed cholesterol, assumed to be 0.2 g/day) arises from pool 2 or pool 3, respectively. Intermediate values for the sizes of pools 2 and 3 were estimated as values midway between the minimum and maximum values for each pool. Summation of the minimum or intermediate values of these masses gave two different estimates of total exchangeable body cholesterol: a minimum estimate and a high one (called M_{total} intermediate).

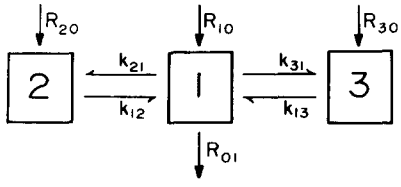

Fig. 1. Three-pool model of cholesterol turnover in humans (see text for definition and discussion of symbols).

Data analysis

The principal aim of this work was to delineate relationships that may exist between the model parameters of whole body cholesterol turnover and various physiological variables that were measured in each subject. One can attempt this by performing a stepwise multiple regression analysis for each of the model parameters as a function of independent physiologic variables. In such an analysis, for each model parameter as the dependent variable, each independent variable would be added to the regression equation until none of the available independent variables caused a significant further improvement in fit. One difficulty with such a procedure is that only linear relationships are usually examined; the possibility of discovering relationships is thus limited unless one also examines several types of transformations of the independent variables (such as the logarithm of the variable or its square root), as well as possible cross-product terms. Consideration of transformations and

cross-products leads to a very large number of possible regression equations, some of which would appear to be significant by chance alone. For example, if 100,000 regression equations are examined, 1,000 of them would appear by chance to be statistically significant at the 1% level. Thus, if a large number of correlations are examined, apparent correlations may be found in the sample even if there are no significant relationships in the population from which the test sample was derived. Therefore, a method needs to be developed in such a situation which will minimize the chances of declaring significance when it is not truly present. One approach to this problem might be to reduce the *P*-value that is considered significant, for the relationships examined. Statistically, one correct way to do this is to divide the *P*-value by the number of tests performed. As a result, this approach is not useful when a large number of relationships are being examined, as in the present study. Accordingly, a different method, with greater power, was selected for the present study. In this method (21), the total study population of 54 was divided into two groups, one of which would be used to find the most significant of the relationships to be considered, and the other group for testing the significance of those relationships.

The 54 subjects were divided into two matched groups as described below. The first group (hypothesis-generating group) consisted of 36 subjects in which we looked for significant relationships between model parameters of cholesterol turnover and the set of physiological variables. The second group, of 18 subjects, served as the hypothesis-testing group, in which the significance of the relationships developed with the first group was tested.

In order to be valid, this approach requires that each of the two groups be randomly chosen, yet have approximately the same characteristics as the total study population. To achieve this end, the total study population was first stratified into nine subsets (strata) depending upon serum cholesterol and triglyceride concentrations and on the presence or absence of a familial form of hyperlipidemia. Proportionate samples were then drawn from each stratum into each of the two groups. The nine strata were: i) normal, 15 subjects; ii) hypercholesterolemic, familial, 7; iii) hypercholesterolemic, nonfamilial (or indeterminate), 3; iv) hypertriglyceridemic, with triglyceride between 200 and 300 mg/dl, familial, 4; and v) nonfamilial (or indeterminate), 5; vi) hypertriglyceridemic, with triglyceride above 300 mg/dl, familial, 5; and vii) nonfamilial (or indeterminate), 7; viii) mixed hyperlipidemic, familial, 5; and xi) nonfamilial (or indeterminate), 3. This somewhat arbitrary grouping was performed to ensure that the hypothesis-generating and -testing groups would have similar lipid characteristics.

Subjects were chosen at random from each of the nine strata to form the hypothesis-generating and -testing groups. The mean and standard deviation for the ages, weights, and percent ideal weights of the subjects in each group, as well as their sex distributions, were examined. A computer program was written to randomly choose the hypothesis-testing group of 18 and then determine the squared difference between the mean age, sex, weight, and percent ideal weight of the group and the corresponding means for the total population relative to the population mean squared. That is, $(\bar{x}q - \bar{x}p)^2/\bar{x}p^2$ was computed for each variable (age, weight, etc.), where $\bar{x}q$ is the mean of that variable in the group of 18 and $\bar{x}p$ is the population mean. A similar relative squared difference for the standard deviation of each variable was computed so that the distribution and mean of the variables could be considered. All eight relative squared differences were summed. One hundred random drawings of 18 subjects were performed, and the one with the smallest sum of relative squared differences was selected as the hypothesis-testing group. This group of 18 subjects had mean values for age, sex, weight, and percent ideal weight almost identical to those of the total population of 54, and furthermore had a distribution of these variables very similar to the whole study population. Moreover, because of the stratification on serum lipid levels and familial status, the two groups were very similar with respect to these variables as well. The remaining 36 subjects constituted the hypothesis-generating group.

A number of different forms of the model parameters were examined to search for relationships between model parameters and physiological variables in the hypothesis-generating group. These included the six uniquely determined model parameters (PR, M_1, k_{12}, k_{21}, k_{13}, and k_{31}) as discussed above, and the three estimates (minimum, intermediate, and maximum) of M_2, of M_3, and of M_{total} (total exchangeable body cholesterol, as $M_1 + M_2 + M_3$). In addition to these model parameters, we also considered: k_{01}, the transfer rate from compartment 1 to the outside (PR/M_1); the reciprocals of all rate constants; mass transfer rates between the central pool (pool 1) and the two side pools (pools 2 and 3) in g/day; and all mass transfer rates and compartmental sizes expressed per kilogram body weight (as g/kg·day or g/kg). In all, 50 different forms of the model parameters were used as dependent variables.

Seven physiological variables were determined in each subject: age, sex, height, weight, frame size, serum cholesterol, and serum triglyceride concentrations. From some of these, as previously described

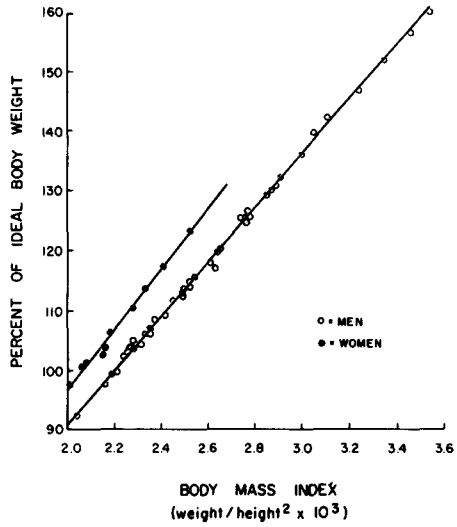

Fig. 2. Relationship between percent of ideal body weight and the body mass index in the study population of 54 subjects. For this plot, the percent of ideal body weight was estimated for each subject by using the ideal weight values for medium-frame persons (22). The body mass index was calculated as weight/height², in terms of kg/cm².

(10), we calculated three other variables related to body size: surface area, percent of ideal body weight (defined as the actual weight divided by the mean desirable weight for the patient's frame as determined from actuarial data (22) times 100), and excess weight (defined as total body weight minus ideal body weight). The latter two variables were used as indices of adiposity, whereas total body weight and surface area were used as indices of overall body size.

We chose deviations from ideal weight as a measure of adiposity rather than the somewhat more commonly employed body mass index (or weight divided by (height)²) for three reasons. First, there was a high linear correlation between percent ideal weight (estimated as observed weight/ideal weight for a medium frame × 100) and the body mass index for each sex (see **Fig. 2**); thus the mass index adds nothing to the percent ideal weight estimate. Second, there was a different relationship between percent ideal weight and body mass index for men and women (see Fig. 2), since there is no adjustment of the body mass index for sex difference. Third, the body mass index does not take differences in frame size into account. Therefore, we chose ideal weight in this work, since it is possible to adjust this variable for differences in frame size and for sex differences, and to obtain a number which appears to better express the degree of adiposity.

In addition to the physiological variables themselves, several non-linear transformations and many cross-products of the physiological variables were also used as independent variables. Each of the primary physiological variables was grouped and assigned a value of 1 if below the mean and 2 if above the mean. Thus, the mean percent ideal weight was 113, so that all subjects with percent ideal weight below this were assigned a value of 1 and above this a value of 2. With serum triglyceride concentrations, three groups were formed and assigned values as follows: 1 if triglyceride ≤200 mg/dl, 2 if >200 and ≤300 mg/dl, and 3 if >300 mg/dl. With serum cholesterol concentrations, the subjects were grouped as below or above 275 mg/dl. The logarithm and the square root of the triglyceride concentration were also used as independent variables. The cross-products included products of each of the six serum lipid variables times each of five body size variables. In all, this led to 53 different independent variables that were used for data analysis.

All possible multiple regression equations were computed in the hypothesis-generating group for each of the 50 forms of the model parameters as a function of the 53 forms of the physiological variables. A BMDP-9R computer program, entitled All Possible Subsets Regression and written at UCLA (23), was used for the computations, which were performed on the IBM 360-75/91 computer system at Columbia University.[2] For each of the 50 forms of the model parameters, the program considered more than 100,000 regression equations and selected a relatively small number (of the order of 100) that comprised the best regression equations for that model parameter. From these results, we chose for further analysis only those regression equations that satisfied two statistical criteria and certain physiological criteria.

First, in order to be selected for further analysis, a regression equation had to provide an r^2 of at least 0.5; that is, it had to account for at least 50% of the total variation in the dependent variable. Second, each of the regression coefficients in the equation had to be significantly different from zero at the 0.05 level (that is, each independent variable in the equation had to be significantly related to the dependent vari-

[2] The BMDP-9R program requires that the set of independent variables being considered not be highly correlated; that is, no independent variable can be expressed exactly as a linear combination of the other independent variables in the study population. Since the hypothesis-generating group had only 36 subjects and since the 53 independent variables were transformations of seven basic physiological variables, no more than about 16 variables could be used at one time. In order to examine all 53 independent variables, the computations for each model parameter were carried out in several stages, using about 16 at a time. At least one form of each physiological variable and cross products of each serum lipid times body size were included in each set of 16. Also, each set of 16 contained all the variables that were selected (i.e., found to be significant) from the 53 forms of the physiological variables by a separate stepwise regression procedure.

TABLE 3. Subjects by groups: unique model parameters (mean ± SD)

Model Parameter	Group (number of subjects)				
	Normal (15)	Hypercholesterolemia (10)	Hypertriglyceridemia (21)	Mixed Hyperlipidemia (8)	All Subjects (54)
PR (g/d)	1.10 ± 0.19	0.97 ± 0.24	1.59 ± 0.49	1.28 ± 0.32	1.29 ± 0.44
M_1 (g)	24.0 ± 3.2	30.3 ± 6.9	24.2 ± 3.9	28.7 ± 3.5	26.0 ± 5.0
k_{12} (d^{-1})	0.083 ± 0.025	0.075 ± 0.046	0.099 ± 0.051	0.074 ± 0.038	0.086 ± 0.042
k_{21} (d^{-1})	0.058 ± 0.018	0.035 ± 0.011	0.060 ± 0.020	0.046 ± 0.011	0.053 ± 0.019
k_{13} (d^{-1})	0.019 ± 0.006	0.017 ± 0.005	0.017 ± 0.006	0.016 ± 0.007	0.018 ± 0.006
k_{31} (d^{-1})	0.026 ± 0.016	0.024 ± 0.010	0.032 ± 0.014	0.024 ± 0.015	0.028 ± 0.014
R_2[a] (g/d)	1.39 ± 0.43	1.05 ± 0.47	1.42 ± 0.45	1.32 ± 0.33	1.33 ± 0.44
R_3[a] (g/d)	0.61 ± 0.38	0.73 ± 0.33	0.79 ± 0.40	0.68 ± 0.36	0.71 ± 0.37

[a] R_2 and R_3 are the mass transfer rates from pool 1 to pools 2 and 3, respectively, defined as M_1k_{21} and M_1k_{31}.

able at this level). In addition to these statistical criteria, we applied certain physiological criteria to the selection process. First, usually only one variable of body size was included in any one equation. For example, if weight and surface area were both in the equation, another equation was looked for which had a comparable r^2 and which included only weight or surface area. If none existed, then an equation with two body size variables was used. Similarly, only one variable involving cholesterol and one variable involving triglyceride was usually included. Age and sex were also allowed to enter. Thus, the maximum number of terms that an equation selected for further testing (in the hypothesis-testing group of 18 subjects) could have was five: one variable expressing dependency on body size, one cholesterol term, one triglyceride term, and age and sex. In fact, sex entered into none of the equations and age only in three. Discussion of the equations found and of the results of their analysis in the hypothesis-testing group of subjects will be presented in the Results section.

RESULTS

Model

As described previously (6), the data for each subject were analyzed to determine the best fit obtainable with a three-pool mammillary model, and also with a two-pool model and with models containing more than three pools. In 53 of the 54 subjects (including all 30 subjects described in Table 1) the three-pool model provided a significantly better fit to the data than did a two-pool model. In one previously reported subject (10), where data collection was incomplete because of a period of absence from this country, the parameters of the three-pool model could be estimated satisfactorily, although the fit was not significantly better than that obtained with a two-pool model. No further improvement in fit was obtained for any subject with a four-compartment model. These data thus confirm and greatly strengthen our previous conclusion (10) that the three-pool model appears to be generally valid for the study of cholesterol turnover in humans.

Relationships between model parameters and physiological variables

The means and standard deviations of the model parameters, for each subgroup according to plasma lipid classification and for the total study population, are presented in **Tables 3** and **4.** Statistical comparisons of the parameters between these subgroups were not made, since the extensive multiple regression analyses carried out provide a better way to investigate relationships between the model parameters and physiological variables (including plasma lipid levels). It should be noted, moreover, that a simple statistical comparison of group means for any one parameter may be misleading, since the groups differed with regard to physiological variables in addition to plasma lipid levels.

As described above, a very large number of regression equations were computed and examined, in the hypothesis-generating group of 36 subjects, to search for possible relationships between the model parameters and physiological variables. Many cross-product (interaction) terms, and transformations of the parameters and variables were employed in this search for relationships. As a result of this extensive analysis, a total of 21 regression equations emerged that met the statistical and physiological criteria described in the Methods section. These 21 equations involved 18 different forms of the model parameters (dependent variables), as listed in **Table 5.** In this table, the physiological variables (independent variables) found in the regression equations for the corresponding dependent variables are listed, for all 21 equations. In every instance, the set of independent variables accounted for at least 50% of the total variation in the dependent variable (i.e., r^2 at least 0.5).

TABLE 4. Subjects by groups: M_2 and M_3 under various assumptions (mean ± SD)

Model Parameter[a]	Group (number of subjects)				
	Normal (15)	Hypercholesterolemia (10)	Hypertriglyceridemia (21)	Mixed Hyperlipidemia (8)	All Subjects (54)
M_2min	19.5 ± 10.8	17.4 ± 8.5	16.8 ± 6.7	21.0 ± 9.4	18.3 ± 8.6
M_3min	29.3 ± 12.4	42.2 ± 12.5	44.5 ± 13.0	41.8 ± 13.5	39.4 ± 14.0
M_2inter	25.4 ± 12.8	23.7 ± 10.7	25.6 ± 10.6	29.2 ± 12.2	25.7 ± 11.3
M_3inter	58.6 ± 27.0	66.9 ± 13.9	90.8 ± 27.2	78.2 ± 13.4	75.6 ± 26.8
M_2max	31.4 ± 15.0	30.1 ± 13.2	34.3 ± 14.8	37.5 ± 15.2	33.2 ± 14.4
M_3max	88.0 ± 53.1	91.6 ± 20.3	137.1 ± 48.8	114.5 ± 20.1	111.7 ± 47.5
M_2+M_3 inter	84.1 ± 33.1	90.6 ± 19.6	116.3 ± 35.7	107.4 ± 16.7	101.3 ± 32.8

[a] Min, minimum; inter, intermediate; max, maximum.

The 21 regression relationships summarized in Table 5 were then tested for significance in the hypothesis-testing group of 18 subjects. This analysis was conducted by performing a *t*-test on the regression coefficient of each independent variable to determine whether the coefficient differed significantly from zero. In this way we tested the significance of each independent variable in each regression equation. A total of 59 coefficients were tested and 31 were found to be significant ($P < 0.05$, see Table 5). These 31 independent variables related to 15 different forms of the dependent variables (see Table 5). The corresponding relationships between these variables can thus be considered as confirmed, since they were found in the hypothesis-generating group, and confirmed to be significant in the hypothesis-testing group.

TABLE 5. Multiple regression relationships tested in the hypothesis-testing group[a]

Dependent Variable[b]	Independent Variable[b]
PR	Wt[c], EWt
	Wt[d], TGGP
M_1	Wt[d], Chol[d], TGGP[d]
M_1/Wt	Chol[d], TGGP[d]
k_{01}	EWt[c], Chol, TGGP[c]
$1/k_{01}$	Wt, Chol[d], TGGP[d]
M_3min/IDL	IWt[c], Age[c], Chol[d]
M_3min	Age[c], Wt[c], Chol[c]
	Age[c], EWt[d]
M_2+M_3min	Chol, EWt[d]
M_3inter	Wt[c], TG, TG·IDL
M_3max	Wt[c], TG, TG·IDL
M_2+M_3inter	Wt[c], TG, TG·IDL
M_3inter/M_1	Wt, TG, TG·IDL
M_3max/M_1	Wt, TG, TG·IDL
M_2+M_3inter/M_1	Wt, TG, TG·IDL
M_{tot}min	Wt[d], Chol[d], EWt
	Chol·Wt[d], EWt[d]
M_{tot}inter	Wt[c], TG, TG·IDL, CholGP
k_{00} min	Wt, Chol[d], TGGP
$1/k_{00}$min	Chol[d], Chol·Wt, TGGP

[a] These 21 relationships were chosen by rigorous statistical and physiological criteria from the regression analyses carried out in the hypothesis-generating group of 36 subjects. The relationships were tested in the hypothesis-testing group of 18 subjects.

[b] Symbols and abbreviations used (not already defined in the text): min, minimum; inter, intermediate; max, maximum; tot, total; Chol, serum cholesterol concentration (mg/dl); CholGP, variable equal to 1 if serum cholesterol concentration is ≤275 and 2 if cholesterol >275; Chol·Wt, serum cholesterol concentration times body weight; EWt, excess weight (observed weight minus ideal weight) (kg); IDL, ideal body weight (for height and frame, in kg); IWt, percent of ideal body weight; TG, serum triglyceride concentration (mg/dl); TGGP, variable equal to 1, 2, or 3 depending on serum triglyceride concentration (<200, 200–300, or >300); TG·IDL, serum triglyceride concentration times ideal body weight; Wt, observed body weight (kg).

[c] $P < 0.05$ and

[d] $P < 0.01$ by *t*-test of the regression coefficient of the independent variable, compared to zero, in the hypothesis-testing group.

Examination of the list of dependent variables in Table 5 demonstrates that no equations were found for M_2 or for any of the intercompartmental exchange rate constants (k_{12}, k_{21}, k_{13}, k_{31}). For the unique model parameters, highly significant and confirmed relationships were found only for PR and for M_1. Highly significant and confirmed relationships were also found for M_3 and for M_{total} (total exchangeable body cholesterol), particularly for the minimum estimates of these parameters. Most of the other confirmed relationships listed in Table 5 were for model parameters which are transformations of these four parameters (PR, M_1, M_3 min, and M_{tot} min). The remainder of the confirmed relationships (such as for M_3 max) are not independent of these four parameters. Accordingly, from the generated and tested relationships, we selected as independent and confirmed, the six relationships of physiological variables with PR, M_1, M_3 min, and M_{tot} min. These relationships were physiologically meaningful and provided a set of physiological variables that predicted the four model parameters quite well. In addition, as a result of the method of data analysis employed in this study, we are able to conclude that, in general, no further improvement in fit can be obtained with the current set of physiological variables, and that any improvement in prediction for model parameters will require other, new independent variables.

TABLE 6. Regression coefficients and correlation coefficients for production rate, M_1, M_3 (minimum), and total M (minimum)[a]

Dependent Variable	Independent Variable and Regression Coefficient	Intercept	Multiple r	Residual Error
PR	0.024 Wt	−0.580	0.80	0.26
M_1	0.287 Wt + 0.0358 Chol − 2.40 TGGP	−1.72	0.87	2.5
M_3min	0.622 Wt + 0.0500 Chol + 0.550 Age	−49.57	0.78	8.9
	0.842 EWt + 0.480 Age	7.77	0.73	9.7
M_{tot}min	0.884 Wt + 0.0991 Chol	−11.88	0.83	9.0
	0.831 EWt + 0.00118 Chol·Wt	51.48	0.83	9.1

[a] The coefficients listed were determined for the entire study population of 54 subjects. See footnotes to Table 5 for definition of abbreviations and symbols.

Confirmed equations for model parameters

The coefficients of these six equations were similar for the hypothesis-generating and -testing groups. Accordingly, the final values of the coefficients were determined on the entire set of 54 subjects, and the results are given in **Table 6.** All of the multiple correlation coefficients shown are 0.73 or higher, so that the sets of physiological variables shown in the table can account for 55 to 75% of the observed variation in the four model parameters listed. Body weight entered into the equations for all four model parameters. Serum cholesterol level entered into equations for cholesterol mass, i.e., for the sizes of pool 1 (M_1), pool 3 (M_3 min), and total exchangeable cholesterol (M_{tot} min). Age influenced significantly the size of pool 3. Serum triglyceride level only had an effect on the size of pool 1, and this was best shown by a discontinuous transformation of the serum triglyceride concentration. Neither serum cholesterol nor triglyceride concentration significantly influenced cholesterol production rate, nor did the triglyceride level show a relationship with the sizes of pools other than pool 1.

The equations shown in Table 6 can be used to predict values for the four model parameters for a given individual if the values for the relevant physiological variables are available. The residual errors listed in the last column of Table 6 give the standard deviations of the predicted parameter values if the values of the physiological variables are close to the observed population means (see Table 2). If the physiological variables are far from the population mean values, but in the range of the values observed in our study population, then the standard deviations of the predicted parameter values increase by no more than 10–20%. Accordingly, the residual error values listed in Table 6 provide information about the confidence limits of the predicted parameter values.

It may be noted that the residual error for production rate listed in Table 6 is larger than the standard deviation of PR found in normal and hypercholesterolemic subjects (Table 3). The residual error listed in Table 6 is an average value for the whole study population, and includes large residuals found among hypertriglyceridemic subjects and small residuals found among others. The residual error values for each of the four lipid groups are given in **Table 7.** These within-group residual errors were computed from the deviations from the overall regression line (regression equation) for PR. The results shown in Table 7 indicate that, in fact, the overall regression equation reduces within-group residual error in all lipid groups, and in the total study population as well.

Another question to consider in each of the six equations given in Table 6 is: what is the relative importance of each of the variables in determining the parameter values? It is not possible to compare directly the coefficients, since each variable has a different absolute range. The most useful way to judge the relative importance of each variable is to scale all of the regression coefficients between −1 and +1, thus allowing them to be compared directly. This can be achieved by multiplying each regression coefficient by the ratio of the standard deviation of the independent variable (see Table 2) to the standard deviation of the dependent variable (see Tables 3 and 4). The results of these computations are given in **Table 8.** In the case of a

TABLE 7. Residual error values for production rate for each group of subjects

Group	Observed Standard Deviation[a]	Residual Error Around Regression Line[b]
Normal	0.19	0.14
Hypercholesterolemia	0.24	0.21
Hypertriglyceridemia	0.49	0.34
Mixed hyperlipidemia	0.32	0.16
All subjects	0.44	0.26

[a] From Table 3.
[b] Using the regression equation for PR given in Table 6.

TABLE 8. Relative importance of each variable in the final equations as judged by standardized regression coefficients[a]

Model Parameter	Standardized Regression Coefficient for Each Independent Variable					
	Wt	EWt	Chol	Age	TGGP	Chol·Wt
PR	0.80					
M_1	0.82		0.74		−0.40	
M_3min	0.64		0.37	0.43		
		0.56		0.37		
M_{tot}min	0.79		0.64			
		0.49				0.52

[a] Each standardized regression coefficient was computed as the estimated regression coefficient (Table 6) times the ratio of the standard deviation of the independent variable to that of the dependent variable. See footnotes to Table 5 for definition of abbreviations and symbols.

simple regression involving only one independent variable, as with PR versus weight, the standardized regression coefficient equals the correlation coefficient (r). In Table 8, the relative importance of each independent variable in each equation is given by the absolute value of the standardized regression coefficient, and the direction of the effect by the sign. Thus, for M_1, weight is most important, cholesterol next, and the triglyceride-group variable third. The effect of increasing triglyceride-group is to decrease M_1.

Figs. 3 to 6 illustrate some of the relationships found. **Fig. 3** shows the relationship between PR and body weight. The absence of a relationship between serum triglyceride concentration and PR adjusted for differences in body weight (according to the first equation in Table 6) is shown in **Fig. 4. Figs. 5** and **6** show

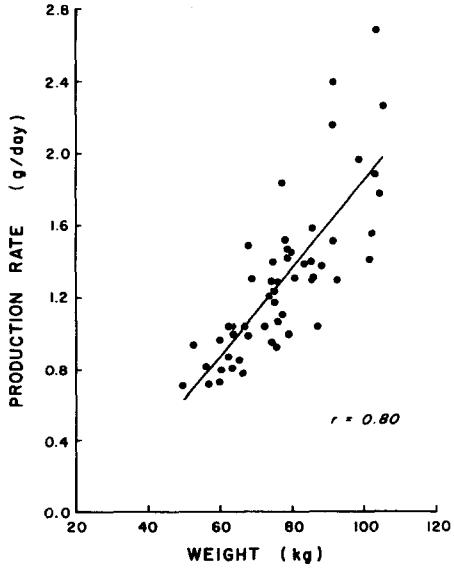

Fig. 3. Relationship between PR and body weight.

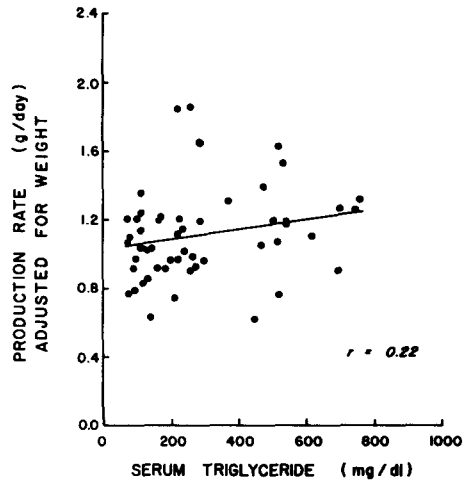

Fig. 4. Relationship between PR, adjusted for differences in body weight, and the concentration of serum triglyceride.

the relationships between the serum cholesterol concentration and the sizes of pools 1 and 3, respectively, when these pool sizes are first adjusted for the other independent variables given in the second and third equations in Table 6.

In order to ensure that no significant relationships between the physiological variables and the intercompartmental rate constants (the k's) or the three estimates of pool 2 were overlooked in the group of 36 subjects, we examined all possible regressions for these model parameters in the whole set of 54 subjects. No r^2 value was found to be greater than 0.34, and hence no significant relationship between these model parameters and the physiological variables could be found in the whole study population.

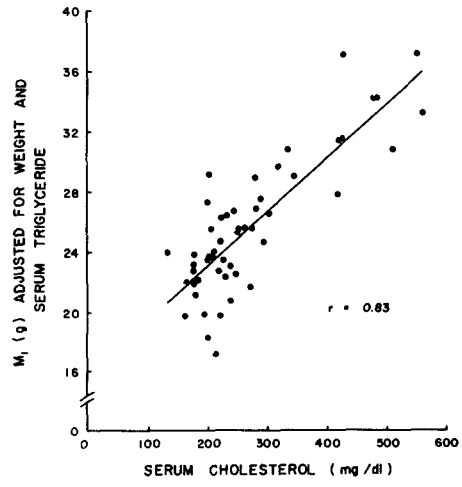

Fig. 5. Relationship between M_1, adjusted for differences in body weight and in the triglyceride-group variable, and the concentration of serum cholesterol.

DISCUSSION

These studies were conducted with the objective of obtaining detailed and quantitative information about body cholesterol metabolism in intact humans, and about the effects of serum lipid levels and other physiological variables upon the major parameters of body cholesterol metabolism. In order to achieve this objective, analyses were carried out to delineate relationships that may exist between the model parameters of whole body cholesterol metabolism and the various physiological variables that were measured in each subject. These analyses aimed to relate the model parameters to such physiological variables as serum lipid levels and measurements of body size, plus age and sex.

The work reported here greatly advances the information available from our previous, 1976 report (10). This advance comes from the fact that with the larger series of subjects reported here (total number 54), we were able to explore literally hundreds of thousands of possible relationships between the parameters of the three-pool model and physiological variables. Previously (10), because of the limited data available (24 subjects), only a small number of simple, linear relationships could be examined. Moreover, since only two of the subjects had hypertriglyceridemia alone, relationships between model parameters and plasma triglyceride concentrations could not be explored effectively. Thus the conclusions, while of interest, were necessarily of limited scope and confidence. In the present paper, by having a large enough population to enable us to use two groups to generate and test hypotheses, we were able to explore a large number of more complex relationships, and to reach much stronger conclusions.

The method of data analysis employed here involved the division of the total study population into two matched groups, one of which (hypothesis-generating group, 36 subjects) was used to find the most significant of the relationships to be considered, and the other group (hypothesis-testing group, 18 subjects) for testing the significance of these relationships. This method was used in order to guard against declaring statistical significance for relationships when, in fact, none was present. This statistical approach has been used effectively in studies in other fields (24, 25), but has not to our knowledge been used in metabolic studies of the type reported here. Since the general approach is quite powerful, and permits the exploration and evaluation of a very large number of possible relationships, it might well be worthy of consideration by investigators with similar problems working in other areas.

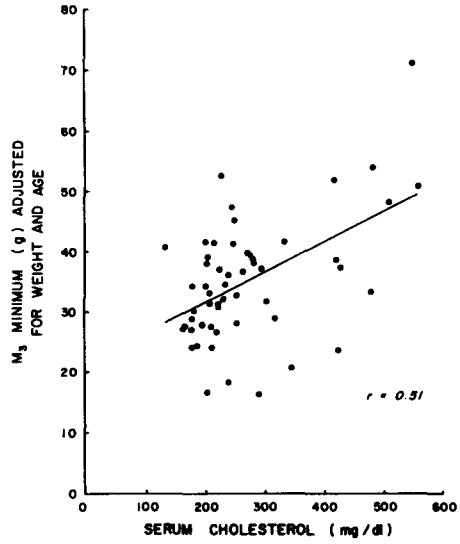

Fig. 6. Relationship between minimum M_3, adjusted for differences in body weight and in age, and the concentration of serum cholesterol.

As described in the Methods section, the population studied in this project was quite heterogeneous. Even with this heterogeneity, however, the three-pool model provided the best fit to the long-term data in every subject (except one for whom complete data were not available). Thus, this model seems to be generally valid for the study of body cholesterol turnover in intact humans, and for comparison of hyperlipidemic patients with normals and with each other.

As discussed previously (6), it must be recognized that the three pools in the model represent mathematical constructs and do not have precise physical meaning. The finding that the long-term turnover of plasma cholesterol conforms to a three-pool model in humans means that the various tissue pools of exchangeable body cholesterol fall into three groups in terms of the rates at which they equilibrate with plasma cholesterol. As discussed before (6), pool 1, which consists of cholesterol in fairly rapid equilibrium with plasma cholesterol, mainly consists of cholesterol in plasma, blood cells, liver, and intestines. Pool 2 consists of cholesterol which equilibrates at an intermediate rate with plasma cholesterol, and probably includes some of the cholesterol in viscera, together with some of the cholesterol in peripheral tissues. Adipose tissue cholesterol appears to be an important part of the most slowly turning over compartment, pool 3 (9). Cholesterol in other peripheral tissues, particularly connective tissue and skeletal muscle (26–28), and including arterial walls (29), also equilibrates slowly with plasma cholesterol; cholesterol in these tissue sites probably also constitutes a significant portion of pool 3.

In thinking about body cholesterol metabolism, it is necessary to distinguish between the kind of information that can be obtained from long-term studies of body cholesterol metabolism, such as those reported here, and the information that can be obtained from short-term kinetic studies of a subsystem within the body. Long-term studies with the three-pool model provide insight into the quantitative aspects of whole body cholesterol production, of the movement of cholesterol between different compartments in the body, and of the size of those compartments. In contrast, short-term studies of cholesterol kinetics in different lipoproteins (30–32), or in hepatic or biliary compartments (32, 33), all deal with the 'fine structure' of pool 1, with the sub-compartmentation of cholesterol in pool 1. Such rapid, short-term studies cannot address questions of total body cholesterol production or of the pool sizes (and movement) of cholesterol in the whole body. Conversely, long-term studies of body cholesterol metabolism cannot provide information about possible sub-compartments within any of the three pools of the three-pool model (such as possible hepatic cholesterol compartments associated with the metabolism of bile acids and biliary cholesterol (33)). Thus, these two kinds of studies operate on quite different time-scales, address different questions, and are complementary to each other.

As a result of the data analysis carried out, we have obtained a confirmed set of highly predictive equations that describe some of the major body cholesterol kinetic parameters in intact humans. Of the unique model parameters, highly significant and confirmed relationships were found only for production rate and the size of pool 1 (M_1). Such relationships were also found for the size of pool 3 (M_3) and for total exchangeable body cholesterol, particularly for the minimum estimates of these parameters. No significant relationships were found for any of the estimates of the size of pool 2 (M_2), or for any of the four intercompartmental rate constants.

For the confirmed relationships, production rate was found to be a function of body weight alone. In our total study population of 54 subjects, the observed mean production rate was 1.29 ± 0.44 (SD) g/day; from the final equation in Table 6, the expected value of PR for a 70 kg person would be 1.10 g/day. After adjusting the production rate values for variation in body size, no relationship was found between PR and the serum concentration of either cholesterol ($r = -0.14$) or triglyceride ($r = 0.22$, see Fig. 4). The absence of a relationship between PR and either the serum cholesterol level itself, or any transformation of the serum cholesterol level, confirms and extends previous studies that have demonstrated very similar turnover rates of cholesterol in patients with hypercholesterolemia and in normal subjects (2, 8, 10). It was previously suggested (2, 10) that hypercholesterolemia is not associated with abnormal rates of formation or excretion of cholesterol in the body, but with some impediment to the "clearance" or removal of cholesterol from the plasma pool. In patients with familial hypercholesterolemia, the mechanism responsible for this phenomenon has been shown to involve a decreased fractional rate of low density lipoprotein degradation (34), resulting from the reduced numbers of receptors for this lipoprotein present on the surface of fibroblasts and other types of cells in such patients (35, 36).

As discussed previously (10), studies by others have not provided consistent findings on the possible relationship between hypertriglyceridemia and the turnover rate of cholesterol. Some workers have observed high turnover rates of cholesterol (37), or of bile acids (38–40) (representing the major catabolic product of cholesterol) in patients with hypertriglyceridemia. These findings have not, however, been generally confirmed in other studies (41, 42). In our own data, when PR and triglyceride were examined alone, without reference to other variables, PR did show a significant relationship with the serum triglyceride level. On further analysis, however, this was found to be due to the positive correlation between hypertriglyceridemia and body weight. Hence, when PR was adjusted for body weight, no relationship between adjusted PR and triglyceride concentration was observed. In view of the very extensive search for relationships that was conducted in the present study, we can conclude that a general and consistent relationship between PR and the serum triglyceride level does not exist. It is possible, however, (10, 41), that a subgroup of hypertriglyceridemic patients may exist who show an increased synthesis of cholesterol, although this is not found in most patients with hypertriglyceridemia. Such a subgroup would not have been detected in the present study if it constituted only a small proportion of our patients with hypertriglyceridemia.

In contrast to production rate, the serum triglyceride level did have a significant effect on the size of the rapidly exchangeable compartment (M_1), and this was best shown by a discontinuous transformation of the serum triglyceride concentration. Thus, M_1 was found to be a function of both body weight and of the serum concentrations of both cholesterol and triglyceride. In our total study population, the observed mean M_1 was 26.0 ± 5.0 (SD) g; from the final equation in Table 6, the expected value of M_1 for a 70 kg

person with serum cholesterol level of 220 mg/dl and a normal triglyceride level (<200 mg/dl) would be 25.6 g. As discussed previously (2, 10), and as indicated by the equation for M_1 in Table 6, the increase in the size of M_1 in patients with hypercholesterolemia mainly reflects the increased cholesterol content of the plasma compartment in such patients. A comparably clear interpretation is not available for the new finding of a significant and confirmed negative relationship between M_1 and the serum triglyceride level. This finding does, however, suggest that a metabolic linkage exists between serum triglyceride and cholesterol within the rapidly exchangeable compartment (pool 1). Future studies will be needed to explore the nature of this linkage.

In the mammillary model shown in Fig. 1, synthesis of cholesterol may take place in each of the three pools, while output is constrained to occur only through the rapidly miscible central pool, pool 1. The model has eight unknown parameters. Since a sum of three exponentials has only three coefficients and three exponential constants, as discussed previously (6, 10), only six model parameters can be determined uniquely from an analysis of the plasma cholesterol turnover curve. The remaining ambiguity, in which the masses and synthesis rates of the side pools (pools 2 and 3) cannot be determined uniquely, may be termed indeterminacy. A general analysis of indeterminacy in pool models has been developed (43).

One approach to the problem of indeterminacy is to inject a labeled precursor of cholesterol in addition to cholesterol itself. This approach was used by Kekki, Miettinen, and Wahlström (11), who injected [^{3}H]-mevalonate and [^{14}C]cholesterol into each of two subjects, and used the resulting data to try to estimate the synthesis of cholesterol in body pools other than the rapidly miscible pool 1. Unfortunately, because of the short duration of the studies carried out (42 and 63 days, respectively, in the two subjects), along with limitations in the mathematical analysis, the conclusions drawn from these studies cannot be accepted as valid.

Another approach to this problem is to determine the ranges of possible values for the indeterminate parameters. As reported here, we computed both minimum and maximum values for M_2 and for M_3, and intermediate values of these masses as well. These several estimates of the sizes of pool 2 and 3 were computed, and are reported, in order to be completely rigorous on theoretical grounds. Physiologically, however, as previously discussed (6, 10), it is highly probable that the true values for M_3 and M_2 are much closer to the minimum than to the maximum values. Studies in baboons have suggested that the assumption that the entry of new cholesterol into the body occurs almost entirely via the rapidly exchangeable compartment (leading to minimum values for pools other than pool 1) may not be too unreliable (28). Furthermore, in studies where a three-pool model was fit simultaneously to both plasma and adipocyte cholesterol specific activity-time curves in six patients, no synthesis of cholesterol in pool 3 was found using the mammillary model of Fig. 1 (9). Accordingly, it is likely that the confirmed equations reported here, for the minimum values of M_3 and of total body exchangeable cholesterol, can provide information about these body pool sizes that is both physiologically meaningful and quantitatively fairly reliable (with a relatively small coefficient of variation).

Two equations were found for the minimum value of M_3. These showed that minimum M_3 is significantly related to body size (total weight in one equation and excess weight in the other), age, and (in one equation) the serum cholesterol concentration. The relationship between M_3 and adiposity (excess weight) is consistent with the finding that adipose tissue cholesterol appears to be an important component of pool 3 (9). Similarly, the observed relationship with age may in part reflect the changes in body composition, particularly the relative increase in the amount of fat tissue in the body, which occur with increasing age. The relationship of M_3 with age is also consistent with the increase in cholesterol concentration in connective tissue (also an important part of pool 3) with age reported by Crouse, Grundy, and Ahrens (44). The finding of a significant relationship with the serum cholesterol level as well as age suggests that, with time, cholesterol deposits in slowly exchanging tissue sites and that the extent of such deposition increases with increasing serum cholesterol level. The results also suggest (see also (10)) that the kinetic analysis as used here may be able to provide a method for estimating the size of pathological accumulations of cholesterol in slowly equilibrating tissue sites (presumably including arteries) in patients with hypercholesterolemia.

In conclusion, we have obtained a confirmed set of predictive equations which describe some of the major body cholesterol kinetic parameters. These equations account for a great deal of the variation found between people in the model parameters of production rate, M_1, and the minimum values of M_3 and of total body exchangeable cholesterol. In future work, these verified equations can be used both to predict these parameters of total body cholesterol metabolism in intact humans, and as the basis for studies of abnormalities in selected families and patients.

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