Comparison of cholesterol turnover by sterol balance and input–output analysis, and a shortened way to estimate total exchangeable mass of cholesterol by the combination of the two methods

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Abstract Daily turnover of cholesterol obtained by the balance method was compared to daily input rates calculated by input–output analysis in 43 experiments. The mean value of input rates for kinetic data of 10.1–16.4 weeks' duration (14 experiments) was 1.05 g/day vs. the chemical turnover of 0.94 g/day (difference 10.9%). For decay curves of 4.8–9.9 weeks' duration (29 experiments) the mean results were 1.67 g/day vs. 1.31 g/day, respectively (difference 20.1%). A combination of the balance method with input–output analysis is proposed to estimate the size of M (minimum value of the total exchangeable mass of cholesterol) in short-term experiments. Using this method, the analysis of curves of 10–12 weeks' duration showed a mean difference of 7.5% with the analysis of curves of 50–66 weeks' duration in 17 patients. However, because of considerable variations that can occur in individual cases, it is urged that a standard correction factor not be used, either in estimating turnover data or M from 10–12 weeks' kinetic data; rather, the proposed combined method will alert the investigator to the occurrence of discrepant results.

Supplementary key words intravenous tracer · computer analysis · hyperlipidemia · atherosclerosis · coronary artery disease

In the past decade the sterol balance method (1, 2) was developed to measure a number of important parameters of cholesterol metabolism. One of these parameters is the daily turnover of cholesterol (biosynthesized plus absorbed dietary cholesterol) (g/day) representing the sum of endogenous fecal neutral sterols and fecal bile acids (3). In recent years the use of tracer kinetic methods has permitted the calculation of body masses and turnover of cholesterol (4–6). The calculation of input rates (I_T) by input–output analysis yields the daily turnover (g/day), representing the sum of absorbed dietary and biosynthesized cholesterol (5). Thus these two sets of data, obtained by sterol balance on one hand and kinetic analyses on the other, describe the same parameter. As a result, any data derived by kinetic analysis can be verified in the same patients by the sterol balance technique. For example, in an earlier paper from this laboratory, Grundy and Ahrens (3) compared the results simultaneously derived from kinetic analysis and sterol balance in 10 patients and found that the means of the two sets of measurements agreed within 15%. In the present paper these comparisons have been extended; turnover data obtained by sterol balance and input–output analysis are compared in a total of 43 experiments in 38 patients. Results obtained by the sterol balance method were 10.9% lower, on average, than those obtained by kinetic analysis when the radioactive decay curves were followed for 10–16 weeks. When the decay curves were analyzed only over a 5–10 week period, the mean difference between the two methods was 20.1%.

We have recently reported from this laboratory (7, 8) that the rapidly miscible pool M_a can be precisely determined from the data points of the initial 7–10 days of the decay curves, using input–output analysis. The most precise determination of I_T and M (total exchangeable mass of cholesterol), however, requires long-term tracer studies (45 weeks or longer); values for I_T derived from curves obtained over 50–66 weeks were, on the average, 13.8% lower and, for a minimum estimate of M, were 20–26% larger than results obtained from the analysis of curves of 10–12 weeks' duration (7). The sterol balance method, on the other hand, allows the determination of I_T (which is the same as daily cholesterol turnover) in only a few weeks.

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Thus, in studying any one patient, if the sterol balance and kinetic analysis methods are applied together, it is possible to determine the true value of I_T (by sterol balance) and M_a (by kinetic analysis) in 3–4 weeks. The present study also demonstrates that a good approximation of the minimum value of M can be achieved in only 10–12 weeks if I_T is obtained by sterol balance and kinetic analysis methods combined.

METHODS

Patients

Thirty-eight patients were hospitalized on the metabolic ward of The Rockefeller University Hospital. The age, sex, habitus, and clinical diagnosis of each are included in **Tables 1** and **2**. During all the studies, the patients were considered to have attained the metabolic steady state, i.e., body weight, plasma lipid levels, fecal sterol excretion data, and clinical course were stable.

Cholesterol balance studies in many of these patients have been reported elsewhere (3, 10–12); the objectives, however, were different from those of the present study. In 10 patients (11 experiments) comparisons similar to the present study were made previously (3). The total number of experiments reported in this study is 43; in five patients two studies were carried out and, in one patient, three studies.

Diets

All patients were maintained at constant body weight on liquid formula feedings throughout the

TABLE 1. Cholesterol input rates derived by sterol balance and isotope kinetic methods in 14 patients, where specific activity–time curves of 10–16 weeks' duration were obtained

	Patient	Age	Sex	Weight	% Ideal Weight[a]	Diagnosis[b]	CH[c]	TG[c]	Length of curve[d]	Turnover[e] (by sterol balance)	Input Rate[f] (I_T)	% Diff.[g]	M_a[h]
				kg			*mg/dl*	*mg/dl*	*weeks*	*g/day*	*g/day*		
1	D.A.	55	M	64	104	CombHL; IHD	403 ± 18	2089 ± 266	16.4	1.24	1.06	−16.9	25.4
2	J.R.	36	F	53	98	HyperTC Xanth; IHD	464 ± 24	188 ± 31	14.0	0.61	0.78	+21.8	26.4
3	N.A.	30	M	67	102	IHD; HyperTC	257 ± 6	179 ± 9	12.4	0.73	0.96	+23.9	34.2
4	J.W.	72	F	54	124	IHD	325 ± 14	96 ± 20	12.1	0.65	0.75	+13.3	26.5
5A	H.S.	54	M	78	117	CombHL	416 ± 19	1529 ± 140	12.1	1.51	1.80	+16.1	39.6
6	J.T.	69	M	72	119	CVD	261 ± 8	139 ± 18	12.0	0.89	1.02	+12.7	25.2
7	J.H.	39	M	74	104	CombHL	447 ± 13	293 ± 33	12.0	1.11	1.14	+2.6	38.1
8	F.G.	62	F	67	94	HyperTG	215 ± 13	606 ± 118	11.6	0.82	1.06	+22.6	14.7
9	M.R.	60	M	67	109	CombHL Xanth	310 ± 8	546 ± 68	11.1	0.76	1.09	+30.3	25.7
10	C.Z.	63	M	94	122	IHD	227 ± 17	120 ± 20	11.0	1.39	1.29	−7.8	25.2
11	I.G.	46	F	56	112	IHD	234 ± 14	131 ± 19	10.7	0.90	0.71	−26.8	16.8
12	A.B.	70	F	49	113	IHD	287 ± 14	152 ± 29	10.6	0.71	0.94	+24.5	20.1
13	R.C.	48	F	58	92	CombHL Xanth	330 ± 41	541 ± 87	10.1	0.39	0.81	+51.9	51.2
14	J.J.	39	F	71	131	HyperTG	197 ± 19	600 ± 99	10.1	1.46	1.27	−14.9	25.4
Mean ± SD									11.9	0.94 ± 0.35	1.05 ± 0.28	10.9 ± 21.5	28.2 ± 9.7

[a] From 1959 life insurance tables (9).
[b] IHD, ischemic heart disease; CVD, cerebrovascular disease; xanth, xanthomatosis; combHL, combined hyperlipidemia; hyperTC, hypercholesteremia; hyperTG, hypertriglyceridemia.
[c] Plasma cholesterol (CH) and triglyceride (TG) concentrations during the metabolic steady state (mean ± SD).
[d] Number of weeks during which plasma cholesterol specific activity data were analyzed after i.v. administration of labeled cholesterol at time zero.
[e] Daily turnover of cholesterol by sterol balance methods, according to Eq. 7, ref. 3, where turnover is equal to synthesis plus absorption.
[f] Daily input rate of cholesterol by input–output analysis (7), where input is equal to synthesis plus absorption.
[g] % difference = $[(I_T - \text{turnover}) \div I_T] \times 100$.
[h] Mass (g) of pool a, the most rapidly turning over pool of cholesterol.

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TABLE 2. Cholesterol input rates derived by sterol balance and isotope kinetic methods in 29 experiments in 24 patients, where specific activity–time curves of 5–10 weeks' duration were obtained

Patient	Age	Sex	Weight	% Ideal Weight[a]	Diagnosis[b]	CH[c]	TG[c]	Length of curve[d]	Turnover[e] (by sterol balance)	Input Rate[f] (I_T)	% Diff.[g]	M_a[h]
			kg			*mg/dl*	*mg/dl*	*weeks*	*g/day*	*g/day*		*g*
15A R.H.	40	M	90	113	CombHL	333 ± 47	590 ± 78	9.9	1.30	2.81	+53.7	34.3
16 H.T.	57	M	46	84	HyperTC	307 ± 14	148 ± 14	9.8	0.50	0.74	+32.4	15.0
15B R.H.	40	M	90	113	CombHL	207 ± 24	265 ± 41	9.6	1.28	2.77	+43.6	35.1
17 J.O.	41	M	83	103	IHD	209 ± 12	180 ± 33	9.1	1.70	2.18	+22.0	31.0
18 A.G.	48	M	95	126	HyperTG; IHD	199 ± 15	239 ± 26	9.0	2.10	2.32	+9.5	21.9
19 P.L.	33	M	175	271	Ob	171 ± 12	184 ± 24	9.0	2.43	2.44	0	31.2
20 N.C.	59	F	150	310	Ob	194 ± 10	125 ± 14	9.0	1.71	1.91	+10.5	23.0
21 A.M.	44	M	73	92	HyperTC Xanth; IHD	390 ± 12	123 ± 16	8.7	0.52	1.05	+50.5	42.5
22 S.B.	52	M	86	121	HyperTG; IHD	258 ± 8	520 ± 170	8.7	1.09	1.71	+36.3	28.3
23 J.G.	55	M	63	79	IHD	194 ± 9	84 ± 16	8.6	0.62	0.87	+28.7	26.9
24A A.G.	50	M	70	110	HyperTG Xanth; IHD	201 ± 26	337 ± 40	8.6	0.79	1.28	+38.3	16.8
25A T.N.	39	M	84	105	CombHL Xanth	237 ± 35	76 ± 17	8.4	1.26	1.80	+30.0	25.1
26 R.T.	48	M	65	108	CombHL; IHD	377 ± 40	2048 ± 74	8.1	0.77	1.07	+28.0	25.1
27 R.B.	59	F	52	73	IHD; RHD	242 ± 25	74 ± 19	8.1	0.81	0.74	−9.5	15.6
24B A.G.	50	M	70	110	HyperTG Xanth; IHD	201 ± 9	325 ± 25	8.0	1.19	1.21	+1.7	18.0
28 R.S.	49	F	102	182	Ob	218 ± 16	170 ± 20	8.0	1.58	1.35	−17.0	26.0
29 S.Z.	44	F	120	260	Ob	215 ± 22	133 ± 14	8.0	2.30	3.65	+36.9	34.1
30 C.P.	46	F	147	295	Ob	185 ± 9	121 ± 17	8.0	2.67	2.08	−28.4	17.9
31 R.G.	58	F	61	120	CombHL Xanth; IHD	407 ± 13	256 ± 46	7.7	0.98	1.54	+36.4	30.9
32 J.L.	52	M	83	117	HyperTG; IHD	255 ± 14	230 ± 33	7.7	0.74	1.28	+42.2	28.9
33A L.M.	58	F	50	97	HyperTC Xanth	403 ± 34	192 ± 26	7.6	0.69	0.72	+4.2	24.3
34 G.W.	48	M	81	114	HyperTC Xanth	502 ± 53	126 ± 18	7.2	0.71	1.10	+35.5	34.7
33B L.M.	58	F	50	97	HyperTC Xanth	401 ± 19	200 ± 22	7.0	1.08	1.02	−5.9	23.6
25B T.N.	39	M	84	105	CombHL Xanth	266 ± 19	212 ± 17	6.9	1.10	1.77	+37.9	26.7
36 D.B.	47	M	63	93	PVI	170 ± 7	190 ± 13	6.4	0.89	0.88	0	21.9
5B H.S.	57	M	79	102	CombHL	365 ± 13	825 ± 232	6.4	1.33	1.90	+30.0	32.5
5C H.S.	57	M	79	102	CombHL	567 ± 42	1908 ± 174	6.1	1.87	2.50	+25.2	37.2
37 H.S.	50	F	78	137	IHD	200 ± 10	155 ± 19	5.9	0.81	0.99	+18.2	18.7
38 J.O.	41	M	83	103	IHD	209 ± 12	180 ± 33	4.8	3.08	2.88	−6.9	22.5
Mean ± SD								**7.9**	**1.31 ± 0.68**	**1.67 ± 0.77**	**20.1 ± 21.5**	**26.5 ± 7.0**

[a] From 1959 life insurance tables (9).
[b] IHD, ischemic heart disease; Xanth, xanthomatosis; Ob, obesity; RHD, rheumatic heart disease; PVI, peripheral vascular insufficiency; combHL, combined hyperlipidemia; hyperTC, hypercholesteremia; hyperTG, hypertriglyceridemia.
[c–h] See Table 1.

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study, as described previously (13). In patients studied several times, the formula was varied from one study to another. However, in any one experiment, the same formula diet was given throughout the entire study, and balance and kinetic studies were carried out simultaneously. Thus, the results obtained by kinetic and balance methods could not have been affected by differences in dietary intakes. When any intervention was imposed subsequent to tracer administration (i.e., change of formula or initiation of drug therapy), the experiment was considered to be terminated at that point (for the purposes of this study).

Plasma lipids

In the earlier studies, plasma cholesterol was measured by the method of Abell et al. (14) and plasma triglycerides were measured by a microgravimetric procedure (15). In later studies, measurements were made on a Technicon Auto Analyzer (Model I) (16, 17). Cholesterol analyses by the two procedures agreed within ±7%, but, in every case, the earlier triglyceride measurements were usually higher by about 100 mg/100 ml plasma. The methods used in any one patient were the same throughout his study.

Isotopic sterols

[4-^{14}C]Cholesterol and [1,2-^{3}H]cholesterol (New England Nuclear Corp., Boston, MA) were purified by thin-layer chromatography on Florisil, using ethyl ether–heptane 55:45. Only that portion of the tracer was used that migrated with the same R_f value as the cholesterol standard. A measured quantity (30–100 μCi) was administered intravenously as a single dose dissolved in 1 ml of ethanol and dispersed in 150 ml of physiologic saline; any residual radioactivity remaining in the infusion set was determined after ethanol extraction.

Concentration and specific activity of total plasma cholesterol were determined biweekly; radioactivity was measured on an aliquot of the same extract made for determination of concentration, with the radioactivity being measured in a Packard Tri-Carb scintillation counter (Model 3003) as previously described (2).

Patients were studied for varying periods; specific activity decay curves were followed for 10.1–16.4 weeks (mean 11.9 weeks) (Table 1) in 14 experiments, and for 4.8–9.9 weeks (mean 8.2 weeks) (Table 2) in 29 experiments.

Fecal steroid analysis

Fecal neutral and acidic steroids were isolated separately, and their masses and specific activities were measured by methods presented previously (1, 2). These procedures permit the essential distinction to be made between plant sterols and cholesterol, and between the two families of bacterial conversion products derived from plant sterols and cholesterol during intestinal transit (5β,3β-OH and 5β,3-keto compounds).

The amounts of neutral steroids excreted were corrected for losses occurring during intestinal transit and for variations in fecal flow rates, with dietary β-sitosterol as an internal standard (18). Chromic oxide was used as a marker for correction of day-to-day variations in the fecal flow of acidic steroids (19).

Daily excretion of endogenous fecal neutral steroids (mg/day) was derived as equal to the total radioactivity in the neutral steroid fraction (corrected with β-sitosterol recoveries) (dpm/day) divided by the specific activity of plasma cholesterol 1–2 days previously (3). Daily turnover was calculated as the sum of endogenous fecal neutral steroids and fecal bile acids (corrected for chromic oxide recovery). The small fraction of newly synthesized cholesterol that is directly secreted into the bile as cholesterol without first exchanging with the plasma will escape measurement by this isotopic balance method.

Kinetic analyses

The theoretical basis of input–output analysis is described elsewhere (5). The results of plasma analyses were expressed as percent dose of radioactivity given per one gram of total plasma cholesterol times specific activity divided by the injected dose of radioactive cholesterol. If $w(t)$ denotes specific activity per unit dose at any time t(days), then the input rate I_T (g/day) of body cholesterol is given

$$I_T = 100 \Big/ \int_0^\infty w(t)\mathrm{d}t \qquad \text{Eq. 1}$$

The rapidly exchangeable mass M_a (g) is given by

$$M_a = 100/w(0) \qquad \text{Eq. 2}$$

All computations were done on an XDS Sigma 7 computer. The computer program, which includes Eq. 1 and 2, uses the trapezoidal rule for area integration. The initial and final slopes of the curves were determined by exponential curve fitting for the purpose of extrapolation back to time zero and forward to infinity.

Combination of sterol balance and input–output analysis

We postulated that the combination of the two methods, sterol balance and input–output analysis, permits a reliable estimation of a minimum value for

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total exchangeable body mass of cholesterol (M) in a much shorter period than the kinetic method alone. To test this point we have carried out a series of special mathematical analyses on a group of patients (other than the above-described) who had been previously studied by us (7). The specific radioactivity decay curves of 17 patients were analyzed. Clinical data and plasma lipid levels of these patients are included in **Table 3**. The methods used were reported previously (6). A single intravenous injection of purified [7α-^{3}H]-cholesterol or [4-^{14}C]cholesterol (75–83 μCi) was given as described previously (6), and the specific activity decay curves were followed for 50–66 weeks.

Eq. 1 and 2 (see above) were used to calculate I_T and M_a.

The mean transit time $\bar{t}_p$ (days) of tracer cholesterol is given by

$$\bar{t}_p = \int_0^\infty tw(t)\mathrm{d}t \Big/ \int_0^\infty w(t)\mathrm{d}t \qquad \text{Eq. 3}$$

The total exchangeable body mass M (gms) is given by

$$M = I_T \cdot \bar{t}_p \qquad \text{Eq. 4}$$

We used the value obtained by sterol balance as the true I_T. The computer then calculated $\bar{t}_p$ correspond-

TABLE 3. Calculation of the mean transit time of tracer cholesterol ($\bar{t}_p$), and of the minimum value of total exchangeable body mass of cholesterol (M) by the combined method described in text

								50–66 Weeks' Kinetic Study			10–12 Weeks' Study: Kinetic Analysis Alone			10–12 Weeks' Study: Proposed Combined Method		
Patient	Age	Sex	Weight	% Ideal Weight[a]	Diagnosis[b]	CH[c]	TG[c]	M[d]	I_T[e]	$\bar{t}_p$[f]	M[d]	% Diff[g]	$\bar{t}_p$[f]	M[d]	% Diff[h]	$\bar{t}_p$[f]
			kg			*mg/dl*	*mg/dl*	*g*		*days*	*g*		*days*	*g*		*days*
39 B.R.	53	M	81	113	MS	232 ± 17	168 ± 14	162.4	1.81	89.8	97.4	40.0	46.5	137.7	15.2	76.1
40 A.B.	52	M	72	98	IHD	220 ± 18	125 ± 24	79.1	1.15	68.9	59.5	24.8	47.6	72.9	7.9	63.6
41 B.P.	64	F	56	102	IHD; HyperTC	307 ± 18	110 ± 17	65.9	0.86	76.8	71.3	8.2	88.7	56.1	15.0	65.3
42 J.L.	49	M	74	110	IHD; HyperTC	318 ± 19	99 ± 20	90.8	1.06	86.0	76.0	16.2	68.9	82.0	9.6	77.7
43 N.A.	50	F	54	92	IHD; HyperTC Xanth	542 ± 26	121 ± 28	95.0	1.17	81.3	79.0	16.9	63.8	87.5	7.9	74.9
44 A.W.	46	M	75	108	IHD; HyperTC Xanth	631 ± 14	148 ± 14	129.7	1.49	87.0	101.3	21.9	62.6	116.9	9.9	78.4
45 S.K.	67	F	52	73	IHD	234 ± 12	122 ± 14	60.5	0.68	89.4	46.6	22.9	61.6	56.0	7.4	82.8
46 H.H.	59	M	61	108	MS	224 ± 20	90 ± 33	72.6	1.18	61.5	61.2	15.7	48.6	70.4	3.0	59.7
47 N.S.	72	M	77	114	IHD	197 ± 23	100 ± 17	88.9	0.87	101.6	71.4	19.7	73.2	86.1	3.1	98.4
48 S.P.	49	F	83	130	IHD; EH	272 ± 32	84 ± 12	119.4	1.22	98.1	80.1	32.9	53.8	121.8	−2.1	100.1
49 G.K.	63	M	76	112	IHD; HyperTC	407 ± 31	113 ± 21	97.8	1.27	76.7	54.3	44.5	43.3	93.1	4.8	73.0
50 T.B.	45	M	92	122	IHD; HyperTC Xanth	580 ± 33	128 ± 16	144.1	1.30	111.0	101.4	29.7	62.9	137.4	4.7	105.8
51 D.B.	43	F	76	136	EH	224 ± 15	94 ± 19	88.5	1.09	81.2	84.3	4.7	75.3	76.6	13.9	69.9
52 M.R.	53	F	64	122	PVI	289 ± 24	104 ± 19	110.6	0.89	123.8	88.4	20.1	90.0	97.0	12.3	108.6
53 A.K.	18	M	54	86	IHD; HyperTC Xanth	699 ± 45	78 ± 15	101.0	1.26	80.0	96.1	4.9	76.4	95.3	4.1	76.2
54 C.A.	41	F	51	98	MS	228 ± 11	44 ± 10	64.7	1.10	58.9	49.0	24.3	40.8	59.5	8.1	54.1
55 E.A.	60	F	52	100	IHD; PE	224 ± 17	86 ± 11	74.0	1.06	70.0	76.6	−3.6	79.1	71.8	3.0	67.9
Mean								96.8	1.14	84.8	76.1	20.2	63.7	89.3	7.5	78.4

[a] From 1959 life insurance tables (9).
[b] IHD, ischemic heart disease; Xanth, xanthomatosis; PVI, peripheral vascular insufficiency; PE, pulmonary emphysema; EH, essential hypertension; MS, multiple sclerosis; hyperTC, hypercholesteremia.
[c] Plasma cholesterol (CH) and triglyceride (TG) concentrations during the metabolic steady state (mean ± SD).
[d] M is minimum value for total exchangeable mass of cholesterol.
[e] I_T is input rate (sum of absorbed and synthesized cholesterol).
[f] $\bar{t}_p$ is mean transit time of tracer cholesterol.
[g] % difference = $[(M_{50-66\ wks} - M_{10-12\ wks}\ \text{Kinetic}) \div M_{50-60\ wk}] \times 100$.
[h] % difference = $[(M_{50-66\ wks} - M_{10-12\ wks}\ \text{Proposed}) \div M_{50-60\ wks}] \times 100$.

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ing to the area obtained from I_T, as described in the Appendix. We could then compute M according to Eq. 4.

RESULTS

Calculation of input rates and cholesterol turnover

For the kinetic data of 10.1–16.4 weeks' duration, the mean value of input rates (I_T) was in good agreement with that obtained by sterol balance; in 14 patients the mean value of turnover was 0.94 g/day, whereas that of I_T was 1.05 g/day (Table 1 and **Fig. 1**). We consider the mean difference of 10.9% to be gratifyingly small; possible reasons for the lower values by sterol balance are discussed elsewhere (3).

It must be emphasized, however, that individual differences were sometimes large, ranging from −26.8% to +51.9%, although the correlation coefficient between the two sets of data was $r = 0.808$ ($P < 0.001$). Therefore, we conclude that, in the metabolic steady state, the turnover data by sterol balance methods can usually be assumed to approximate the input rates calculated by kinetic analysis over periods of at least 10 weeks. However, in view of the magnitude of the individual variations noted above, it seems reasonable to rely most heavily on the experiments in which the difference is below 30%. Of the 14 experiments included in Table 1, the results in 12 agreed within 30%.

Table 2 includes the data of kinetic curves of 4.8–9.9 weeks' duration in 29 experiments. In these experiments of shorter duration, the agreement between the means obtained by sterol balance and kinetic analysis were less satisfactory (1.31 vs. 1.67 g/day, respectively, a difference of 20.1% between the means). The results are graphically shown in **Fig. 2**. The correlation coefficient between the two sets of data was r = 0.785 ($P < 0.001$).

Calculation of the total exchangeable mass of cholesterol (M)

When the value of I_T is known from sterol balance data (as outlined above), a minimum value of M can be calculated from Eq. 4 if the mean transit time ($\bar{t}_p$) can be estimated validly from kinetic curves (see Appendix). We have analyzed our data to determine the shortest time in which a kinetic curve would yield values for $\bar{t}_p$ with an acceptably small error limit. Table 3 includes data on the analysis of die-away curves carried out for 50–66 weeks, as well as an analysis of the first 10–12 weeks of these same curves, using the presently described "combined method" vs. the kinetic

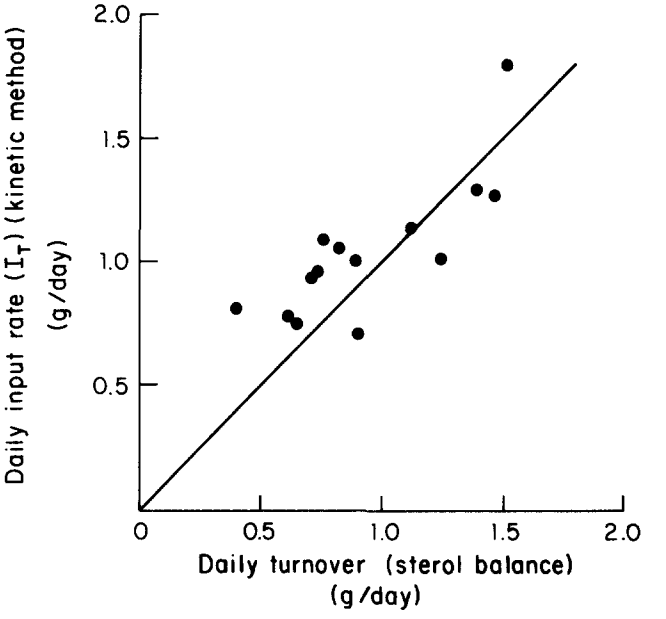

Fig. 1. Daily turnover of cholesterol by two methods in 14 patients plotted against the line of identity, where specific activity–time curves of 10–16 weeks' duration were obtained. Mean for kinetic method is 1.05 g/day; mean for balance method is 0.94 g/day; $r = 0.81$ ($P < 0.001$).

method alone. The mean difference in the value of M was 7.5% between results obtained by the combined method and the full-curve analysis, reflecting a close approximation of the mean values of $\bar{t}_p$ by the two

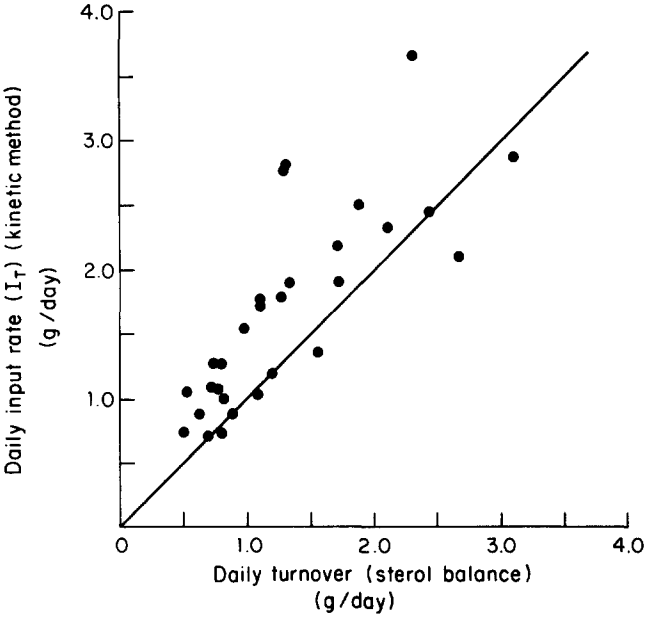

Fig. 2. Daily turnover of cholesterol by two methods in 29 experiments in 24 patients plotted against the line of identity, where specific activity–time curves of 5–10 weeks' duration were obtained. Mean for kinetic method is 1.67 g/day; mean for balance method is 1.31 g/day; $r = 0.79$ ($P < 0.001$).

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approaches (78.4 vs. 84.8 days). Thus, the error in M of only 7.5% represents a significant improvement over the 20.2% difference calculated on the short-duration curves by the kinetic method alone. When the length of the curve analysis was cut still further to 8 weeks, the mean difference in M rose to 10.6%, and for 4-week curves to 22.2%.

DISCUSSION

Of all the parameters that can be calculated by kinetic analysis, the only one that can be verified in intact man by an independent method (i.e., sterol balance) is the value of daily turnover. The purpose of the present paper is to compare daily turnover data obtained by the balance method to the input rates (I_T) obtained by kinetic analysis in the same patients; to date, we have made such comparisons in 38 patients. The second objective is to propose a new method (called the combined method) in which sterol balance data are combined with input–output data to estimate a minimum value of the total exchangeable mass of body cholesterol (M) in a relatively short time period.

The present paper indicates that the mean difference in I_T between the results obtained by kinetic analysis and by sterol balance procedures averaged 10.9% when the decay curves were followed for 10–16 weeks. However, the individual variations, which are sometimes very large, do not allow a single correction factor to be applied to short-term experiments. We know from previous short-term studies that one of the possible reasons for the large individual variations is that the final slope of the decay curves cannot be extrapolated accurately to infinity. Consequently we need (and have used) an independent method (sterol balance) to obtain the area under short-term decay curves extrapolated to infinity in order to calculate more reliably the value of M by the combined method.

In a previous paper from this laboratory it was shown that kinetic analyses of 50- to 66-weeks' duration yielded results for I_T that were, on average, 13.8% lower than results obtained from curves of analyses 10–12 weeks long (7). We can therefore postulate that results for I_T by the kinetic method would agree extremely closely with those obtained by the balance method, providing the kinetic analysis is carried to 50–66 weeks. By contrast, when the length of the decay curves represented only 5–10 weeks, the difference in results grew to 20.1%. This follows the same logic—the longer the curves, the closer we approach the truer values obtained by the balance method.

It should be emphasized that the kinetic analysis assumes that all synthesized cholesterol passes through the plasma at least once. Any newly synthesized cholesterol that bypasses plasma, such as that synthesized in the liver that passes directly via the biliary tract into feces, would not be reflected in the kinetic calculations. However, that very portion would be included in the measurement of turnover by the sterol balance method. In recent experiments[2] we have found that the amounts of biliary cholesterol and bile acids bypassing the plasma pool are relatively small and contribute little to the discrepancies encountered, i.e., the size of the error thus incurred in kinetic analysis is probably below the sensitivity of the method.

In the course of carrying out an input–output analysis of 12 weeks' duration, it is often feasible to obtain sterol balance data for the minimum desirable period of 4 weeks (six 4-day stool collections). Since the size of the rapidly exchangeable mass (m_a) is yielded by input–output analysis, the only parameter that remains to be determined in this relatively short time period is a figure for the minimum value of M (the total exchangeable mass of cholesterol in the body). We suggest that this minimum value of M is acceptably determined by the combined method described in this report; the calculation of M by the combined method incurred less error than its derivation from short-term kinetic curves.

Thus, the present approach combining kinetic and sterol balance methods allows us to calculate M with (theoretically) a precision of 7.5% in a period of 10–12 weeks. The same precision by kinetic analysis alone can be reached only if the study is continued for 30 weeks or longer (7). It is clear, then, that the proposed combined method can always be applied when balance data are being gathered simultaneously, but it also stands on its own merits as a valid way to estimate M in one-third the time now required by kinetic analysis alone.

At present there is no direct way of measuring M in man or determining its precise limits in vivo. However, two reports on laboratory animals support the conclusion that the kinetic method for estimating M is acceptably accurate. First, comparative kinetic and chemical data obtained at time of killing in squirrel monkeys by Lofland, Clarkson, and Bullock (20) indicated that, in six animals on control diets, the mean value for the whole body pool of cholesterol by direct chemical measurement was 1402 ± 43 mg, and that the mean value of M by kinetic derivation was 1418 ± 70 mg. Second, Pertsemlidis, Kirchman, and Ahrens (21) reported that, in six dogs, the mean value for

[2] Samuel, P., and E. H. Ahrens, Jr. Unpublished data.

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body mass of cholesterol measured by direct chemical analysis was 28.5 g, whereas the mean value for M for these animals calculated from isotope kinetic data was 27.2 g.

In summary, then, we have described a combined kinetic and sterol balance method in which specific activity decay curves are followed for only 10–12 weeks. Balance data are obtained during this period in order to obtain a chemical estimate of cholesterol turnover. The value of M_a (rapidly exchangeable mass of cholesterol) is dependably calculated from the decay curves, and a minimum value of M can be estimated by the proposed combined method with a precision of 7.5%.

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APPENDIX

In order to evaluate the integrals in Eq. 1 and Eq. 3, it is necessary to assume that the final slope of the decay curve has been reached and to extrapolate this curve to infinity. It is also necessary to extrapolate the initial portion of the curve back to time zero. It has been shown that the final slope (if indeed it is the final slope) may not be reached for as long as 43 weeks. If, however, one uses the sterol balance method to obtain I_T, it is then no longer necessary to obtain the final slope and to extrapolate the decay curve $w(t)$ out to infinity. The reciprocal of I_T (from balance data) is a measure of the area under the curve taken to infinity in Eq. 1.

Consider the following short-term experiment. A decay curve is obtained for some relatively short period of time, say 4–12 weeks. Denote the last data point obtained as (t_{max}, w_{max}) where w_{max} equals w (t_{max}). At the same time determine I_T by the chemical balance method. This gives, by Eq. 1, the theoretically correct value for the area under the full decay curve taken to infinity. Call this area A_{theo}. Compute the area under the experimentally given decay curve up to t_{max} by numerical integration. Call this area $A_{t\,max}$.

Then a unique curve is invoked of the form ae^{-bt} that passes through the point (t_{max}, w_{max}) and with the property that

$$\int_{t\,max}^{\infty} ae^{-bt}dt = A_{theo} - A_{t\,max}$$

From the above conditions we find that a and b are then given by

$$a = w_{max} \exp[(w_{max}t_{max})/(A_{theo} - A_{t\,max})] \qquad \text{Eq. 5}$$

$$b = w_{max}/(A_{theo} - A_{t\,max}) \qquad \text{Eq. 6}$$

The integral in the numerator of the expression for $\bar{t}_p$ Eq. 3, can then be determined in the following manner

$$\int_0^{\infty} tw(t)dt = \int_0^{t\,max} tw(t)dt + \int_{t\,max}^{\infty} tw(t)dt \qquad \text{Eq. 7}$$

The first term on the right side of Eq. 7 can be determined by numerical integration, while the second term can be evaluated analytically by using ae^{-bt} for $w(t)$ where a and b are given by Eq. 5 and 6. The denominator of Eq. 2 is known from I_T and M can then be determined from Eq. 4.

In order to check the value of M obtained by this procedure, it was compared to the value of M obtained from long-term tracer experiments. Assume that the values of M, M_a, and I_T derived from the long-term (50–66 weeks) experiments are true values of these parameters. Assume further that, had data obtained by the sterol balance method been available, they would have yielded results for I_T equal to those obtained from the long-term kinetic studies. Values of M were obtained with curves representing 4–12 weeks. At the same time, the curve was artificially shortened by removing successive data points from the end of the curve, and a simple tracer kinetic analysis was performed with the curve assumed to represent 4–12 weeks. The values thus obtained for M were also compared to the long-term results, percent differences were calculated and compared to the percent difference obtained by the combined kinetic–balance method.

While it is true that no one knows the proper curve that is to be extrapolated from t_{max} to infinity, and that the function ae^{-bt} is only one of an infinite number of choices, there is good reason for choosing this form for the final portion of the decay curve. From compartment analysis one expects the decay curve to be made up of a sum of exponentials and that the last one will therefore be of this form. One could, of course, choose the final portion of this curve to be made up of sums of exponentials, but then there would not be sufficient information to determine them uniquely.

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