Production of plasma esterified cholesterol in lean, normotriglyceridemic humans

Philip J. Barter

Department of Internal Medicine and Clinical Research Center, The University of Iowa, Iowa City, Iowa 52242

Abstract The rate of production of plasma esterified cholesterol was measured both in vivo and in vitro in seven subjects and in vivo alone in eight subjects. All subjects were lean, clinically healthy, and had triglyceride concentrations less than 1.5 μmoles/ml. In vivo production was calculated from the labeling of free and esterified cholesterol in plasma samples collected at 1-hr intervals for 8 hr after an intravenous injection of [^{3}H]mevalonic acid, on the assumption that plasma free cholesterol was the sole immediate precursor of esterified cholesterol. In vitro production was measured in serum samples collected 1 hr after the injection of [^{3}H]mevalonic acid (when radioactivity in esterified cholesterol was very low relative to that in free cholesterol); these samples were incubated for 1 hr at 37°C. The rates measured in vivo and in vitro were very similar in the seven subjects, strengthening the confidence in the techniques. In vivo production was measured during the postabsorptive state in all 15 subjects and in 5 of them also during the last 8 hr of a 32–56-hr period when all calories were taken in three hourly meals of an 80% carbohydrate, fat-free formula. In the postabsorptive state there was no apparent relationship between the production of esterified cholesterol and the concentration of either free or esterified cholesterol. Rather, despite a wide range of cholesterol concentrations, esterified cholesterol production was similar in all subjects. During the carbohydrate consumption the esterified cholesterol concentrations were significantly lower than during the postabsorptive state, but there was virtually no change in rate of production. It has been concluded that the differences in concentration of esterified cholesterol in lean, normotriglyceridemic subjects cannot be explained solely on the basis of differences in its production.

Supplementary key words hypercholesterolemia · dietary carbohydrate · [^{3}H]mevalonic acid

Although a relationship between the plasma cholesterol concentration and the occurrence of atheromatous disease has been well established (1, 2), the metabolic regulation of the plasma cholesterol remains obscure. Cholesterol balance studies and isotope kinetic analyses have added considerably to the knowledge of overall cholesterol metabolism, but they have provided little specific information about the plasma compartment. Interpretation of the plasma kinetics of isotopically labeled cholesterol is greatly limited by the rapid and reversible exchanges of unesterified (free) cholesterol between the plasma and many tissue pools (3).

Indirect evidence suggests that most of the cholesterol entering the plasma is in the free form (4); the plasma esterified cholesterol appears to be formed within the plasma as a product of the lecithin:cholesterol acyltransferase (LCAT) reaction (4–6). Unlike free cholesterol, the plasma esterified cholesterol exchanges little or not at all with that in tissue pools (3), which implies an active control of its transport in the plasma.

In humans, approximately 70% of plasma cholesterol is in the esterified form (7). Although this percentage may be lower in certain conditions such as hypertriglyceridemia (3), in most subjects the ratio of free to esterified cholesterol is relatively constant over a wide range of plasma cholesterol concentrations (7). Therefore, although the ready exchangeability of free cholesterol implies that its concentration in tissues and plasma may be passively determined by a phase distribution between tissue membrane and plasma hydrophobic phases, the constant free-to-esterified ratio in the plasma indicates that the regulation of the plasma free cholesterol concentration cannot be completely dissociated from that of esterified cholesterol. Consequently, an understanding of the active regulation of plasma esterified cholesterol transport may provide insight into the control of the total cholesterol concentration.

Nestel and Monger (8) have described and partially validated a technique for measuring the rate of production of plasma esterified cholesterol in vivo. Studies using this technique have revealed positive correlations between the esterified cholesterol production and the concentrations of cholesterol and triglyceride (9) and body weight (8, 9). It has been noted, however, that the rate of production in

Abbreviations: LDL, low density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; TLC, thin-layer chromatography; EC, esterified cholesterol; FC, free cholesterol.

lean, normotriglyceridemic subjects with elevated cholesterol concentrations may be no greater than in their normocholesterolemic counterparts (8). It is this final observation that has been further examined in these present studies.

Studies in a group of clinically healthy, lean, normotriglyceridemic subjects possessing a wide range of cholesterol concentrations revealed little variation in the rate of production of plasma esterified cholesterol in vivo. Nor was the production changed significantly in five of these subjects whose cholesterol concentrations had been acutely reduced by a fat-free, carbohydrate-enriched formula. It has been concluded that differences in production could not account for the wide range of esterified cholesterol concentrations in these subjects.

METHODS

Clinical data on the subjects studied are presented in Table 1.

TABLE 1. Clinical data[a]

Subject	Age	Sex	Weight	Height	Lipoprotein Phenotype[b]
	yr		*kg*	*cm*	
1	23	M	80	174	Normal
2	21	M	72	184	Normal
3	27	M	80	177	Normal
4	25	M	79	185	Normal
5	25	M	84	178	Normal
6	23	M	96	194	Normal
7	46	M	79	179	Normal
8	35	M	83	181	Normal
9	32	M	64	182	Normal
10	21	M	63	169	Normal
11	35	M	73	174	Normal
12	29	M	75	180	Type IIa
13	39	M	79	183	Type IIa
14	62	F	58	159	Type IIa
15	39	M	75	179	Type IIa

[a] All subjects were asymptomatic.
[b] According to Ref. 26.

Dietary preparation

(a) Postabsorptive studies (all subjects). Studies were begun at 9 a.m. after a 14-hr overnight fast that continued until the study was completed at 5 p.m.

(b) Absorptive studies (subjects 7, 9, 11, 12, and 14). After a 14-hr overnight fast, a base-line plasma sample was collected at 8 a.m. before beginning the consumption of a fat-free, 80% mixed carbohydrate, 20% protein formula. Equal aliquots of this formula, each providing one-eighth of the judged daily caloric requirements, were taken at 3-hr intervals for 33 or 57 hr (Table 4), and the studies were performed during the last 8 hr of this absorptive period.

Of the five subjects undergoing both postabsorptive and absorptive studies, three had the postabsorptive study first; studies were separated by about 6 wk. Subjects 2, 9, and 14 had been on a diet containing 100 mg of cholesterol, 40% carbohydrate, 40% fat, and 20% protein for 2–3 wk before each study; the other subjects had been consuming their habitual diets prior to study.

Esterified cholesterol production

(a) In vivo studies. DL-[5-^{3}H]Mevalonic acid (DBED salt) was obtained from New England Nuclear Corp., Boston, Mass. The dibenzylethylenediamine was freed by the addition of sodium bicarbonate and removed by extraction with ether. The aqueous solution of sodium mevalonate was neutralized with an equimolar amount of HCl, and individual doses were made up to a volume of 10 ml with normal saline. Intravenous injections of the [^{3}H]mevalonic acid were given at 9 a.m. Subjects studied once received approximately 150 μCi; subjects studied twice received 40 μCi on the first occasion and 160 μCi on the second occasion. At 1-hr intervals for 8 hr, blood samples were collected from an indwelling catheter (kept patent by a slow infusion of saline) into tubes containing dipotassium EDTA (1 mg/ml of blood) as anticoagulant and *p*-chloromercuriphenyl sulfonate (final concentration 0.002 M) to inhibit in vitro LCAT activity. Plasma was separated by centrifugation at 4°C and a 2-ml aliquot (or 4 ml in the low isotope dose studies) was extracted in a solution of isopropyl alcohol–heptane–1 N sulfuric acid 40:10:1, as described by Dole (10). The lipids contained in the heptane phase of the extracts were separated by thin-layer silicic acid chromatography (TLC) on plates developed in hexane–diethyl ether–methanol–acetic acid 90:20:3:2 in order to isolate the free and esterified cholesterol and triglyceride. The concentrations of both cholesterol fractions and triglyceride were measured in a Technicon AutoAnalyzer, model II, using free cholesterol, cholesteryl oleate, and triolein as standards. Radioactivity in the free and esterified cholesterol fractions was measured in a Packard Tri-Carb liquid scintillation counter (model 3380, Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with external standardization, using 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene as scintillator. Quenching was minimal. Recovery of both cholesterol mass and radioactivity after TLC was 90–95%.

(b) In vitro studies. 1 hr after the injection of [^{3}H]mevalonic acid, a blood sample was collected into a tube containing no additives and allowed to clot for 45 min at room temperature before separating the serum. Duplicate or triplicate aliquots of serum were placed in flasks in a shaking water bath at 37°C. 2-ml aliquots were taken from each flask after 5 min (zero time) and 65 min (1 hr) of incubation and were extracted immediately with Dole's solution (10). In three studies the in vitro incubations

were continued for 2–4 hr, with aliquots of plasma taken each hour (Fig. 1). The specific activity of free cholesterol and the total radioactivity in esterified cholesterol were measured in all samples. The esterified cholesterol fractions were counted twice for as long as was necessary to achieve a standard deviation of 1%.

CALCULATIONS

In vivo production of esterified cholesterol

The technique was basically that described by Nestel and Monger (8), which utilized a mathematical analysis outlined by Zilversmit (11). Two major assumptions are made: (*a*) plasma free cholesterol (or a precursor with the same specific activity as plasma free cholesterol) is the sole immediate precursor of plasma esterified cholesterol, and (*b*) the fraction of the esterified cholesterol pool removed from the plasma has a specific activity the same as that measured in the total pool (i.e., assume that the esterified cholesterol pool is homogeneous). These assumptions are examined in detail in the Discussion section. The calculations in the present study were based on the labeling of the free and esterified cholesterol during the first 8 hr after the [^{3}H]mevalonic acid injections rather than during the much longer time periods utilized by Nestel and Monger (8).

After the injection of [^{3}H]mevalonic acid, the plasma free cholesterol is rapidly labeled; the labeling of esterified cholesterol is much slower. On the basis of the above assumptions, at any given time the amount of radiolabel entering the esterified cholesterol pool is equal to the mass of esterified cholesterol produced per unit time (P_{EC}) multiplied by the free cholesterol specific activity, and the amount of label being removed is equal to the mass of esterified cholesterol removed per unit time (R_{EC}) multiplied by the esterified cholesterol specific activity. The rate of change of label in the esterified cholesterol pool at any given time is therefore equal to

$$(P_{EC} \times FC \text{ sp act}) - (R_{EC} \times EC \text{ sp act})$$

Integrating,

$$EC \text{ cpm}\,(T_2) - EC \text{ cpm}\,(T_1) = \int_{T_1}^{T_2} (P_{EC} \times FC \text{ sp act}) - (R_{EC} \times EC \text{ sp act})\,dt$$

This may be rewritten:

$$EC \text{ cpm}\,(T_2) - EC \text{ cpm}\,(T_1) = P_{EC} \int_{T_1}^{T_2} (FC \text{ sp act} - EC \text{ sp act})\,dt + \int_{T_1}^{T_2} (P_{EC} - R_{EC}) \times EC \text{ sp act}\,dt$$

Therefore,

$$P_{EC} = \frac{EC \text{ cpm}\,(T_2) - EC \text{ cpm}\,(T_1)}{\int_{T_1}^{T_2} (FC \text{ sp act} - EC \text{ sp act})\,dt} - (\text{error factor}) \qquad \text{(Eq. 1)}$$

Where

$$(\text{error factor}) = \frac{\int_{T_1}^{T_2} (P_{EC} - R_{EC}) \times EC \text{ sp act}\,dt}{\int_{T_1}^{T_2} (FC \text{ sp act} - EC \text{ sp act})\,dt}$$

Under metabolic steady state conditions, when production and removal are equal (i.e., $P_{EC} - R_{EC} = 0$), the error factor is zero, and

$$P_{EC} = \frac{EC \text{ cpm}\,(T_2) - EC \text{ cpm}\,(T_1)}{\int_{T_1}^{T_2} (FC \text{ sp act} - EC \text{ sp act})\,dt} \qquad \text{(Eq. 2)}$$

In the present studies the plasma esterified cholesterol production has been calculated from Eq. 2. The error introduced by the assumption of steady state conditions is probably small (see Appendix); if production exceeds removal there will be an overestimation of production and if removal exceeds production, production will be underestimated.

To provide a test of the validity of this approach (see Discussion section), each study has been divided into eight 1-hr segments, and a value of P_{EC} has been calculated for each 1-hr period. The integral in the denominator of Eq. 2 was taken as the area between the rectilinear plots of the specific activity–time curves and was measured by counting squares. The production of esterified cholesterol was expressed as micromoles/liter/hour. When a single value for the in vivo esterified cholesterol production is presented (Tables 3 and 4), it represents the mean of the values calculated for the five separate 1-hr periods between 3 and 8 hr after the [^{3}H]mevalonic acid injections.

On the assumption of steady state conditions, the fractional removal rate (hour^{-1}) of esterified cholesterol was calculated from the ratio:

$$\frac{\text{production } (\mu\text{moles/liter/hr})}{\text{concentration } (\mu\text{moles/liter})}$$

The turnover time is the reciprocal of the fractional removal rate.

In vitro production of esterified cholesterol

The increment in esterified cholesterol cpm during a 1-hr incubation divided by the free cholesterol specific activity provides a measure of the in vitro production of esterified cholesterol and, as with the in vivo studies, has been expressed in micromoles/liter/hour.

TABLE 2. Specific activities of plasma free[a] and esterified[b] cholesterol after injection of [^{3}H]mevalonic acid

Subject		Hours after Injection 0	1	2	3	4	5	6	7	8
Postabsorptive state[c]										
3	EC concentration (μmoles/ml)		2.39	2.35	2.33	2.40	2.38	2.38	2.33	2.34
	EC sp act (cpm/μmole)		15	42	74	107	128	148	168	184
	FC sp act (cpm/μmole)		669	1246	1406	1326	1154	1029	948	867
	EC production (μmoles/l/hr)	108	70	58	67	41	52	45	54	
6	EC concentration (μmoles/ml)		2.41	2.43	2.40	2.46	2.46	2.47	2.43	2.47
	EC sp act (cpm/μmole)		13	36	58	79	99	116	133	143
	FC sp act (cpm/μmole)		550	908	953	964	896	822	754	684
	EC production (μmoles/l/hr)	118	81	58	64	57	55	58	49	
8	EC concentration (μmoles/ml)				3.07	3.05	3.09	3.11	3.09	3.07
	EC sp act (cpm/μmole)				56	72	87	102	111	121
	FC sp act (cpm/μmole)				849	846	751	690	642	588
	EC production (μmoles/l/hr)				65	64	76	48	58	
10	EC concentration (μmoles/ml)		3.38	3.35	3.43	3.48	3.43	3.41	3.38	3.41
	EC sp act (cpm/μmole)		27	60	93	131	158	183	213	228
	FC sp act (cpm/μmole)		833	1381	1582	1523	1429	1311	1210	1117
	EC production (μmoles/l/hr)	228	104	77	88	60	65	81	77	
15	EC concentration (μmoles/ml)		6.58	6.73	6.66	6.68	6.73	6.79	6.73	6.68
	EC sp act (cpm/μmole)		6	13	22	28	36	43	48	55
	FC sp act (cpm/μmole)		396	698	766	764	742	667	641	605
	EC production (μmoles/l/hr)	209	89	78	64	74	75	47	77	
Carbohydrate consumption[d]										
9	EC concentration (μmoles/ml)		2.61	2.50	2.66	2.61	2.50	2.50	2.55	2.61
	EC sp act (cpm/μmole)		13	33	59	87	106	128	142	156
	FC sp act (cpm/μmole)		417	850	876	916	839	770	677	623
	EC production (μmoles/l/hr)	160	84	92	85	47	79	71	93	
12	EC concentration (μmoles/ml)				3.24	3.19	3.21	3.18	3.21	3.18
	EC sp act (cpm/μmole)				59	69	80	91	98	107
	FC sp act (cpm/μmole)				751	704	659	577	522	491
	EC production (μmoles/l/hr)					47	56	60	61	60

[a] FC.
[b] EC.
[c] Studies were performed after an overnight fast.
[d] Studies were performed during the last 8 hr of a 33-hr period, when all calories were given in three hourly meals of a fat-free, 80% carbohydrate formula (see text).

Statistical analyses were performed according to Snedecor (12).

RESULTS

Table 2 presents the concentrations of esterified cholesterol and the specific activities of free and esterified cholesterol in the plasma samples collected at 1-hr intervals for 8 hr after the intravenous injection of [^{3}H]mevalonic acid. Of the seven subjects, five were postabsorptive and two were consuming the fat-free, 80% carbohydrate formula. The in vivo production of plasma esterified cholesterol was calculated on the assumption that plasma free cholesterol was the sole immediate precursor (see Calculations section), and values are presented for each 1-hr period of study. The results in these seven subjects were representative of all the studies during both the postabsorptive state and the carbohydrate consumption.

The esterified cholesterol concentrations were relatively constant during each study, justifying the assumption of a metabolic steady state (see Appendix). The calculated value of esterified cholesterol production was higher during the first 2 hr than later, but after the second hour it remained relatively constant for the duration of the 8-hr studies in all subjects (Table 2). The values given for the in vivo production in Tables 3 and 4 are the means of the five values obtained between 3 and 8 hr.

Serum samples collected 1 hr after the [^{3}H]mevalonate injections in these seven subjects were incubated to measure the production of esterified cholesterol in vitro. The in vitro production of esterified cholesterol had been found to be linear with time for the first 2 hr, though not necessarily beyond 2 hr (Fig. 1). The values presented in Table 3 represent 1-hr incubations performed in duplicate or triplicate in each subject. The free cholesterol specific activity did not change during the 1 hr of incubation so that only a single value is given. The in vitro production of esterified cholesterol agreed closely with the in vivo value in all subjects (Tables 2 and 3).

The in vivo production of esterified cholesterol was also measured in 8 other subjects and the results in all 15

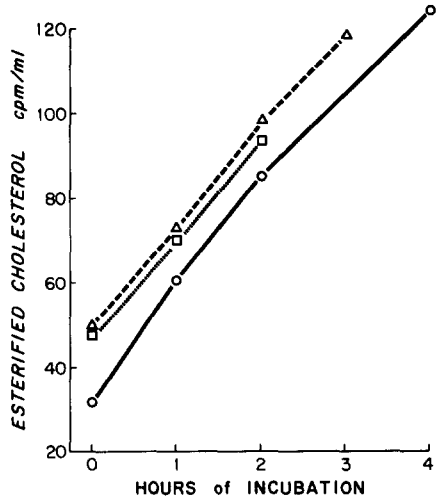

Fig. 1. Serum incubated in vitro. Serum samples collected 1 hr after an injection of [^{3}H]mevalonic acid in three subjects were incubated in vitro at 37°C. The increase in radioactivity in the esterified cholesterol (each point represents the mean of duplicate analyses) was linear with time for the first 2 hr in all three studies.

subjects are shown in Table 4. Studies were performed during the postabsorptive state in all subjects, and, in five of them, also during the sustained consumption of a carbohydrate-enriched formula.

In the postabsorptive studies the esterified cholesterol concentration ranged from 1.65 to 6.70 μmoles/ml, while the production was 52–94 μmoles/l/hr. There was, however, no apparent relationship between esterified cholesterol production and the concentration of either free (r = 0.15) or esterified (r = 0.09) cholesterol. Rather, there appeared to be an inverse relationship between the fractional removal rate of esterified cholesterol and its concentration (Table 4), and there was a highly significant positive correlation between the turnover time (x) and the concentration (y) (r = 0.96, $P < 0.001$, $y = 0.24 + 0.062x$) (Fig. 2). The correlation between turnover time (the reciprocal of the fractional removal rate) and concentration was calculated because the relationship between fractional removal rate and concentration was hyperbolic (Table 4).

Five of the subjects were also studied during the consumption of a carbohydrate-enriched diet, when the esterified cholesterol concentrations were significantly lower than during the postabsorptive studies ($P < 0.025$ by paired t test). Esterified cholesterol production, however, appeared to be unchanged. Rather, the fractional removal rate of esterified cholesterol was greater during the carbohydrate consumption in every subject ($P < 0.01$ by paired t test) (Table 4). The concentrations of cholesterol and triglyceride immediately before the carbohydrate ingestion

TABLE 3. Production of esterified cholesterol in serum incubated in vitro[a]

Subject	Free Cholesterol Specific Activity	Esterified Cholesterol 0 min	Esterified Cholesterol 60 min	Esterified Cholesterol Production In Vitro	Esterified Cholesterol Production In Vivo
	cpm/μmole	*cpm/ml*		*μmoles/l/hr*	
Postabsorptive state[b]					
3	593	50.1	78.9	49	
	595	47.6	74.6	45	52
6	409	48.4	69.5	52	
	437	48.0	73.8	59	
	429	47.3	67.3	47	57
8	469	31.7	60.8	62	
	450	33.0	63.1	67	
	451	31.3	61.5	67	62
10	765	95.6	145.3	65	
	728	94.3	148.0	74	74
15	420	55.5	81.3	61	
	424	55.2	81.5	62	67
Carbohydrate consumption[c]					
9	378	36.1	59.8	63	
	361	37.2	61.1	66	75
12	391	36.2	59.5	60	
	374	34.1	54.9	56	57

[a] The two or three values presented for each subject represent duplicate or triplicate incubations.
[b] Studies were performed after an overnight fast.
[c] Studies were performed on serum samples collected 26 hr after subjects began ingesting a fat-free, 80% carbohydrate formula (see text).

TABLE 4. In vivo production of plasma esterified cholesterol during the postabsorptive state and during carbohydrate consumption

Subject	Triglyceride	Plasma Concentrations Cholesterol Free	Plasma Concentrations Cholesterol Esterified	Esterified Cholesterol Production	Fractional Removal Rate
	μmoles/ml			*μmoles/l/hr*	*hr*$^{-1}$
Postabsorptive state[a]					
1	0.27	0.61	1.65	64	0.039
2	0.57	0.70	1.94	66	0.034
3	0.44	0.84	2.36	52	0.022
4	0.77	0.98	2.36	58	0.025
5	1.43	0.96	2.40	94	0.039
6	0.49	0.83	2.44	57	0.023
7	0.70	1.14	3.05	73	0.024
8	0.71	1.06	3.08	62	0.020
9	0.47	1.06	3.18	64	0.020
10	0.85	1.33	3.41	74	0.022
11	0.97	1.47	4.08	78	0.019
12	0.71	1.52	4.42	63	0.014
13	1.49	2.06	5.39	75	0.014
14	0.70	2.26	6.30	63	0.010
15	0.66	2.48	6.70	67	0.010
Carbohydrate consumption[b]					
7	0.52	1.03	2.79	75	0.027
9	1.06	0.96	2.57	75	0.029
11	1.23	1.11	3.00	78	0.026
12	0.74	1.14	3.20	57	0.018
14	1.14	1.63	4.55	64	0.014

[a] Studies were performed after an overnight fast.
[b] Studies were performed during the last 8 hr of a 33-hr (subjects 7, 9, 11, and 12) or a 57-hr (subject 14) period, when all calories were given in three hourly meals of a fat-free, 80% carbohydrate formula (see text).

(overnight fasted) and those after 28 hr (subjects 7, 9, 11, and 12) or 52 hr (subject 14) of the sustained consumption of the formula are presented in Table 5.

DISCUSSION

To calculate the rate of production of plasma esterified cholesterol in vivo, Nestel and Monger (8) measured the specific activities of free and esterified cholesterol for up to 3 days after the injection of labeled mevalonic acid, and the calculations were based on the time period between the maximum free cholesterol specific activity and the point at which the specific activity–time curves for free and esterified cholesterol crossed. Confidence in the technique was enhanced when it was shown that the sum of the transports in the individual lipoprotein classes closely approximated the total (8, 9). It was also shown that for as long as 40 hr after the mevalonic acid injections the specific activity of esterified cholesterol in the liver was still well below that in the plasma (5), suggesting that hepatic esterified cholesterol was not a major precursor of plasma esterified cholesterol. Other evidence on this point is the very low concentration of cholesteryl esters in the plasma of subjects with familial LCAT deficiency (6). Furthermore, studies in which the esterification of cholesterol in vivo and in vitro was measured have provided a similar range of values (4). This was confirmed in the present studies when simultaneous in vivo and in vitro measurements were made in the whole plasma (serum) of the same subjects (Table 3).

If the in vivo technique described by Nestel and Monger (8) is valid, the values measured for production should be independent of the time period chosen to make the calculations; the value should be the same whether calculated from specific activities during the first 8 hr after the mevalonic acid injections or during the much longer period between the peak free cholesterol specific activity and the point at which the specific activity–time curves cross. This fact was utilized in these studies to provide a necessary test of the validity of the technique. The first 8 hr after the injections of [^{3}H]mevalonic acid were studied because they spanned a period of large and rapid change in the specific activities of both free and esterified cholesterol (Table 2). Although the short time periods may have introduced an added source of error to the individual measurements, a number of consecutive values were used to calculate a mean.

The hour-to-hour values calculated for the production of plasma esterified cholesterol tended to decrease during the first 2 hr after the [^{3}H]mevalonic acid injections but were relatively constant after 3 hr in all subjects (Table 2). The higher early values may be explained either by the existence of a precursor possessing a specific activity higher than that measured in the plasma free cholesterol during the first 2 hr of the studies or by kinetic heterogeneity of the esterified cholesterol pool.

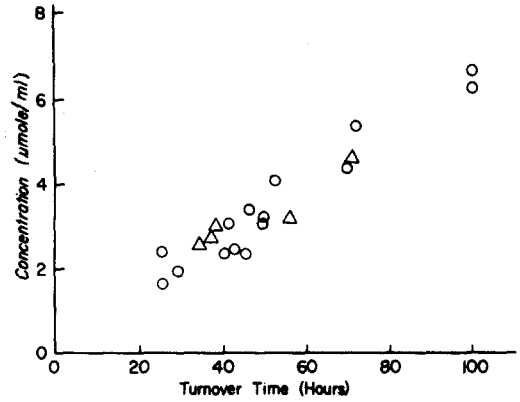

Fig. 2. Relationship between concentration and turnover time of plasma esterified cholesterol in the postabsorptive state (○) and during the sustained consumption of a carbohydrate formula (△).

Although the early specific activity of free cholesterol would have been higher in the liver than in the plasma, available evidence suggests that any hepatic contribution to the production of plasma esterified cholesterol in humans is probably minimal (4–6). A more likely precursor of high specific activity is a discrete pool(s) of free cholesterol within the plasma. Indeed, plasma free cholesterol is not homogeneous, and the exchange of free cholesterol among the different pools is not instantaneous (13). In baboons injected with labeled mevalonic acid, there is evidence of considerable heterogeneity of free cholesterol in the high density lipoproteins (13), which are the preferred substrate for the LCAT reaction (14, 15).

Plasma esterified cholesterol does not comprise a homogeneous pool (8, 9, 16). It is likely, therefore, that esteri-

TABLE 5. Plasma concentrations of triglyceride and cholesterol before and during sustained consumption of a carbohydrate-enriched formula

Subject		Triglyceride	Cholesterol	% Ester[a]
		μmoles/ml		
7	P[b]	0.70	4.19	73
	C[c]	0.52	3.79	73
9	P	0.47	3.96	75
	C	1.06	3.56	73
11	P	0.97	4.58	73
	C	1.23	4.08	72
12	P	0.71	4.75	74
	C	0.74	4.33	74
14	P	0.70	7.89	74
	C	1.14	6.20	74

[a] Percentage of cholesterol in esterified form.
[b] Plasma samples collected after an overnight fast immediately before beginning the carbohydrate formula.
[c] Plasma samples collected 28 hr (subjects 7, 9, 11, and 12) or 52 hr (subject 14) after beginning the carbohydrate formula (see text).

fied cholesterol may be removed from the plasma from one or more pools possessing specific activities different from that measured in the total pool. However, the specific activities of esterified cholesterol measured in the total pool 8 hr after the [^{3}H]mevalonic acid injections were still less than 25% of the prevailing free cholesterol specific activities in all studies. Referring to Eq. 2 in the Calculations section, it is apparent, therefore, that disparities between the specific activities of the removal pools and those actually measured would have to be quite large before they could have more than a minor effect on the calculated production. Nevertheless, the existence of a small, very rapidly turning over pool of esterified cholesterol in which the specific activity increased very much faster than that measured in the total plasma cannot be excluded. The early results are compatible with the existence of such a pool, but its contribution to overall production cannot be quantified.

Which of these suggestions may be the true explanation of the high early production values cannot be determined from these studies. Therefore, although the relative constancy of the hour-to-hour values after the first 2 hr adds somewhat to the confidence in the validity of this approach, the production values should be interpreted with the reservations that they may include some hepatic production and may exclude the contribution of any small, rapidly turning over plasma pools.

The similarity of the values for production measured in vitro and in vivo (Table 3) tends to support the validity of both measurements and does not lend support to a significant contribution of either the liver or a small, rapidly turning over plasma pool. These values for the in vitro production of esterified cholesterol are comparable to those reported elsewhere using heat-inactivated plasma and [^{14}C]cholesterol as substrate (17–19), although higher values have also been reported (20, 21). It should be emphasized, however, that although the LCAT reaction has a broad pH maximum in the range pH 7.0–8.0 (21), an in vitro reaction in unbuffered serum that had been separated from the red blood cells cannot be extrapolated with confidence to the in vivo situation.

The observed rates of production of plasma esterified cholesterol in vivo in these studies are very similar to those reported elsewhere (8, 9, 22) so long as subjects are equated with respect to body weight and triglyceride concentrations and corrections are made for the differing forms in which the results have been expressed. A previous report of a positive correlation between the production of plasma esterified cholesterol and the plasma cholesterol concentration (9) was based on studies in subjects possessing a wide range of body weights and triglyceride concentrations, both of which also correlate positively with the production of esterified cholesterol (8, 9). (A positive correlation between esterified cholesterol production and plasma triglyceride concentration has also been found using this present technique.)[1] In the much more homogeneous group in the present studies, there was no significant correlation between the production of esterified cholesterol and the concentrations of either free or esterified cholesterol.

In a metabolic steady state there must be a mechanism by which the production and removal of esterified cholesterol are balanced. If removal were governed by first-order kinetics, the concentration would stabilize at a level at which the removal sites would be presented with a mass such that removal would equal production. The similarity of the production in the subjects in the present study would then imply that the wide range of concentrations was a function of differences in the efficiencies of the removal mechanisms. However, the similarity of production indicates that the removal of esterified cholesterol (mass per unit time) was also similar in all subjects and perhaps suggests that removal may be governed by zero-order rather than first-order kinetics. In this case, removal would be fixed and production would have to be controlled by some form of negative feedback. If esterified cholesterol itself were an inhibitor of production, the concentration would stabilize at a level such that production would balance removal; the esterified cholesterol concentration would then be determined by the effectiveness of the negative feedback mechanism. In either case, whether removal was governed by zero-order or first-order kinetics, it is apparent that the concentration of esterified cholesterol in these subjects was not a simple consequence of differences in the rates of production.

The reduction in cholesterol concentration during the consumption of a fat-free, 80% carbohydrate diet has recently been well documented (23); the present studies stress the rapidity of this phenomenon. It is unlikely that the fall in concentration was a simple consequence of removing cholesterol from the diet, because two of the subjects had been eating very low cholesterol diets for 3 wk before beginning the carbohydrate formula, including subject 14, who had the greatest reduction in concentration (Table 5). The lower concentrations of esterified cholesterol during the consumption of carbohydrate could not be accounted for by a reduced production (Table 4), even though the falling concentrations may have resulted in an underestimation of production (see Calculations section and Appendix). This implies that either the removal mechanism had become more efficient, removing a larger fraction of the pool per unit time, or, if zero-order kinetics prevailed, the feedback inhibition of production had become more effective. However, because production (and therefore removal) may have been underestimated in the carbohydrate studies, it is possible that the primary event was an increase in the removal of esterified cholesterol, with concentration falling until production, less inhibited

[1] Barter, P. J. Unpublished observation.

by the lower concentration, increased to again balance removal.

Langer, Strober, and Levy (24) reported studies of the metabolism of low density lipoprotein (LDL) apoprotein and concluded that the metabolic defect in patients with hypercholesterolemia was a decreased fractional catabolism of LDL rather than an increase in its production. Relating subjects in the study of Langer et al. (24) to those in this present study who possessed comparable cholesterol concentrations, there is a striking similarity in the fractional removal rates of LDL apoprotein and plasma esterified cholesterol. Considering that most of the plasma esterified cholesterol is transported in the LDL, this similarity is perhaps not surprising. Using a two-pool model for cholesterol metabolism, Nestel, Whyte, and Goodman (25) found that the mass of cholesterol in the exchangeable pools was unrelated to cholesterol production; cholesterol clearance, however, expressed as a fraction of the plasma cholesterol pool, was decreased in patients with hypercholesterolemia.

In conclusion, these studies indicate that the production of plasma esterified cholesterol in lean, normotriglyceridemic subjects is not simply a function of the whole plasma concentration of the substrate free cholesterol. Neither can the differing concentrations of esterified cholesterol be explained on the basis of differences in its production.

APPENDIX

Error introduced by non-steady state conditions

The use of Eq. 2 (see Calculations section) to calculate the in vivo production of esterified cholesterol when steady state conditions do not prevail will be in error by the factor:

$$\frac{\int_{T_1}^{T_2} (P_{EC} - R_{EC}) \times EC \text{ sp act } dt}{\int_{T_1}^{T_2} (FC \text{ sp act} - EC \text{ sp act})\, dt} \quad \text{(Eq. 3)}$$

Expressed as a percentage of the production as calculated from Eq. 2 (see Calculations section), this error factor becomes

$$\frac{\int_{T_1}^{T_2} (P_{EC} - R_{EC}) \times EC \text{ sp act } dt}{EC \text{ cpm } (T_2) - EC \text{ cpm } (T_1)} \times 100 \quad \text{(Eq. 4)}$$

The value of the numerator in Eq. 4 for any given 1-hr period will be closely approximated by

$$\frac{P_{EC} - R_{EC}}{\text{mean } EC \text{ pool size}} \times \int_{T_{n-1}}^{T_n} EC \text{ cpm } dt \quad \text{(Eq. 5)}$$

The 1-hr increments in esterified cholesterol cpm (the denominator in Eq. 4), however, were always greater than 7% of the corresponding $\int_{T_{n-1}}^{T_n} EC$ cpm dt values, and in several cases, even during the eighth hour of the studies, the increments still exceeded 10% of the integral. The value of the denominator in Eq. 4 was therefore always greater than

$$0.07 \times \int_{T_{n-1}}^{T_n} EC \text{ cpm } dt$$

It is possible, therefore, to calculate the magnitude of the error factor for a given imbalance between production and removal. Consider the hypothetical situation in which pool size changed by 8% during the 8-hr studies (1%/hr). It is conceivable that a change of this magnitude may not have been detected by the techniques used. With a change in pool size of 1%/hr, the value of Eq. 5 (the numerator in Eq. 4) is

$$0.01 \times \int_{T_{n-1}}^{T_n} EC \text{ cpm } dt$$

The value of Eq. 4 under these circumstances would therefore be less than 15%. In other words (even during the eighth hour, when an error from this source would have been greatest), the maximum error introduced by ignoring non-steady state conditions sufficient to produce an 8% change in pool size during the 8-hr studies could have been no more than 15% of the calculated value.

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