# Origin of esterified cholesterol transported in the very low density lipoproteins of human plasma

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**Abstract** In two subjects the specific activity of esterified cholesterol in plasma lipoprotein subfractions was measured for up to 9 hr after an intravenous injection of [$^3$H]mevalonic acid. It was found to be consistently higher in larger ($S_f$ > 100) than in smaller ($S_f$ 20–100) very low density lipoproteins (VLDL). Four subjects were given an intravenous injection of heparin so that the VLDL could be studied as its concentration fell and subsequently rose again. During the first hour the relative reduction was greatest for triglyceride, intermediate for free cholesterol, and least for esterified cholesterol. Between 1 and 7 hr postheparin, the VLDL pool was restored, but the pattern of increase of individual lipids was not parallel. The triglyceride increment was much greater during the 1–4-hr period than during the 4–7-hr period; in three of the subjects the free cholesterol increment was also greater during the earlier period. The increase in esterified cholesterol, however, was consistently greater during the 4–7-hr period. In six other subjects the specific activity of VLDL esterified cholesterol was related to that of its possible plasma precursors in samples collected at 1-hr intervals for 8 hr after the injection of [$^3$H]mevalonic acid. Free cholesterol emerged as the most likely immediate precursor with the possibility of a hepatic as well as an intraplasma origin. The results did not support a major in vivo transfer of esterified cholesterol from high density lipoproteins to VLDL.

**Supplementary key words** free cholesterol · heparin · lipoprotein subfractions · [$^3$H]mevalonic acid

Plasma esterified cholesterol in man appears to be produced mainly within the plasma by the lecithin:cholesterol acyltransferase (LCAT) reaction (1–3). High density lipoproteins (HDL) are the preferred substrate for this reaction, but there is also some activity with the low density lipoproteins (LDL) (2, 4). There is no reaction, however, with isolated very low density lipoproteins (VLDL) (4). Although it has been shown in vitro that esterified cholesterol may be transferred from HDL to VLDL in exchange for triglyceride (5, 6), the importance of such a transfer in vivo is uncertain. In rats (7, 8) and in cholesterol-fed rabbits (9), a significant proportion of the VLDL esterified cholesterol is produced in the liver, but the contribution of hepatic production in man is again uncertain. The similarity of the fatty acid composition of cholesteryl esters in the different lipoprotein classes (10), quite different from that in the liver (1), has been cited as evidence for a common, extrahepatic origin of all plasma esterified cholesterol (2), although it should be noted that hepatic esterified cholesterol does not exist as a single homogeneous pool (1).

This present work examines aspects of the metabolism of VLDL esterified cholesterol in man. A simple precursor–product relationship between the pools of esterified cholesterol in HDL and VLDL was not observed. Rather, the results are compatible with formation of VLDL esterified cholesterol primarily by direct esterification of free cholesterol, perhaps in the liver as well as in the plasma.

## METHODS

The clinical details of the subjects are presented in Table 1.

### Experimental

All studies were begun in the morning after the subjects had fasted for 16 hr; this fast continued for the 7–9 hr duration of the studies. The markedly hypertriglyceridemic subjects (nos. 3, 4, and 5) had been given diets containing less than 10% of the calories as fat for 2 days prior to the study. Chylomicrons were not detectable, as assessed by cellulose acetate electrophoresis (11), at the time of study in any subject.

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Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT; lecithin:cholesterol acyltransferase; sp act, specific activity; TLC, thin-layer chromatography; FTR, fractional turnover rate.

TABLE 1. Clinical details of subjects

| Subject | Age | Sex | Wt | Ht | Lipoprotein Pattern[a] | Clinical Status |
|---|---|---|---|---|---|---|
| | *yr* | | *kg* | *cm* | | |
| 1 | 29 | M | 73 | 179 | Normal | Asymptomatic |
| 2 | 23 | M | 43 | 150 | Type IV | Nephrotic syndrome |
| 3 | 49 | M | 76 | 184 | Type IV | Ischemic heart disease |
| 4 | 64 | F | 65 | 163 | Type IV | Ischemic heart disease |
| 5 | 59 | F | 83 | 159 | Type IV | Cerebrovascular disease |
| 6 | 34 | M | 68 | 170 | Normal | Asymptomatic |
| 7 | 26 | M | 84 | 182 | Normal | Asymptomatic |
| 8 | 31 | M | 69 | 181 | Normal | Asymptomatic |
| 9 | 34 | M | 76 | 179 | Normal | Asymptomatic |
| 10 | 25 | M | 84 | 178 | Normal | Asymptomatic |
| 11 | 25 | M | 89 | 185 | Normal | Asymptomatic |
| 12 | 21 | M | 72 | 184 | Normal | Asymptomatic |
| 13 | 26 | M | 78 | 181 | Type IV | Weight gain of 8 kg in past 6 mo |
| 14 | 21 | M | 93 | 178 | Type IV | Obese |
| 15 | 43 | M | 82 | 183 | Type IV | Asymptomatic |

[a] See Ref. 31.

Blood samples were collected into tubes containing dipotassium EDTA (1 mg/ml) as anticoagulant and *p*-chloromercuriphenyl sulfonate (to give a concentration of 0.002 M) as an inhibitor of LCAT activity (3). In the heparin studies (see [*d*] below), paraoxon (0.5 mg/ml) was added to inhibit in vitro lipolysis (12).

Four separate studies were performed:[1]

(*a*) Fasting blood samples were collected from subjects 1–5. Two subclasses of VLDL ($S_f$ > 100 and $S_f$ 20–100) were isolated from 12-ml aliquots of plasma (four separate aliquots of 3 ml) as previously described (13). In subjects 1 and 2, sequential centrifugation at adjusted densities of 1.019 and 1.063 isolated the $S_f$ 12–20 ($1.006 < d < 1.019$) and the $S_f$ 0–12 ($1.019 < d < 1.063$) lipoproteins. The high density lipoproteins were not further separated from the final infranate ($d > 1.063$). All lipoprotein fractions and the $d > 1.063$ infranate (containing the HDL) were extracted in an isopropyl alcohol–heptane–1 N sulfuric acid (40:10:1) solution as described by Dole (14). In subjects 3–5, the low density lipoproteins were precipitated from the $d > 1.006$ infranate using heparin and manganese chloride (15). The supernate (containing the HDL) and the precipitated LDL, after resuspension in saline, were extracted with Dole's solution. No attempt was made to further purify these lipoprotein classes by "washing," and some contamination is therefore possible. Each ultracentrifugal separation lasted 20 hr and was performed at 4°C. The LDL precipitation, and the subsequent centrifugation before the fractions were extracted, took 30–60 min at room temperature.

Aliquots of the lipids contained in the heptane phase of the extracts were taken for the measurement of triglyceride and cholesterol mass in a Technicon AutoAnalyzer after the removal of phospholipids with zeolite. Recovery of lipids in the lipoprotein fractions was 85% and 89% in subjects 1 and 2 and 90–95% in subjects 3–5. A further aliquot of the lipids was separated by TLC on plates developed in hexane–diethyl ether–methanol–acetic acid 90:20:3:2 in order to isolate the free and esterified cholesterol, which were also measured in the AutoAnalyzer.[2] All estimations were performed in duplicate on aliquots sufficient to produce readings in the midrange of the AutoAnalyzer where the coefficient of variation was less than 1.5%. The recovery of radioactivity in both free and esterified cholesterol after TLC was found to be over 90%, and the recovery of total cholesterol mass after TLC in these studies was 90–96%. All values were subsequently corrected for recovery.

(*b*) Subjects 1 and 2 were given an injection of [$^3$H]mevalonic acid. DL-[2-$^3$H]Mevalonic acid lactone (Radiochemical Centre, Amersham, England) was incubated with sterile sodium bicarbonate at pH 8.7 at 37°C for 60 min. 200 $\mu$Ci was injected at 8 a.m., and blood samples were collected after 3, 5, 7, and 9 hr. The plasma was separated into $S_f$ > 100, $S_f$ 20–100, $S_f$ 12–20, $S_f$ 0–12, and HDL as described above. In addition to the mass assays, the radioactivity in free and esterified cholesterol was measured in a liquid scintillation system using 0.3% 2,5-diphenyloxazole (PPO) in toluene as scintillator. Each sample was counted twice for as long as was necessary to achieve a standard deviation of less than 1%; quenching was minimal.

(*c*) Subjects 6–9 were given an intravenous injection of 5000 units of heparin at 8 a.m. after an overnight fast, which continued for the duration of the study. Blood samples were collected immediately before and 1, 4, and 7 hr after the administration of heparin. The VLDL was sepa-

[1] Studies (*a*) and (*b*) were performed in the Department of Clinical Science in Canberra, Australia, and studies (*c*) and (*d*) were performed in the Clinical Research Center in Iowa City, Iowa.

[2] Esterified cholesterol measured against a free cholesterol standard in the Technicon AutoAnalyzer model 11, as used in these studies, read 8–10% higher than it did if it was hydrolyzed first. All values have been corrected for this discrepancy.

TABLE 2. Triglyceride and cholesterol concentrations and cholesterol composition in lipoproteins of different sizes

| Subject | | Lipoprotein Fraction | | | | |
|---|---|---|---|---|---|---|
| | | $S_f > 100$ | $S_f$ 20–100 | $S_f$ 12–20 | $S_f$ 0–12 | HDL |
| | | *μmoles/l of plasma* | | | | |
| 1 | Triglyceride | 550 | 520 | 130 | 160 | 110 |
| | Cholesterol | 210 | 340 | 280 | 2170 | 880 |
| | EC/FC[a] | 1.3 | 1.6 | 1.9 | 2.9 | 4.2 |
| 2 | Triglyceride | 2150 | 1430 | 170 | 290 | 90 |
| | Cholesterol | 620 | 850 | 390 | 2710 | 800 |
| | EC/FC | 1.1 | 1.4 | 2.0 | 3.4 | 4.3 |
| 3 | Triglyceride | 5070 | 3390 | 770[b] | | 70 |
| | Cholesterol | 1940 | 1890 | 1450 | | 540 |
| | EC/FC | 1.2 | 1.4 | 2.9 | | 4.3 |
| 4 | Triglyceride | 7110 | 1300 | 310 | | 150 |
| | Cholesterol | 1730 | 520 | 1550 | | 410 |
| | EC/FC | 0.8 | 1.1 | 3.3 | | 4.2 |
| 5 | Triglyceride | 6180 | 1070 | 540 | | 110 |
| | Cholesterol | 2120 | 880 | 3180 | | 540 |
| | EC/FC | 1.2 | 1.6 | 2.9 | | 4.2 |

[a] EC/FC denotes the molar ratio of esterified to free cholesterol.
[b] The $S_f$ 0–20 were not further separated in subjects 3–5.

rated from 12-ml aliquots of plasma by ultracentrifugation at density 1.006. VLDL concentrations of triglyceride and total, free, and esterified cholesterol masses were measured as described above.

(*d*) Subjects 10–15 were given an intravenous injection of 120–150 μCi of [$^3$H]mevalonic acid (as described in [*b*]), and blood samples were collected at 1-hr intervals for 8 hr. The VLDL, LDL, and HDL were separated as described for subjects 3–5 (see [*a*] above), and the mass and radioactivity in the free and esterified cholesterol in each fraction were determined.

## RESULTS

The data in Table 2 confirm the previous observation (16) that the relative proportion of cholesterol in the esterified form increases progressively with increasing lipoprotein density. Within subfractions of VLDL, the percentage of cholesterol as ester and the ratio of total cholesterol to triglyceride were both lower in the larger than in the smaller VLDL.

After an intravenous injection of [$^3$H]mevalonic acid, label rapidly appeared in plasma free cholesterol and more slowly in esterified cholesterol. The specific activity of free cholesterol was identical in all lipoprotein classes, but the esterified cholesterol specific activity was consistently highest in the HDL, followed in order by the $S_f >$ 100 lipoproteins, the $S_f$ 20–100, and the $S_f$ 12–20, and was lowest in the $S_f$ 0–12 lipoproteins (Fig. 1).

Subjects 6–9 were given an intravenous injection of heparin to produce a rapid removal of VLDL triglyceride (Table 3), and the composition of VLDL was analyzed after 1, 4, and 7 hr.

The percentage reduction in VLDL lipids during the first hour after heparin was greatest for triglyceride, intermediate for free cholesterol, and least for esterified cholesterol, leaving VLDL with increased ratios of total cholesterol to triglyceride and esterified to free cholesterol (Table 3). The VLDL increment during the period between 1 and 4 hr postheparin (period 1) was characterized by a low total cholesterol to triglyceride ratio relative to the preheparin VLDL (Table 4). The esterified to free cholesterol ratio of these accumulating VLDL, however, was only slightly changed from the preheparin value, being slightly reduced in three of the four subjects and increased in the fourth (Table 4). During the period 4–7 hr postheparin (period 2), the ratios of total cholesterol to triglyceride and of esterified to free cholesterol in the VLDL increment were consistently higher than in either the preheparin VLDL or the period 1 increments (Table 4).

In absolute terms, the VLDL triglyceride increment was greater during period 1 than during period 2, as was the free cholesterol increment in three of the four subjects. The esterified cholesterol increment, however, was uniformly greater during period 2.

Subjects 10–15 were given an intravenous injection of [$^3$H]mevalonic acid to gain insight into the origin of VLDL esterified cholesterol. Assuming (*a*) a single precursor, (*b*) a homogeneous VLDL esterified cholesterol pool, and (*c*) a metabolic steady state, the theoretical relationship between the specific activity of VLDL esterified cholesterol and that of its immediate precursor is defined by the equation:

$$k = \frac{\text{Increment in VLDL esterified cholesterol sp act } (T_2 - T_1)}{\int_{T_1}^{T_2} (\text{precursor sp act}) - (\text{VLDL esterified cholesterol sp act})\,dt}$$

(17) where $k$, a constant, is the fractional turnover rate[3] of VLDL esterified cholesterol. Each study has been di-

[3] Fractional turnover rate is the fraction of the pool turning over per unit time.

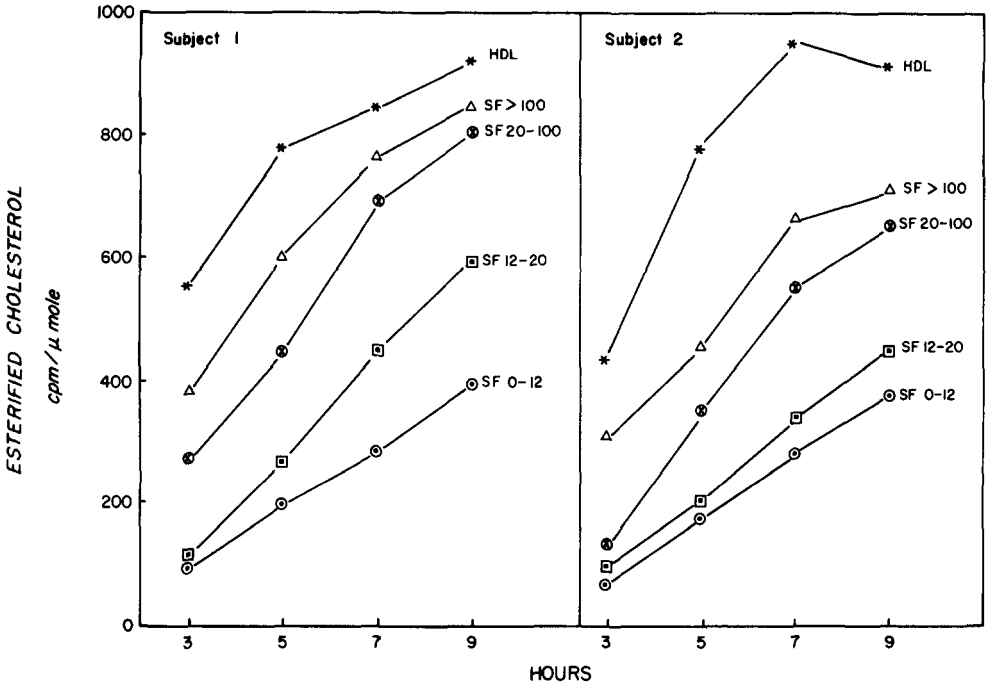


**Fig. 1.** The specific activity of esterified cholesterol in lipoprotein subfractions after an intravenous injection of [$^3$H]mevalonic acid in a normal subject, *1*, and a nephrotic subject with endogenous hypertriglyceridemia, *2*.

vided into eight 1-hr segments, and the value of $k$ was calculated for each hour on the basis of either plasma free cholesterol or HDL esterified cholesterol as the precursor (Table 5). As the true value of $k$ is independent of $T_1$ and $T_2$, it is possible to assess how well a suspected precursor conforms to the theoretical situation. The assumptions inherent in this approach and its limitations are outlined in the discussion section.

Table 5 presents the concentration of VLDL esterified cholesterol and the specific activities of VLDL esterified cholesterol, HDL esterified cholesterol, and plasma free cholesterol (the mean of virtually identical values in the three lipoprotein classes) for each hour of the studies. Also presented are the values of "$k$" calculated on the assumption of either HDL esterified cholesterol or plasma free cholesterol as the sole precursor. The integral in the denominator of the ratio was taken as the area between the rectilinear plots of the specific activity time curves and measured by counting squares.

The assumption that a transfer from HDL provided the only source of VLDL esterified cholesterol (by inserting HDL esterified cholesterol into the equation in place of the precursor) was tested only in subjects 10 and 13–15 and resulted in a value of "$k$" that tended to decrease with time. In subjects 11 and 12, such a calculation was not possible because the specific activity of esterified cholesterol in VLDL was consistently higher than that in HDL (Table 5).

When plasma free cholesterol was assumed to be the sole precursor, the calculated value of "$k$" initially declined with time, but was relatively constant during the last 4 hr in all subjects. In subjects 10–12, with normal VLDL concentrations, the value decreased for 3–4 hr, but in subjects 13–15, who had elevated VLDL concentrations, the value varied little after the second hour.

In all subjects the specific activity of esterified cholesterol in LDL was consistently lower than that in VLDL, as previously noted (18) (Fig. 1).

## DISCUSSION

Plasma esterified cholesterol is not a single homogeneous pool. After the intravenous injection of labeled mevalonic acid, the appearance of radioactivity in the esterified cholesterol of plasma lipoprotein fractions is heterogeneous with the specific activity greatest in HDL, intermediate in VLDL, and lowest in LDL (18, 19). In this present study, the observation is made that when the VLDL concentration is very low, the specific activity in VLDL may be higher than that in HDL (Table 5).

The earlier labeling of esterified cholesterol in larger than in smaller VLDL (Fig. 1) is similar to the pattern of labeling in VLDL triglyceride after [$^{14}$C]palmitic acid injection (13) and is consistent with the observation that larger VLDL are degraded to smaller particles as triglyceride is cleared (13). However, the higher esterified to free cholesterol ratio in smaller than in larger VLDL (16) (Table 2) indicates that the stepwise clearance of triglyceride from VLDL (13) is accompanied by either selective

TABLE 3. VLDL lipid values before and after injection of heparin

| Subject | | Concentrations | | | Ratios | |
|---|---|---|---|---|---|---|
| | | Triglyceride | Cholesterol | | Total Cholesterol/Triglyceride | Esterified Cholesterol/Free Cholesterol |
| | | | Free | Esterified | | |
| | | *μmoles/l of plasma* | | | | |
| 6 | Preheparin | 740 | 137 | 147 | 0.38 | 1.1 |
| | 1 hr | 145 | 49 | 62 | 0.79 | 1.3 |
| | 4 hr | 710 | 114 | 124 | 0.34 | 1.1 |
| | 7 hr | 970 | 163 | 207 | 0.38 | 1.3 |
| 7 | Preheparin | 760 | 158 | 194 | 0.47 | 1.2 |
| | 1 hr | 135 | 65 | 90 | 1.19 | 1.4 |
| | 4 hr | 650 | 116 | 142 | 0.40 | 1.2 |
| | 7 hr | 780 | 152 | 212 | 0.47 | 1.4 |
| 8 | Preheparin | 390 | 72 | 101 | 0.45 | 1.4 |
| | 1 hr | 53 | 18 | 31 | 0.98 | 1.7 |
| | 4 hr | 390 | 65 | 103 | 0.42 | 1.6 |
| | 7 hr | 510 | 98 | 191 | 0.57 | 1.9 |
| 9 | Preheparin | 1510 | 235 | 258 | 0.33 | 1.1 |
| | 1 hr | 830 | 183 | 207 | 0.47 | 1.1 |
| | 4 hr | 1510 | 217 | 240 | 0.30 | 1.1 |
| | 7 hr | 1820 | 266 | 320 | 0.32 | 1.2 |

TABLE 4. Increments in VLDL lipids after heparin injection

| Subject | Hours Postheparin | Increments | | | Ratios | |
|---|---|---|---|---|---|---|
| | | Triglyceride | Cholesterol | | Total Cholesterol/Triglyceride | Esterified Cholesterol/Free Cholesterol |
| | | | Free | Esterified | | |
| | | *μmoles/l of plasma* | | | | |
| 6 | 1–4 | 565 | 65 | 62 | 0.22 | 1.0 |
| | 4–7 | 260 | 49 | 83 | 0.51 | 1.7 |
| | Preheparin | | | | 0.38 | 1.1 |
| 7 | 1–4 | 515 | 51 | 52 | 0.20 | 1.0 |
| | 4–7 | 130 | 36 | 70 | 0.81 | 1.9 |
| | Preheparin | | | | 0.47 | 1.2 |
| 8 | 1–4 | 337 | 47 | 72 | 0.35 | 1.5 |
| | 4–7 | 120 | 33 | 88 | 1.01 | 2.7 |
| | Preheparin | | | | 0.45 | 1.4 |
| 9 | 1–4 | 680 | 34 | 33 | 0.10 | 1.0 |
| | 4–7 | 310 | 49 | 80 | 0.42 | 1.6 |
| | Preheparin | | | | 0.33 | 1.1 |

removal of free cholesterol or the further addition of esterified cholesterol. On theoretical grounds, at least, it has been suggested that both a removal of free cholesterol and an incorporation of esterified cholesterol into VLDL may be linked to the clearance of triglyceride (20). It was logical, therefore, to study the effects of an enhanced triglyceride clearance on the composition of the VLDL.

The intravenous injection of heparin releases lipoprotein lipase activity from its usual tissue sites into the plasma, producing a dramatic intravascular lipolysis (21). This intravascular lipolysis is not the normal physiological mechanism for triglyceride clearance, and changes in VLDL composition resulting from it must be interpreted with reservation. The greater relative reduction in free than esterified cholesterol during the first hour, while compatible with an influence of triglyceride clearance on VLDL cholesterol metabolism, was also consistent with the previous observation of a much greater reduction of $S_f > 100$ than $S_f$ 20–100 VLDL after heparin (22) and may simply reflect preferential metabolism of larger VLDL by postheparin plasma.

Within 7 hr of the heparin injection, the depleted VLDL pool had been restored, with some overshoot in three of the four subjects. The increasing concentration of VLDL lipids between 1 and 7 hr postheparin indicates an imbalance between net inflow and net outflow transport, probably the consequence of a declining lipolytic activity (although this was not measured in vitro) in the presence of a markedly reduced pool size; an increased hepatic triglyceride production from recycled free fatty acids (23) may have further exaggerated the imbalance.

The pattern of increase of the different lipids, however, was not parallel. The greatest increment in triglyceride took place during the 1–4-hr postheparin period, while the increase in cholesterol was more linear. The VLDL increment between 1 and 4 hr postheparin was characterized by a low cholesterol:triglyceride ratio (Table 4). Although this was consistent with a failure of the stepwise clearance of triglyceride to keep pace with the inflow, the ratio of esterified to free cholesterol in the accumulating VLDL was only marginally lower than in the preheparin VLDL in three subjects and was higher in the fourth. During the 4–7-hr period, however, when the smaller triglyceride increment and the higher ratio of cholesterol to triglyceride suggested a relative increase in the stepwise clearance of triglyceride, the ratio of esterified to free cholesterol was increased and the absolute esterified cholesterol increment was greater (Table 4). The interpretation of these observations is limited by the inability to separate the relative influences of inflow and outflow on the changing concentrations.

Evidence currently available in man suggests that cholesterol enters the plasma in the free form and that esterified cholesterol is produced within the plasma by the action of LCAT (1–3). Although isolated VLDL is a poor substrate for this reaction (4), it has been demonstrated in vitro that esterified cholesterol may be transferred to VLDL from HDL (5, 6), the preferred substrate for the LCAT reaction (2, 4). The second group of mevalonic acid studies was performed to assess the in vivo importance of such a transfer and to gain further insight into the origin of the esterified cholesterol transported in the VLDL.

The pattern of labeling of esterified cholesterol in HDL and VLDL did not conform to a simple precursor–product relationship (Table 5). In fact, in the two subjects with the lowest concentrations of VLDL, the specific activity of esterified cholesterol was consistently higher in VLDL than in HDL. However, in baboons injected with [$^{14}$C]mevalonate, the labeling of HDL subfractions is heterogeneous (24), and the possibility of transfer from a subfraction of HDL cannot be excluded in these studies.

When the fractional turnover rate of VLDL esterified cholesterol was calculated on the assumption of plasma

TABLE 5. Specific activities of plasma free cholesterol[a] and VLDL and HDL esterified cholesterol[b] after injection of [$^3$H]mevalonic acid

| Subject | | Time (hours after [$^3$H]mevalonate injection) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 10 | VLDL EC concentration ($\mu$moles/l) | | 351 | 369 | 354 | 378 | 393 | 381 | 366 | 372 |
| | VLDL EC sp act (cpm/$\mu$mole) | | 44 | 113 | 181 | 215 | 267 | 297 | 320 | 338 |
| | HDL EC sp act (cpm/$\mu$mole) | | 50 | 158 | 262 | 337 | 386 | 420 | 441 | 458 |
| | Plasma FC sp act (cpm/$\mu$mole) | | 602 | 1076 | 1167 | 1113 | 1026 | 987 | 855 | 821 |
| | FTR VLDL EC (if HDL EC precursor)[c] | 14.7 | 2.7 | 1.1 | 0.34 | 0.43 | 0.25 | 0.19 | 0.15 | |
| | FTR VLDL EC (if plasma FC precursor)[c] | 0.157 | 0.090 | 0.070 | 0.037 | 0.063 | 0.041 | 0.038 | 0.034 | |
| 11 | VLDL EC concentration ($\mu$moles/l) | | 116 | 103 | 100 | 110 | 123 | 135 | 116 | 123 |
| | VLDL EC sp act (cpm/$\mu$mole) | | 60 | 132 | 183 | 229 | 257 | 273 | 301 | 319 |
| | HDL EC sp act (cpm/$\mu$mole) | | 20 | 64 | 115 | 151 | 193 | 224 | 244 | 264 |
| | Plasma FC sp act (cpm/$\mu$mole) | | 439 | 809 | 884 | 1006 | 1033 | 973 | 879 | 827 |
| | FTR VLDL EC (if HDL EC precursor)[d] | | | | | | | | | |
| | FTR VLDL EC (if plasma FC precursor) | 0.327 | 0.130 | 0.074 | 0.062 | 0.036 | 0.022 | 0.044 | 0.034 | |
| 12 | VLDL EC concentration ($\mu$moles/l) | | 85 | 108 | 69 | 82 | 92 | 92 | 95 | 82 |
| | VLDL EC sp act (cpm/$\mu$mole) | | 78 | 209 | 349 | 488 | 529 | 541 | 575 | 602 |
| | HDL EC sp act (cpm/$\mu$mole) | | 37 | 128 | 208 | 265 | 346 | 384 | 426 | 463 |
| | Plasma FC sp act (cpm/$\mu$mole) | | 845 | 1501 | 1747 | 1780 | 1560 | 1482 | 1354 | 1265 |
| | FTR VLDL EC (if HDL EC precursor)[d] | | | | | | | | | |
| | FTR VLDL EC (if | 0.204 | 0.127 | 0.104 | 0.103 | 0.035 | 0.012 | 0.040 | 0.036 | |

[a] FC
[b] EC
[c] The fractional turnover rate (FTR) of VLDL esterified cholesterol has been calculated on the assumption that first HDL esterified cholesterol and then plasma free cholesterol is the sole immediate precursor of VLDL esterified cholesterol (see text). FTR is expressed in hours$^{-1}$.
[d] Calculation not possible because specific activity of esterified cholesterol was higher in VLDL than in HDL.

free cholesterol as the sole, immediate precursor, the value initially declined with time but was relatively constant during the last 4 hr of the 8-hr studies in all subjects (Table 5); in the three subjects with elevated VLDL concentrations, the value varied little after the second hour. However, before concluding the existence of a precursor–product relationship between free cholesterol and VLDL esterified cholesterol, the findings during the first 2–4 hr must be explained.

The calculation of the fractional turnover rate depends upon three assumptions: (*a*) metabolic steady state conditions exist, (*b*) the VLDL esterified cholesterol is a homogeneous pool, and (*c*) there is a single immediate precursor. The assumption of steady state conditions was justified by the apparent stability of the VLDL esterified cholesterol concentration, but the other assumptions demand further discussion.

The heterogeneous labeling of VLDL subclasses (Fig. 1) indicates that the VLDL esterified cholesterol is not a homogeneous pool. However, during the first 4 hr of these studies the specific activity of VLDL esterified cholesterol was very low relative to that of the free cholesterol, indicating that if indeed free cholesterol was the sole precursor, the amount of label entering the VLDL esterified cholesterol pool greatly exceeded the amount leaving it. Even if there had been no label leaving the VLDL esterified cholesterol pool during this early period (i.e., if the fraction being removed had a specific activity of zero), the value of the fractional turnover rate during the first 4 hr could have been overestimated by a maximum of 20% in any study, much too small an error to account for the findings.

The existence of multiple precursors is possible. The demonstration in vitro of a transfer of esterified cholesterol

TABLE 5. *Continued*

| Subject | | Time (hours after [$^3$H]mevalonate injection) 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|
| 13 | VLDL EC concentration ($\mu$moles/l) | | 1092 | 1092 | 1092 | 1004 | 1092 | 1092 | 1122 | 1092 |
| | VLDL EC sp act (cpm/$\mu$mole) | | 28 | 70 | 112 | 143 | 177 | 211 | 235 | 259 |
| | HDL EC sp act (cpm/$\mu$mole) | | 54 | 194 | 294 | 378 | 401 | 436 | 457 | 480 |
| | Plasma FC sp act (cpm/$\mu$mole) | | 594 | 1122 | 1259 | 1251 | 1202 | 1090 | 1000 | 923 |
| | FTR VLDL EC (if HDL EC precursor) | 2.0 | 0.56 | 0.27 | 0.15 | 0.15 | 0.15 | 0.11 | 0.11 | |
| | FTR VLDL EC (if plasma FC precursor) | 0.097 | 0.052 | 0.038 | 0.028 | 0.032 | 0.035 | 0.028 | 0.034 | |
| 14 | VLDL EC concentration ($\mu$moles/l) | | 708 | 721 | 744 | 563 | 708 | 694 | 607 | 721 |
| | VLDL EC sp act (cpm/$\mu$mole) | | 44 | 81 | 113 | 147 | 171 | 192 | 216 | 231 |
| | HDL EC sp act (cpm/$\mu$mole) | | 46 | 125 | 210 | 264 | 270 | 295 | 309 | 332 |
| | Plasma FC sp act (cpm/$\mu$mole) | | 386 | 772 | 906 | 902 | 802 | 767 | 719 | 638 |
| | FTR VLDL EC (if HDL EC precursor) | 38.0 | 1.51 | 0.46 | 0.32 | 0.24 | 0.21 | 0.24 | 0.17 | |
| | FTR VLDL EC (if plasma FC precursor) | 0.258 | 0.070 | 0.044 | 0.044 | 0.031 | 0.035 | 0.043 | 0.034 | |
| 15 | VLDL EC concentration ($\mu$moles/l) | | 1602 | 1705 | 1627 | 1627 | 1602 | 1602 | 1627 | 1627 |
| | VLDL EC sp act (cpm/$\mu$mole) | | 8 | 14 | 21 | 31 | 42 | 46 | 53 | 62 |
| | HDL EC sp act (cpm/$\mu$mole) | | 30 | 74 | 96 | 113 | 128 | 138 | 145 | 151 |
| | Plasma FC sp act (cpm/$\mu$mole) | | 305 | 526 | 596 | 602 | 571 | 557 | 523 | 495 |
| | FTR VLDL EC (if HDL EC precursor) | 0.69 | 0.15 | 0.10 | 0.13 | 0.13 | 0.04 | 0.08 | 0.09 | |
| | FTR VLDL EC (if plasma FC precursor) | 0.052 | 0.015 | 0.013 | 0.018 | 0.020 | 0.008 | 0.016 | 0.019 | |

from other lipoprotein classes to VLDL has already been mentioned, but this would imply the transfer from a pool of esterified cholesterol in which the specific activity relative to that of free cholesterol was lower during the earlier than during the later periods. Therefore, if such a transfer was significant, the assumption of free cholesterol as the sole precursor would result in a value of the fractional turnover rate that increased rather than decreased with time. The higher values during the early periods, however, could be explained by the existence of a precursor possessing a higher early specific activity than that measured in plasma free cholesterol.

In baboons the specific activity of free cholesterol is higher in HDL than in LDL for the first 2 hr after [$^{14}$C]mevalonic acid injection (24). In those studies the HDL were isolated very rapidly, while in these present studies the separation was much slower and an exchange of free cholesterol between the lipoprotein classes in vitro may have obscured such differences. It is therefore possible that VLDL esterified cholesterol may have been derived from a discrete pool of plasma free cholesterol which possessed an early specific activity 3–10 times higher than the measured value. Another and, perhaps, a more likely high specific activity precursor is hepatic free cholesterol. It is known that plasma and hepatic free cholesterol pools equilibrate fairly rapidly (1, 25), and it is possible that 3–4 hr after the [$^3$H]mevalonic acid injections the specific activities in the plasma and liver may have been similar. The possibility of an hepatic origin of VLDL esterified cholesterol is therefore raised.

The evidence suggesting plasma esterification as the major source of plasma esterified cholesterol in man has been drawn from the similarity of the esterification measured in vitro and in vivo (2). Furthermore, the order of labeling of esterified cholesterol in different lipoprotein classes in vitro has closely resembled that found in vivo (2, 18). In the recently described condition of familial LCAT deficiency, the absence of plasma cholesterol esterifying activity was associated with very low plasma esterified cholesterol concentrations (3). The cholesteryl esters found in the VLDL of these patients were assumed, on the basis of fatty acid composition, to be of intestinal origin (26).

The magnitude of any hepatic contribution is therefore likely to be small relative to the intraplasma production.

In rats (7, 8) and in cholesterol-fed rabbits (9), there is good evidence that a significant proportion of VLDL esterified cholesterol is formed in the liver. Unlike these animals, however, human liver does not appear to possess acyl CoA:cholesterol acyltransferase activity (27). In rats the composition of cholesteryl esters in VLDL resembles that in the liver and may differ markedly from the other lipoprotein classes (7). In postabsorptive humans the cholesteryl ester composition is similar in all lipoprotein classes (10) and is quite different from the liver, where there is a much higher proportion of saturated and a lower proportion of polyunsaturated fatty acids (1). Although the slightly lower linoleate and the slightly higher oleate in VLDL than in other plasma lipoproteins (10) is consistent with some hepatic origin of VLDL esterified cholesterol, it appears unlikely that the liver is the only source.

The results in this study are compatible with a combined hepatic and plasma origin of VLDL esterified cholesterol. A greater relative plasma contribution in the subjects with elevated concentrations of VLDL may explain the better precursor–product relationship between the plasma free cholesterol and VLDL esterified cholesterol in these subjects (Table 5). The similarity of the fractional turnover rates of VLDL esterified cholesterol in all subjects (approximately 0.03 $hr^{-1}$) suggests that the production was greater in subjects with higher concentrations. However, there is little evidence that the hepatic production of triglyceride is significantly increased in subjects with hypertriglyceridemia (28, 29), suggesting that the increased production of VLDL esterified cholesterol in such subjects may reflect a greater intraplasma esterification.

The in vivo findings in this present study are not necessarily incompatible with the in vitro studies. The transfer of *some* esterified cholesterol from HDL to VLDL is not excluded; it is only shown that a simple precursor–product relationship does not exist. The observation in vitro that isolated VLDL is a poor substrate for the LCAT reaction (4) may indicate only that other cofactors, such as one of the HDL proteins (30), are necessary. Whatever the mechanism, these in vivo results do suggest a direct esterification of free cholesterol, perhaps in the liver as well as in the plasma, as the major source of the esterified cholesterol in VLDL.

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