Regulation of Human Plasma Lecithin:Cholesterol Acyltransferase Activity by Lipoprotein Acceptor Cholesteryl Ester Content*

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Very low density lipoproteins and low density lipoproteins attain maximal cholesteryl ester contents during the incubation of human plasma and, under these conditions, both lecithin:cholesterol acyltransferase and transfer proteins are inhibited. These lipoproteins provide the major part of free cholesterol for the lecithin:cholesterol acyltransferase reaction, and are the major acceptors of cholesteryl ester generated by lecithin:cholesterol acyltransferase and transported to the lipoprotein accepters by the transfer protein. The results obtained indicate that the concentration of acceptor limits esterification and transfer in plasma, and that in vivo these acceptors contain close to their maximal cholesteryl ester content. Human plasma end product acceptor lipoproteins have a composition similar to that of the ester-rich low density lipoprotein characteristic of primate models of experimental atherosclerosis.

In single-walled vesicles of lecithin and cholesterol, the activity of isolated lecithin:cholesterol acyltransferase is limited by the accumulation of cholesteryl ester in the lipid bilayer (1). Inhibition is relieved by addition of nonsubstrate acceptor liposomes which, in the presence of cholesteryl ester transfer protein (2), can store the transported cholesteryl ester (1). Beyond this point both LCAT and transfer reactions are inhibited in the medium. Two reports (3, 4) have indicated structural association of LCAT and transfer protein in human plasma. The major part of cholesteryl ester is located in the low density lipoprotein class which is not a direct substrate for LCAT, but which accepts preformed esters via transfer protein activity. In the present paper we have investigated whether in plasma LCAT-mediated synthesis of cholesteryl esters is similarly limited by the ability of acceptor lipoproteins to bind and store cholesteryl esters and, if so, what is the maximal cholesteryl ester content of these end product lipoproteins.

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† The abbreviations used are: LCAT, lecithin:cholesterol acyltransferase; apo B, apoprotein B; LDL, low density lipoprotein; VLDL, very low density lipoprotein; DTNB, dithiobis-2-nitrobenzoic acid; HDL, high density lipoprotein.

EXPERIMENTAL PROCEDURES

Assay of LCAT—Transferase activity was determined as the rate of decrease of plasma-free cholesterol mass as a function of time during incubation at 37°C. Free and ester cholesterol were measured enzymatically (5) as previously described (3). Loss of free sterol under these conditions was inhibited >95% by the LCAT inhibitor, dithiobis-2-nitrobenzoic acid (6).

Assay of Cholesteryl Ester Transfer Activity—Transfer activity was determined as the rate of increase of cholesteryl ester mass in very low and low density lipoprotein classes, precipitated with heparin-MnCl2 (7) when plasma was incubated at 37°C, as previously described (3).

Immunoadfinity Chromatography of Apoprotein B—Apo B, the major apoprotein of LDL, was obtained from LDL isolated centrifugally from human plasma (8). The apo B protein was precipitated with tetramethyl urea (5%), v/v. Antibody to apo B was raised in rabbits (3) and the y-globulin fraction, purified by DEAE-cellulose chromatography (9), was complexed with Sepharose-CNBr (10). Columns (1 × 20 cm) of immobilized anti-apo B were equilibrated with 0.15 M NaCl-0.001 M disodium EDTA, pH 7.4. Plasma from freshly drawn blood was passed through at a flow rate of 5-10 ml/h; fractions of eluate containing detectable protein were pooled and removal of antigen was determined by radial immunodiffusion (11). The assay was previously described (3).

RESULTS

LCAT and Transfer Activities of Human Plasma—When plasma from freshly drawn blood was incubated at 37°C, the initial linear rate of cholesteryl esterification was maintained for 30–40 min (Fig. 1). This initial rate of LCAT activity was 0.9–1.2 μg of cholesterol esterified min⁻¹ ml⁻¹ plasma (mean 1.05 μg, 12 experiments). The initial rate of transfer of cholesteryl ester to VLDL and LDL was 80–95% (mean 90%, 5 experiments) that of LCAT activity in the same plasma. Both LCAT and transfer rates decreased proportionately with further incubation, to the point that after 3–4 h there was essentially no further decrease in plasma-free cholesterol, increase in plasma cholesteryl ester, or further transfer of cholesteryl ester to VLDL and LDL (Fig. 2). When net esterification was inhibited with 1.4 mM DTNB, transfer protein activity was unchanged (Table I) and the cholesteryl ester transferred to VLDL and LDL under these conditions was derived from the supernatant of heparin-MnCl2-precipitations, which contained the high density lipoprotein fractions (3). Inhibition by DTNB was fully reversible (Fig. 1).

When plasma was incubated until esterification and transfer were inhibited and then mixed with VLDL and LDL from unincubated plasma, the initial rate was completely restored (Table II), indicating that inhibition was not the result of irreversible inactivation of the esterification and transfer systems. On the other hand, addition of the same lipoproteins to fresh plasma during the initial linear phase of esterification and transfer was without discernible effect. The release from inhibition might have resulted from either an increase in the supply of substrate, free cholesterol, and lecithin, or addition of acceptor for product cholesteryl ester. To distinguish between these possibilities, VLDL and LDL were enriched with cholesteryl ester, without depletion of substrate lipids, by incubation in the presence of DTNB (Table I). These lipoproteins were without ability to relieve the inhibition of LCAT and transfer protein activities in incubated plasma.

Effects of Acceptor Concentration on LCAT and Transfer Protein Activities—The apo B content was varied from 10 to 90% of the initial plasma value by immunoadfinity chromatography.
obtained in the presence and absence of DTNB, respectively. Each man plasma. Citrated plasma-0.01 VLDL lipoprotein was added at a concentration sufficient to double the within 2%) obtained by incubation of plasma at 37°C for 30 min in 25 of the rate of decrease of plasma-free cholesterol (FC) assayed enzymatically in quadruplicate.

PLASMA

Esterification and Transfer of Cholesterol in Human Plasma

TABLE I

Values are means of quadruplicate determinations (reproducible within 2%) obtained by incubation of plasma at 37°C for 30 min in the presence or absence of DTNB as inhibitor of LCAT activity. Initial and final samples were mixed with heparin-MnCl₂, samples of the soluble fraction taken for determination of HDL cholesteryl ester, and the precipitate washed as described under "Experimental Procedures" redissolved, and assayed as for HDL. All values relate to free or esterified cholesterol mass/μl of plasma.

| Reaction conditions | Loss of plasma free cholesterol μg/ml | Gain in HDL cholesteryl ester | Gain in VLDL cholesteryl ester |
|---------------------|--------------------------------------|-------------------------------|-------------------------------|
| − DTNB              | 20.7                                 | +2.9                          | +17.7                         |
| + DTNB (1.4 mM)     | 2.7                                  | −13.3                         | +16.0                         |

TABLE II

Effects of initial and end product lipoproteins on the rate of lecithin:cholesterol acyltransferase activity in plasma

Values shown are the means of quintuplicate determinations. VLDL + LDL was obtained by precipitation from initial or incubated plasma and after washing, resuspended as described under "Experimental Procedures." Ester/free cholesterol mass ratios were 2.2 for the initial precipitate and 2.4 and 3.0 for the end product lipoproteins obtained in the presence and absence of DTNB, respectively. Each lipoprotein was added at a concentration sufficient to double the endogenous level of apo B in the plasma. Final VLDL and LDL were obtained after incubation for 3 h at 37°C.

| Added lipoprotein at 180 min | 0-30 min | 180-210 min |
|-----------------------------|----------|-------------|
|                             | μg FC esterified/μl | 30 min |

| None                        | 20.3      | 1.5         |
| + Initial VLDL + LDL        | 19.1      | 22.7        |
| + Final VLDL + LDL          | 16.5      | 1.3         |
| + Final VLDL + LDL (from incubation in the presence of DTNB) | 18.9 | 1.1 |

* FC, free cholesterol.

Fig. 1 (left). Initial rate of cholesterol esterification in human plasma. Citrated plasma-0.01 M Tris-HCl (pH 7.4) was incubated at 37°C either directly (○—○) or after inhibition of activity with DTNB (final concentration 1.4 mM). The plasma was maintained for 30 min on ice, then the inhibition reversed by addition of a 5-fold molar excess of 2-mercaptoethanol (6), and incubated at 37°C under the same conditions (●—●). Esterification was measured in terms of the activity in plasma-free cholesterol (FC) assayed enzymatically in quadruplicate.

PLASMA

TABLE III

Effects of partial removal of lipoproteins that contained apo B on the maximum of cholesteryl ester accumulated in plasma

Plasma was passed through immobilized anti-apo B antibody. The initial apo B content after dilution with citrate and Tris buffer was 625 μg/ml and after affinity chromatography, was 302 μg/ml (corrected to the same plasma protein content). Original and apo B-depleted plasmas were incubated for 3 h at 37°C. Initial and final levels of free and ester cholesterol were determined and after precipitation of plasma samples with heparin-MnCl₂, the ester-free weight ratio was determined on the lipoproteins of the precipitated fraction.

| Plasma fraction | Cholesteryl ester concentration (as ester) μg/ml | Increment of Cholesteryl ester μg/ml | Ester/free cholesterol weight in VLDL + LDL |
|-----------------|-----------------------------------------------|-------------------------------------|--------------------------------------------|
| Whole plasma    | 889                                           | 967                                 | 98                                         |
| Apo B-depleted plasma (50%) | 519                                           | 565                                 | 46                                         |

raphy, and the mass of cholesteryl ester synthesized and transferred to acceptor lipoproteins before inhibition of LCAT activity was determined. Removal of apo B was without effect on the level of either LCAT or transfer proteins in plasma (3). As shown in Table III, the ability of plasma to generate and transfer cholesteryl ester was reduced in proportion to its apo B content. However, the cholesteryl ester content of the end product lipoprotein acceptors was the same in each case.

Source of Free Cholesterol for the LCAT Reaction—The experiments above indicated that in human plasma, VLDL and LDL receive the major part of cholesteryl ester generated by the LCAT reaction. The source of free cholesterol for LCAT was determined by assaying the free cholesterol content of lipoproteins during the linear initial phase of the esterification reaction, i.e. when incubation time was short relative to the known rates of exchange of cholesterol between these lipoproteins (12). As shown in Fig. 3, essentially all of the substrate for LCAT in plasma was derived from VLDL and LDL. These fractions contain no LCAT (3) and such substrate must therefore be esterified by the minor lipoprotein
**TABLE IV**
Composition of initial and end product VLDL and LDL from human plasma

Triglyceride, protein, and phospholipid were assayed as previously described (16). Values given are the means of duplicate analyses which differed <5.0% for all procedures; VLDL and LDL were precipitated from plasma before and after incubation (4 h, 37°C) with heparin-MnCl₂ as detailed under "Experimental Procedures" then washed precipitate was redissolved and separated into VLDL (d < 1.009 g/cm³) and LDL (1.019 < d < 1.063 g/cm³). In the experiment illustrated the initial plasma contained 232 μg ml⁻¹ of LDL-free cholesterol and 61 μg ml⁻¹ of VLDL-free cholesterol, and ester cholesterol contents of 701 and 78 μg ml⁻¹, respectively, based on this separation. Loss of VLDL free cholesterol was 30 and the equivalent loss and gain for LDL was 68 and 85 pg ml⁻¹, respectively. In three other experiments, the cholesteryl ester content of VLDL was initially 9.8%, 13.8% and 22.0%, and finally 20.9%, 23.1%, and 28.1%; and of LDL was initially 38.3%, 40.2%, and 46.8%, and finally, 46.8%, 50.0%, and 50.3%, respectively. Other lipid changes were comparable to those illustrated.

| Lipoprotein | Triglyceride | Cholesteryl ester | Unesterified cholesterol | Phospholipid | Protein |
|-------------|--------------|------------------|-------------------------|--------------|---------|
| Initial VLDL | 50.4 | 13.2 | 10.2 | 20.0 | 6.2 |
| End product VLDL | 48.0 | 23.1 | 5.8 | 16.8 | 6.2 |
| Initial LDL | 2.0 | 43.8 | 8.4 | 24.5 | 21.2 |
| End product LDL | 3.2 | 47.1 | 6.0 | 21.3 | 22.3 |

fraction containing LCAT which can be recovered in the lower lipoprotein density ranges.

Further information on the limiting composition of human plasma VLDL and LDL, and on their contributions to LCAT substrate and product acceptor functions, was obtained by chemical analysis of the initial and end product acceptor lipoproteins. As shown in Table IV, LDL cholesteryl ester increased about 10% above its proportion in fresh plasma before further transfer of cholesteryl ester was inhibited. The major increase of cholesteryl ester mass was in LDL, although a greater change in composition was found in the smaller mass of VLDL in the plasma. The mass of free cholesterol supplied by each lipoprotein class for the esterification was almost the same as that taken back, as ester, via the transfer protein reaction.

**DISCUSSION**

The central core of cholesteryl ester in plasma lipoprotein particles, particularly LDL, is much greater than can be dissolved in the lipid vesicle bilayer. Nevertheless, there is convincing evidence that this core may be in equilibrium with an ester pool in the lipoprotein surface (13) accessible to transfer. We previously showed that in a defined vesicle system with pure LCAT and transfer protein, these activities became limited by the capacity of the acceptors to take up cholesteryl ester. The present studies strongly suggest that a similar mechanism limits the accumulation of cholesteryl ester in human plasma. LCAT activity in incubated plasma was stimulated by acceptor lipoproteins but not by substrate, and the extent of esterification was a function of acceptor concentration in plasma. The results of these experiments support the concept of end product lipoproteins, which contain a maximal cholesteryl ester content and accordingly no longer permit ongoing esterification of cholesterol in the complex containing LCAT and transfer protein. The increase in LDL and VLDL cholesteryl ester content that can occur in plasma before inhibition is comparatively small, about 10% of original mass of this lipid fraction. This requires that the level of acceptor lipoproteins must be closely integrated with the rate of esterification of cholesterol in plasma. The composition of end product LDL obtained in this study is very similar to that of the atherogenic "large LDL" found in cholesterol fed pri-

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