Lecithin: Cholesterol Acyltransferase of Human Plasma

ROLE OF CHYLOMICRONS, VERY LOW, AND HIGH DENSITY LIPOPROTEINS IN THE REACTION*

YVES L. MARCEL AND CAMILLA VEZINA†

From the Laboratory of Lipid Metabolism and Atherosclerosis Research, The Clinical Research Institute of Montreal, Montreal 130, Quebec Province, Canada

SUMMARY

The effects of chylomicrons, VLDL (very low density lipoprotein), IDL (high density lipoprotein), and its subfractions HDL₁ and HDL₂, on lecithin: cholesterol acyltransferase reaction in total plasma have been studied. Plasma cholesterol esterification was measured by the decrease in unesterified cholesterol which was determined by gas-liquid chromatography. Addition of HDL or HDL₂ had no effect on lecithin: cholesterol acyltransferase reaction in plasma, but HDL₁ was inhibitory to the reaction. On the contrary, both chylomicrons and VLDL significantly increased the initial rate as well as the net yield of the reaction in plasma. When increasing concentrations of chylomicrons or VLDL were added to normal plasma, the initial rate of cholesterol esterification increased progressively and the response curve was that of a sigmoid.

When normal subjects were given an oral fat load, the initial velocity of lecithin: cholesterol acyltransferase reaction in their plasma increased significantly together with the plasma concentration of chylomicrons and VLDL, which supports the assumption that the results described above have a physiological role. The mechanism of this stimulation of lecithin: cholesterol acyltransferase reaction by triglyceride-rich lipoproteins may result from the transfer of lipid substrates from these lipoproteins to the HDL or from the activation of this reaction by transfer of peptides between HDL and the triglyceride-rich lipoproteins, or both.

The role of lecithin: cholesterol acyltransferase in the metabolism of plasma lipoproteins is still a matter of speculation. Earlier studies with semipurified systems have ruled out a direct participation of this enzyme in the metabolism of triglyceride-rich lipoproteins, since it was found that this enzyme reacts preferentially with the cholesterol and phosphatidylcholine transphosphatidylylcholine.

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† Recipient of a studentship from the Medical Research Council of Canada.

METHODS

Biological Materials—Blood from fasting subjects was collected in precooled, evacuated tubes which contained disodium EDTA (1 mg per ml of blood), and chilled in crushed ice. The plasma obtained after centrifugation at 4° was used immediately for determination of lecithin: cholesterol acyltransferase or for preparation of plasma lipoprotein fractions.

Three normal volunteers, two males and one female, ingested a liquid fat meal of 275 ml of cream containing about 35% fat after an overnight fast. Blood samples were taken from the subjects just prior to the cream ingestion and at intervals up to 6 hours after.

Plasma lipoprotein fractions were separated from freshly obtained plasma of normal or hyperlipoproteinemic subjects by

1 The abbreviations used are: VLDL, very low density lipoprotein; IDL, low density lipoprotein; HDL, high density lipoprotein; HDL₁, high density lipoprotein of d 1.063 to 1.215 g per ml; HDL₂, high density lipoprotein of d 1.215 to 1.219 g per ml; apo C represents the minor fast migrating peptides of HDL which include, as designed by their COOH-terminal, R-Ser, R-Glu, R-Ala₁, R-Ala₂ (differing in their sialic acid content); DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).
Hatch and Lees (5). Chylomicrons and VLDL were washed by serial preparative ultracentrifugation according to the method of Hatch and Lees (5). Chylomicrons and VLDL were washed by recentrifugation under a layer of distilled water and 0.9% NaCl solution (d = 1.006 g per ml, respectively. High density lipoproteins from a normal plasma pool were isolated and washed once at d = 1.063 g per ml and once at d = 1.21 g per ml. In some experiments the high density lipoproteins were subfractionated by centrifugation at d = 1.125 g per ml. The high density lipoprotein solutions were dialyzed against 0.01 M phosphate-1 mM Na2EDTA (pH 7.4). Purity of the lipoprotein fractions was assessed by immunodiffusion (5), paper (5), and agar-agarose gel electrophoresis (6).

Lipid Analyses—Plasma total cholesterol (7) and triglyceride (8) were measured with an autoanalyzer. Plasma lipoprotein distributions in the cream ingestion studies were estimated by densitometer scanning (Vitatron Densitometer TLD 100) of oil red O-stained gels obtained from agar-agarose electrophoresis according to the technique of Noble (6).

For determination of unesterified cholesterol concentrations in endogenous high density lipoproteins of plasma, the plasma high density lipoproteins were prepared by dextran sulfate precipitation of β-lipoproteins in the presence of calcium chloride (5). The unesterified cholesterol concentration of the high density lipoproteins was then assayed by gas-liquid chromatography (9).

Determination of Lecithin:Cholesterol Acyltransferase Activity—Cholesterol esterification was determined by measuring the decrease in the concentration of plasma unesterified cholesterol before and after an in vitro incubation. In the studies of the effect of added lipoproteins on plasma cholesterol esterification, freshly obtained plasma was incubated in the presence of 0.01 M sodium phosphate buffer, pH 7.4, containing 1 mM EDTA, or in the presence of the lipoprotein fraction. In some experiments DTNB (dissolved in 0.1 M sodium phosphate, pH 7.4, containing 1 mM EDTA, final concentration 1.4 mM) was added to inhibit plasma cholesterol esterification during certain manipulations. The reaction was then initiated by the addition of mercaptoethanol (in 0.01 M sodium phosphate, pH 7.4, containing 1 mM EDTA, final concentration 15 mM).

For each determination of unesterified cholesterol, five aliquots of the same reaction mixture were incubated in stoppered test tubes in a Dubnoff shaking incubator at 37°C. The reaction was stopped by addition of methanol (5 ml), and an internal standard, 5α-cholestanol (Sigma Chemical Co), β-sitosterol (Lilly Laboratories), or cholesteryl butyrate (Nu-Chet-Prep, Inc.), was added and the lipids were extracted (9). The concentration of unesterified cholesterol, as its trimethylsilyl ether derivative, was then determined by gas-liquid chromatography as previously described (9, 10).

RESULTS

Effect of HDL on Esterification of Cholesterol in Plasma—We had previously reported that addition of HDL in varying concentrations did not increase the initial rate of cholesterol esterification in normal plasma (10). The effect of added HDL on the time course of the reaction has been investigated here (Table I). In this experiment, the endogenous concentration of HDL based on measurement of unesterified cholesterol was increased about 2.5-fold. This addition of exogenous HDL did not cause any change in the time course of the reaction up to 120 min, and especially did not increase the span of linearity which had been previously shown to be less than 40 min in normal plasma (10). Since it had been shown that HDL was the best substrate for the lecithin:cholesterol acyltransferase reaction and that HDL was an inhibitor of this reaction (11), we investigated the effect of these two subfractions of HDL on the esterification of cholesterol in plasma (Table II). The addition of HDL had no effect on the time course of cholesterol esterification in plasma whereas the added HDL fraction significantly inhibited the reaction. These results concerning the effects of HDL are in general agreement with those of Fielding and Fielding (11).

Effect of Chylomicrons upon Reaction of Lecithin:Cholesterol Acyltransferase in Plasma—When aliquots of chylomicrons, which were obtained from a type V patient and which contained 100 µg of unesterified cholesterol, were added per ml of a normal plasma, containing itself 464 µg per ml of unesterified cholesterol, the initial velocity of the lecithin:cholesterol acyltransferase reaction significantly increased from 119 to 171 nmol of cholesterol per hour per ml of plasma (Fig. 1). In addition the linearity of the reaction, which could not be shown in this plasma incubated alone, was obtained up to 40 min of incubation in the presence of chylomicrons. Over the complete time period of the incubation, cholesterol esterification in chylomicron-containing plasma was higher than that in plasma alone. Similar results were obtained in four separate experiments in which chylomicrons, obtained either from a pool of type V plasma or from a pool of type I plasma, were added to normal plasma.

Because it could have been possible that the chylomicrons isolated from plasma of types I and V subjects contained some lecithin:cholesterol acyltransferase activity, this point had to be verified. Chylomicrons were incubated alone and in the presence of purified HDL, and the cholesterol esterification was measured.

### TABLE I

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>HDL-UC&lt;sup&gt;b&lt;/sup&gt; in incubation mixture</th>
<th>Cholesterol esterified/ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
<td>nmoles</td>
</tr>
<tr>
<td>Plasma (250 µl) + buffer</td>
<td>67</td>
<td>48 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma (250 µl) + HDL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171</td>
<td>46 ± 6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The incubation procedure was as described under "Methods." Total incubation volume was 600 µl (adjusted with 0.01 M sodium phosphate-1 mM EDTA, pH 7.4).

<sup>b</sup> HDL-UC: amount of unesterified cholesterol in the total HDL fraction.

<sup>c</sup> ± S.D., n = 5.

### TABLE II

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Cholesterol esterified/ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Plasma (150 µl) + buffer</td>
<td>37 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma (150 µl) + HDL&lt;sub&gt;1&lt;/sub&gt;</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>Plasma (150 µl) + HDL&lt;sub&gt;2&lt;/sub&gt;</td>
<td>33 ± 7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Total incubation volume was 300 µl (adjusted with 0.01 M sodium phosphate-1 mM EDTA, pH 7.4). Both HDL<sub>1</sub> and HDL<sub>2</sub> added to the incubation mixture contained 25 µg of unesterified cholesterol.

<sup>b</sup> ± S.D., n = 5.
In either case, the presence of enzymatic activity could not be shown in the chylomicron preparation (data not illustrated). Therefore the activating effect of chylomicrons on the reaction of lecithin:cholesterol acyltransferase appears to be an endogenous property of this lipoprotein fraction.

**Effect of Chylomicrons on Lecithin:Cholesterol Acyltransferase Reaction**—In order to determine the optimum ratio of chylomicrons to plasma which would give a maximum initial velocity for the reaction of cholesterol esterification, different amounts of chylomicrons from either a type I or a type V plasma were added to a normal plasma (Fig. 2). There was no linear relationship between the chylomicron concentration and the reaction rate. Rather, there was a critical range of the ratio of chylomicron unesterified-cholesterol to plasma unesterified-cholesterol which stimulated sharply the lecithin:cholesterol acyltransferase reaction. This observation, which was verified with chylomicrons and plasma isolated from different subjects, may be a result of transfers of lipid and proteins induced by the high concentration of chylomicrons.

It was also found that removal of chylomicrons from the plasma of three type I patients caused a decrease in the initial reaction rate of lecithin:cholesterol acyltransferase in these plasmas. Thus it appears that endogenous chylomicrons have an enhancing effect on plasma cholesterol esterification but the nature of this effect remains to be characterized.

The first explanation, which can be offered for the effect of chylomicrons on lecithin:cholesterol acyltransferase activity, is that cholesterol and phosphatidylcholine of chylomicrons transfer to HDL and thus stimulate the reaction. In order to study this hypothesis, we investigated the effect of the preincubation of a mixture of normal plasma and chylomicrons on the reaction (Table III). There was no effect of preincubation either on the initial rate of reaction or on the amount of cholesterol esterified at 60 min. Therefore if the stimulation of the lecithin:cholesterol acyltransferase reaction by chylomicrons is due to transfer of lipids or apoprotein from chylomicrons to HDL, these transfers are not rate-limiting for the reaction.

**Effect of VLDL on Reaction of Lecithin:Cholesterol Acyltransferase in Plasma**—Similarly to chylomicrons, the addition of

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**Table III**

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Cholesterol esterified/ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>1. Plasma (200 μl) + buffer</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>2. Plasma (200 μl) + chylomicrons</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>3. Plasma (200 μl) + chylomicrons (pre-incubated for 1 hour)</td>
<td>38 ± 6</td>
</tr>
</tbody>
</table>

* Each incubation mixture contained 200 μl of plasma, 40 μl of DTNB (dissolved in 0.1 M sodium phosphate, pH 7.4, final concentration 1.4 mM) and 120 μl of buffer (0.01 M sodium phosphate-1 mM EDTA, pH 7.4) or 120 μl of chylomicrons (containing 30 μg of unesterified cholesterol). Mercaptoethanol, 40 μl (in 0.01 M sodium phosphate-1 mM EDTA, final concentration 15 mM), was added to initiate the reaction in Experiments 1 and 2 without prior incubation and in Experiment 3 after preincubation at 37°C for 1 hour.

* *S.D., n = 5.*
FIG. 3. Effect of very low density lipoproteins on the time curve of the lecithin:cholesterol acyltransferase reaction in human plasma. The incubation mixtures consisted of 250 µl of normal plasma and (---) 150 µl of 0.01 M sodium phosphate buffer, pH 7.4, or (O---O) 150 µl of phosphate buffer containing a very low density lipoprotein preparation (26 µg of unesterified cholesterol) pooled from type IV subjects. Each point is the mean of five determinations ± S.D.

FIG. 4. Effect of very low density lipoprotein concentration on lecithin:cholesterol acyltransferase activity in human plasma. The incubation mixtures consisted of 250 µl of normal plasma and 450 µl of 0.01 M sodium phosphate buffer, pH 7.4, containing increasing amounts of a very low density lipoprotein preparation from a type IV pool (0, 20, 45, 90, 175, and 340 µg of unesterified cholesterol). The incubation time was 20 min. Each point is the mean of five determinations ± S.D.

decreased (□—□), as compared to the control experiment in which the plasma fractions d < 1.063 and d > 1.063 g per ml are incubated together (---□). The cholesterol esterification in this reconstituted plasma (□—□) was consistently lower than that in the original plasma (■—■), an effect which can be attributed to a loss of enzyme activity during the operations of centrifugation and dialysis. When VLDL are added back to the plasma fraction d > 1.063, the yields of esterification are partially restored (△—△) as compared to the control experiment (□—□). Therefore these results provide a second evidence that VLDL participate in the reaction of lecithin:cholesterol acyltransferase in plasma.

Effect of Fat Ingestion on Plasma Cholesterol Esterification—In order to study in vivo whether or not the increase in plasma concentration of chylomicrons and VLDL would also increase plasma lecithin:cholesterol acyltransferase activity, normal volunteer subjects, who had been fasting overnight, were administered an oral fat load which consisted of 275 ml of heavy cream containing about 35% fat. Blood samples were taken from the subjects prior to the ingestion of cream (0 hour sample), and 1, 2, 4, and 6 hours after. Lecithin:cholesterol acyltransferase activity, plasma lipid values, and lipoprotein distribution in agar-agarose electrophoresis were studied in each plasma sample, and the results obtained with one male subject are summarized in Table IV. The plasma levels of triglycerides started to rise 2 hours after the ingestion of cream, and were maximum 4 and 6 hours after, which coincided with the highest plasma concentration of chylomicrons. At the same time, plasma cholesterol esterification was significantly increased at 4 and 6 hours after the ingestion of cream as compared to the control values (0 hour). Similar results were obtained with another male subject, whereas plasma cholesterol esterification did not change with a female subject. In the latter case, chylomicrons did not appear in the plasma and the increase in the concentration of chylomicrons was minimal. Therefore we have assumed that in this particular subject the increase in plasma chylomicrons and VLDL was not sufficient to induce an increase in lecithin:cholesterol acyltransferase activity.

FIG. 5. Effect of the removal or addition of very low density lipoproteins on the plasma lecithin:cholesterol acyltransferase reaction in the absence of low density lipoproteins. A normal plasma sample, in which the lecithin:cholesterol acyltransferase reaction was inhibited by DTTNB (final concentration 1.4 mM), was divided into three aliquots. Two of the aliquots were subjected to ultracentrifugation, one at d = 1.006 g per ml and the other at d = 1.063 g per ml. The incubation mixtures consisted of (■—■) 250 µl of the original plasma and 280 µl of 0.01 M sodium phosphate buffer, pH 7.4; (O---O) 350 µl of the d > 1.063 g per ml fraction (volume adjusted to contain an enzyme concentration equivalent to that in the original plasma) and 180 µl of the d < 1.063 g per ml fraction (112 µg of unesterified cholesterol); (△—△) 350 µl of the d > 1.063 g per ml fraction and 180 µl of phosphate buffer. The reaction was initiated in each incubation mixture by the addition of 20 µl of mercaptoethanol (0.01 M sodium phosphate buffer, pH 7.4), final concentration 15 mM. Each point is the mean of five determinations ± S.D.

DISCUSSION

The lack of effect of HDL on the lecithin:cholesterol acyltransferase reaction in total plasma was most unexpected in view of the information available in the literature (1, 2). Not only the addition of HDL fails to increase the initial rate of reaction in normal plasma (10), but it also fails to increase the span of linearity of the reaction (Table I). Also, the reincubation of the plasma in the presence of HDL gave the same results which
Table IV
Effect of fat ingestion on plasma cholesterol esterification, lipid values, and lipoprotein concentrations in plasma of normal male subject

<table>
<thead>
<tr>
<th>Time after cream ingestion</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>4 hrs</th>
<th>6 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol esterified at 20 min*</td>
<td>18 ± 4</td>
<td>26 ± 8</td>
<td>5 ± 10</td>
<td>430 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>Cholesterol esterified at 40 min*</td>
<td>44 ± 5</td>
<td>69 ± 10</td>
<td>559 ± 55</td>
<td>466 ± 46</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>184 ± 184</td>
<td>184 ± 184</td>
<td>184 ± 192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unesterified cholesterol</td>
<td>56 ± 58</td>
<td>56 ± 58</td>
<td>58 ± 59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>59 ± 56</td>
<td>90 ± 121</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>1 ± 0</td>
<td>1 ± 1</td>
<td>2 ± 5</td>
<td>2 ± 3</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>5 ± 2</td>
<td>6 ± 2</td>
<td>5 ± 2</td>
<td>5 ± 2</td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>31 ± 43</td>
<td>33 ± 429</td>
<td>0.35 ± 429</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>23 ± 29</td>
<td>23 ± 19</td>
<td>219 ± 198</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Nanomoles of cholesterol esterified per ml of plasma, ±S.D. (n = 5).
† Milligrams per 100 ml of plasma.
‡ The concentration of lipoproteins is expressed in square centimeters obtained by integration of the densitometry recording of the electrophoresis on agar-agarose gel, ±S.D. (n = 3).

indicates that the lack of effect of exogenous HDL does not depend on its equilibration with plasma. When the HDL were subfractionated in HDLs and HDLs, we found that the addition of HDL fraction was inhibitory to the reaction which is in agreement with the results of others (11), whereas HDLs had no effect on the reaction. Therefore it appears that we should propose for HDLs a role of operator in the reaction of lecithin: cholesterol acyltransferase rather than a role of true substrate: the reaction has an absolute requirement for HDLs, which contains a peptide activator of the reaction (12), and which binds the apo C peptides from HDL, to chylomicrons, or VLDL, or both, may be accompanied by the transfer of other peptides which would stimulate the reaction of lecithin: cholesterol acyltransferase with the HDL fraction.

In a preliminary experiment, we have attempted to study the effect of HDL preincubated with an artificial emulsion of triglycerides stabilized by phospholipids (Intralipid) on the lecithin: cholesterol acyltransferase reaction in plasma. These HDL preincubated with Intralipid did stimulate the reaction (data not illustrated) but this effect could not be ascribed entirely to the transfer of apo C as described by Havel et al. (20), since we could also demonstrate transfer of phosphatidylcholine from the lipoprotein to the HDL fraction.

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