EFFECTS OF LIPOPROTEIN LIPASE ON THE STRUCTURE OF CHYLOMICRONS

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ABSTRACT

Chylomicrons isolated from rat lymph were complexed with lipoprotein lipase of postheparin plasma (chylomicrons-LPL) in order to study the effects of lipolysis on the structure of chylomicrons. Triglyceride in the chylomicron core was readily hydrolyzed to free fatty acids (FFA) and glycerol when chylomicrons-LPL were incubated at pH 8.3 in medium containing albumin. Although most of the FFA were immediately released to the medium, some were retained within chylomicrons when FFA-binding sites on albumin were not available. These observations suggest that albumin may have a specific role in the transfer of FFA across the chylomicron surface film. Chylomicrons-LPL assumed many different shapes as they were depleted of triglyceride by the lipolytic action of the enzyme, and total removal of core triglyceride resulted in empty sacks of surface film. The surface film was visualized in sections of OsO4-fixed chylomicrons-LPL as a thin electron-opaque line, 25-30 Å wide, in areas where the underlying electron-opaque core had been replaced by zones of decreased electron opacity, and in folds of surface film extending outward from chylomicrons partially depleted of core lipid. The findings demonstrate that chylomicrons consist of a core of liquid triglyceride enveloped by a pliable and durable monolayer surface film, and that lipoprotein lipase reduces the triglyceride core without disrupting the surface film.

INTRODUCTION

Chylomicrons are particles in blood which carry fatty acids in the form of triglyceride from the intestines to other tissues in the body (25, 32). They consist of a core of triglyceride, with traces of cholesterol ester, enclosed by a surface film of phospholipid, cholesterol, and protein (40-42). Triglyceride in chylomicrons is hydrolyzed by lipoprotein lipase in capillary endothelium of extrahepatic tissues to free fatty acids (FFA) and partial glycerides (3, 32, 33). The FFA produced are released to the blood stream, to be taken up by other tissues, while the partial glycerides are taken into vacuoles and microvesicles of the endothelium for further hydrolysis and transport across the capillary wall (3, 32, 33). The rest of the chylomicron, the surface film and residual glyceride, is removed from blood by the liver (1, 23).

This paper describes a study of the morphological and biochemical changes induced in chylomicrons in vitro by the action of lipoprotein lipase. The chylomicrons were complexed and incubated with lipoprotein lipase of postheparin plasma (3).
METHODS

Animals

Adult female Charles River rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) fed ad libitum Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) were used for these studies.

Preparation of Chylomicrons

Doubly labeled chylomicrons were isolated from thoracic duct chyle collected for 6 h from 18-h fasted rats tube-fed corn oil (34) containing [1,14C]palmitic acid (58 mCi/mmol; CFA 23, batches 50-53, Amersham/Searle Corp., Arlington Heights, Ill.) and trioleyl-[2-3H]glycerol (143 mCi/mmol, TRA 172, batch 5 and 6, Amersham/Searle Corp.).

The chyle was centrifuged in a swinging head rotor (SW-39) at 24,000 rpm for 60 min at 3°C with a Spinco model L-2-65 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The compact floating layer of chylomicrons, which formed during centrifugation, was collected and suspended, as described elsewhere (34), in 4% albumin-Tyrode’s solution (bovine serum albumin, 40 mg/ml, in glucose-free Tyrode’s buffer solution) at a triglyceride concentration of 70–110 mM. The suspension was stored at 4°C and used for experiments within 7 days after being collected.

Chylomicrons complexed with lipoprotein lipase (chylomicrons-LPL) were prepared as follows: 1 ml of a suspension of doubly labeled chylomicrons containing 70–110 µmol of triglyceride and 4 ml of lipoprotein lipase-rich postheparin rat plasma (3) were gently mixed at 24°C for 1 min and quickly chilled to 1–4°C. Chylomicrons complexed with lipoprotein lipase were then concentrated by centrifugation in a swinging head rotor (SW-39) at 24,000 rpm for 60 min at 3°C. The compact floating chylomicrons-enzyme fraction was centrifuged again, and resuspended in 1 ml of albumin-Tris solution.

Incubation of Chylomicrons

Suspensions of chylomicrons-LPL and control chylomicrons were diluted after 30–90 min with 7–20 vol of 4% albumin-Tris solution (pH 8.3) and incubated for 30–60 min at 38°C. Aliquots of the incubation media were taken at appropriate intervals for morphological and biochemical studies. In some experiments, suspensions of chylomicrons-LPL were diluted with 80–100 vol of 4% albumin-Tris solution (pH 8.3), incubated 85–100 min at 38°C, and then centrifuged in a swinging head rotor (SW-25-1 or SW-39) at 24,000 rpm for 60 min at 3°C. Aliquots of the supernate, infranate, and sediment were taken for morphological and biochemical studies.

Biochemical Analyses

Lipids in chylomicrons and incubation mixtures were extracted into hexane by modification (4) of the method of Dole and Meinertz (5), and separated into triglyceride, diglyceride, monoglyceride, and FFA fractions by thin layer chromatography (37). The lipids were dissolved in 15 ml of toluene containing 4.2% Liquifluor (New England Nuclear, Pilot Chemicals Inc., Boston, Mass., cat. no. NEF-903) for measurement of 3H and 14C content. The amount of doubly labeled triglyceride hydrolyzed to glycerol was measured either by decrease in the ratio of 3H to 14C in the hexane extract of the incubation mixture (11) or by the amount of [3H]glycerol present in the isopropanol-aqueous phase of the extraction mixture (21). Radioactivity in lipids and glycerol was measured in a liquid scintillation spectrometer (Packard Tricarb model 314-EX, Packard Instrument Co., Inc., Downers Grove, Ill.). The gain and discriminators were set so that 3H was counted with an arbitrary efficiency of 100% in channel A and less than 1% in channel B, and 14C was counted with an arbitrary efficiency of 100% in channel B and 28–30% in channel A. Appropriate internal standards were used to determine corrections for quench. The triglyceride content in chylomicrons was estimated by the method of Rapport and Alonzo (22).

The molar ratio of FFA to albumin in the incubation mixture was calculated from the amounts of 14C-FFA and albumin (mol wt = 67,000) present and the specific activity of triglyceride-fatty acids in chylomicrons.

Morphological Analyses

Osmium Tetraoxide-Fixed Specimens:

Samples were fixed for 2–18 h in ice-cold 2% solu-
tion of OsO₄ in 0.1 M sodium cacodylate buffer (pH 7.4). Sometimes aliquots of OsO₄-fixed samples were placed directly on a grid coated with a Formvar film, air dried, and viewed as a whole mount with the transmission electron microscope. In most experiments, however, the osmicated samples were prepared and viewed in sections as follows: The suspension of chylomicrons in OsO₄ solution was centrifuged in a Beckman Microfuge (Beckman Instruments, Inc., Spinco Div.) at 12,000 rpm for 1 min. The resultant pellet, which contained fixed chylomicrons and surface films, was then washed 1 h in 0.1 M sodium cacodylate buffer (pH 7.4), dehydrated rapidly with acetone, and embedded in Epon (3). Sometimes, however, the suspension of chylomicrons in OsO₄ solution was mixed with an equal volume of warm (38°C) 5% gelatin solution for 1 min, and then poured into ice-cold silicone-rubber embedding molds (3 X 4 X 12 mm, Ladd Research Industries, Inc., Burlington, Vt.). As

### TABLE I

**Hydrolysis of Chylomicron Triglyceride during Preparation and Incubation of Chylomicron-LPL**

<table>
<thead>
<tr>
<th>Chylomicrons</th>
<th>No. of experiments</th>
<th>During preparation*</th>
<th>During incubation†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial triglyceride concentration</td>
<td>Initial triglyceride concentration</td>
</tr>
<tr>
<td></td>
<td>mM</td>
<td>% of total [14C]</td>
<td>mM</td>
</tr>
<tr>
<td>Complexed with lipoprotein lipase</td>
<td>1</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>14-22</td>
<td>0</td>
</tr>
</tbody>
</table>

* 1 ml of a suspension of chylomicrons containing 70-110 µmol of triglyceride (labeled with [14C]palmitic acid and [3H]glycerol) was mixed with 4 ml of lipoprotein lipase-rich (postheparin) or control (normal) plasma at 24°C for 1 min and centrifuged at 3°C for 1 h. The chylomicrons concentrated in the supernatant layer were added to 5 ml of 4% albumin-Tris solution (pH 8.3), mixed for 3-4 min at 24°C, and centrifuged at 3°C for 1 h. The chylomicrons recovered in the supernatant layer were added to 5 ml of 4% albumin-Tris solution, mixed again for 3-4 min at 24°C, and then stored at 3°C until used.

† The suspensions of chylomicrons were diluted with different amounts of 4% albumin-Tris solution (pH 8.3) and incubated 30 min at 38°C.

‡ Calculated from the amount of [14C]palmitic acid released from triglyceride and the specific activity of triglyceride-fatty acids in chylomicrons.

### TABLE II

**Products of Hydrolysis of Triglyceride during Incubation of Chylomicron-LPL**

<table>
<thead>
<tr>
<th>Chylomicrons</th>
<th>Incubation time</th>
<th>Distribution of [3H]glycerol</th>
<th>[14C]palmitic acid present as FFA % of total [14C]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of experiments</td>
<td>Triglyceride</td>
<td>Diglyceride</td>
</tr>
<tr>
<td>Complexed with lipoprotein lipase</td>
<td>0</td>
<td>3</td>
<td>92.7 ± 1.5</td>
</tr>
<tr>
<td>Complexed with lipoprotein lipase</td>
<td>30</td>
<td>3</td>
<td>69.4 ± 4.6</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>3</td>
<td>95.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>96.0 ± 0.8</td>
</tr>
</tbody>
</table>

Suspensions of chylomicrons-LPL and control chylomicrons, prepared as described in Table I, were diluted with different amounts of 4% albumin-Tris solution (pH 8.3) and incubated 30 min at 38°C. Values are means ± SE.

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soon as the mixture was solidified, the blocks were removed, washed 30 min in ice-cold 0.1 M sodium cacodylate buffer (pH 7.4), fixed 15 min in ice-cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, dehydrated rapidly with acetone, and embedded in Epon (3). Thin sections of the embedded samples were cut, stained with lead citrate (38), and examined in a Philips EM 300 electron microscope.

**Glutaraldehyde-fixed specimens:** Samples were also fixed in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for ½–1 h. The suspension was then centrifuged in a swinging bucket-rotor (SW-39) at 24,000 rpm for 60 min at 3°C. The chylomicrons, which formed a compact floating layer during centrifugation, were washed by suspending them in ice-cold 0.1 M sodium cacodylate buffer (pH 7.4) and centrifuging at 24,000 rpm for 60 min at 3°C. The chylomicrons recovered in the floating layer were postfixed, either as a clump or dispersed, for 2 h in ice-cold 2% OsO4 in 0.1 M sodium cacodylate buffer and then processed as described above for viewing in sections.

**Sodium phosphotungstate-stained specimens:** Samples were spread directly on grids coated with Formvar, stained with 2% sodium phosphotungstate (pH 6.8) for 1 min, rinsed with water, air-dried, and viewed directly as whole mounts with the electron microscope (8). Sometimes the specimens were combined with sodium phosphotungstate before being applied to the grid. Similar results were obtained with both preparations.

**RESULTS**

**Biochemical Studies**

Chylomicrons-LPL were prepared by mixing chylomicrons with postheparin plasma at 24°C (3) and isolating them twice by centrifugation. Since activity of the enzyme is arrested by cold (3), the procedure was carried out mostly at 3°C. However, dispersion of chylomicrons in albumin solution after each centrifugation required warming the mixture to 24°C for 3–4 min. Consequently, about ¼ of the triglyceride in chylomicrons-LPL was hydrolyzed during the preparative procedure (Table I).

Chylomicrons complexed with the enzyme were suspended in different amounts of 4% albumin-Tris solution (pH 8.3) and incubated at 38°C for 30 min (Table I). Triglyceride in the chylomicrons was hydrolyzed during incubation to mostly FFA and glycerol (Table II). The percent of triglyceride hydrolyzed in 30 min ranged from 6 to 36 and was inversely related to the...
FIGURE 7 Control chylomicrons incubated 30 min. These chylomicrons are similar in appearance to the unincubated control chylomicrons shown in Fig. 3. The chylomicron core (C) is smooth, circular, and uniformly electron-opaque. FFA/albumin < 0.7. × 35,000.

FIGURE 8 Chylomicrons-LPL incubated 30 min. These chylomicrons have irregular peripheries due to multiple curved-indentations (arrow) of the core (C). FFA/albumin was not measured. × 35,000.
initial concentration of triglyceride in the incubation medium (Table I). The moles of fatty acids released per mole of albumin in the medium ranged from 0.5 to 2.8 at the start and from 3.9 to 5.6 at the end of the incubation (Table I). The initial ratio was directly related to the medium triglyceride concentration, and the final ratio was related to the enzyme activity during incubation (Table I). In one experiment in which chylomicrons-LPL (at a triglyceride concentration of 4 mM) were incubated for 95 min (Figs. 25–27), the final ratio of FFA to albumin in the incubation mixture was 11.0.

Control chylomicrons were prepared by mixing chylomicrons with normal plasma and isolating them twice by centrifugation. There was no hydrolysis of triglyceride in control chylomicrons during the preparative procedure or during incubation at 38°C in albumin solution (pH 8.3) (Tables I and II).

Morphological Studies

CHYLOMICRONS ISOLATED FROM LYMPH: The structure of chylomicrons isolated from rat thoracic duct lymph and suspended in 4% albumin-Tris solution is shown in Figs. 1 and 2. The chylomicron core appears smooth, circular, and uniformly electron-opaque in sections of specimens fixed with OsO4. Electron-opaque granules, sometimes arranged in concentric lines, are seen at the periphery of the core. The granules are more prominent in chylomicrons fixed with glutaraldehyde and OsO4 (Fig. 1) than in those fixed with only OsO4 (Fig. 2).

CONTROL CHYLOMICRONS: The structure of control chylomicrons, which were mixed with normal plasma during preparation, is shown in Fig. 3. The core appears as in lymph chylomicrons (Fig. 1 and 2), smooth, circular, and uniformly electron-opaque in sections of OsO4-fixed specimens. The electron-opaque granules described above, however, are not seen, at the periphery, in control chylomicrons (compare Figs. 1 and 2 with Fig. 3).

Incubation of control chylomicrons in 4% albumin-Tris solution (pH 8.3) at 38°C for 30 min had no effect on their structure (compare Figs. 7, 9, and 15 with Fig. 3). When control chylomicrons were stained with sodium phosphotungstate and examined as whole mounts, they appeared as homogeneous spheres with smooth contours (Fig. 20).

CHYLOMICRONS COMPLEXED WITH LIPOPROTEIN LIrase: The structure of chylomicrons complexed with lipoprotein lipase (Figs. 4, 5, and 6) was markedly different from that of control chylomicrons (Fig. 3). Since 25% of the core triglyceride was hydrolyzed during the preparation of chylomicrons-LPL (Table I), the following description will emphasize the nature rather than the incidence of morphological changes that occurred as the core triglyceride was decreased by the action of lipoprotein lipase.

The periphery of chylomicrons-LPL fixed with OsO4 and sectioned was irregular due to multiple indentations of the chylomicron core (Fig. 4). At higher magnifications (Figs. 5 and 6) the indentations appeared to result from coalescence of smaller circular indentations, giving the periphery a lacy appearance (Fig. 6). The indentations were usually larger in chylomicrons-LPL incubated 30 min at 38°C (Figs. 8 and 13). Bizarre shaped chylomicrons (Figs. 8, 11, and 13) and irregular structures devoid of osmiophilic core material (Fig. 19) were also seen, especially after incubation.

The indented core of chylomicrons-LPL fixed with OsO4 and sectioned was often surrounded by a peripheral zone of decreased electron opacity (Figs. 5 and 6). Sometimes the peripheral zone was irregular in shape, conforming to the indented contours of the chylomicron core (Fig. 6), whereas other times it appeared uniform, especially after incubation (Fig. 14). Both the core and the peripheral zone were enclosed by a thin discontinuous electron-opaque line, 25–30 Å in width (Figs. 4 and 5). Thin double electron-opaque lines, 55 Å in width, were sometimes seen extending outward from the periphery of the core (Figs. 10–12 and 35).

Chylomicrons fixed with OsO4 were usually centrifuged and the pellet formed was processed for electron microscopy. In order to avoid any possible effect of centrifugation on either sampling or shape of chylomicrons, chylomicrons-LPL fixed with OsO4 were mounted directly in gelatin blocks, fixed a short time in glutaraldehyde solution, and then processed for electron microscopy.

The structure of chylomicrons-LPL mounted in gelatin (Figs. 16 and 17) was similar to that of chylomicrons-LPL prepared as pellets (Figs. 6, 8, and 13). Chylomicrons-LPL silhouetted by gelatin were circular, with an irregularly shaped electron-opaque core and a peripheral zone of decreased electron opacity between the surface and the core (Figs. 16 and 17). Sections of incu-
bated chylomicrons-LPL also contained bizarre-shaped chylomicrons (Fig. 18) and irregular structures devoid of osmiophilic core lipid (Fig. 19).

Suspensions of chylomicrons-LPL were negatively stained with sodium phosphotungstate and examined as whole mounts. With this technique core lipid is electron lucent and thus appears whitish in electron micrographs (Figs. 21–26). The negatively stained preparations contained electron-lucent chylomicrons with small electron-opaque protrusions on the surface (Fig. 26), dumbbell-shaped chylomicrons (Figs. 21–23), and electron-opaque disks (Fig. 21), as well as smooth spherical chylomicrons (Fig. 21). Some of the dumbbell-shaped chylomicrons consisted of electron-lucent globules connected by a short thin electron-lucent segment (Figs. 21 and 22), while others consisted of an electron-lucent globule attached to an electron-opaque portion depleted of core lipid (Figs. 21). Some of them, however, had small patches of electron-lucent lipid at the periphery (Fig. 25). The disks (Figs. 21 and 25) resembled in substructure the electron-opaque areas in dumbbell-shaped chylomicrons (Fig. 23). These electron-opaque structures seen in negatively stained preparations are most reasonably patches or folds of chylomicron surface film.

Lipid-free disks were also seen in whole mount preparations of chylomicrons-LPL fixed with OsO₄ (Fig. 27). In these preparations core lipid was electron-opaque and, thus, the disks appeared electron-lucent.

A sediment containing disks similar to those described above (Fig. 21) was obtained when a suspension of chylomicrons-LPL incubated 85 min was centrifuged at 50,000 g for 1 h (Fig. 28). The disks, which averaged 0.3 µm in diameter, were tightly packed, sometimes in rows (Fig. 28). A few of the disks contained tiny remnants of electron-lucent lipid. Sections of the sediment after it was fixed with OsO₄ contained numerous slightly curved linear electron-opaque structures that were 0.2–0.4 µm long and arranged lengthwise in single rows (Figs. 29–31). The linear structures appeared in some areas as single lines, with a minimal width of 55 Å, and in other areas as double lines, suggesting that the structures were collapsed vesicles (Figs. 31 and 34). Electron-opaque rings about 0.2–0.4 µm in diameter and electron-opaque circular structures less than 0.2 µm in diameter were also discernable (Figs. 32 and 33). The curved linear structures (Figs. 29 and 31) may be sections perpendicular to the diameter, and the rings and circular structures (Figs. 32 and 33), sections through the rim and dome, respectively, of concave disks. The findings suggest that these disks may be collapsed surface films of chylomicrons completely depleted of core triglyceride.

**DISCUSSION**

Triglyceride in chylomicrons is readily hydrolyzed to FFA and glycerol by lipoprotein lipase when incubated in medium containing albumin.
FIGURES 15–19  Chylomicron suspensions fixed with OsO₄, mixed with gelatin, and sectioned.

FIGURE 15  Control chylomicrons incubated 30 min. The chylomicron core (C) appears smooth, circular, and uniformly electron-opaque. Electron-opaque granules, as seen in Figs. 1 and 2, are not present at the periphery. FFA/albumin = 0.3. × 175,000.

FIGURE 16  Chylomicrons-LPL incubated 30 min. The electron-opaque core (C) is very irregular in shape. Note the peripheral zones (P) of decreased electron opacity. FFA/albumin = 6.3. × 150,000.

FIGURE 17  Chylomicrons-LPL incubated 30 min. Note the electron-lucent peripheral zone (P) between the core (C) and the surface film (SF). FFA/albumin = 6.3. × 125,000.

FIGURE 18  Chylomicrons-LPL unincubated. This chylomicron is pear-shaped. FFA/albumin = 1.8. × 150,000.

FIGURE 19  Chylomicrons-LPL incubated 30 min. This irregular profile represents the remnant of a core-depleted chylomicron. FFA/albumin = 6.3. × 170,000.
(10, 11, 14, 15, 24–26). Most of the FFA produced bind immediately to albumin while the glycerol disperses in the water. Although the pH (8.3) and temperature (38°C) of the incubation medium are favorable for the formation of water-soluble soaps, virtually none of the FFA produced by hydrolysis is found, unbound to albumin, in the aqueous phase. Since the number of FFA-binding sites on albumin is limited to six or seven per molecule (9, 31, 36), the amount of triglyceride hydrolyzed could be restricted by the amount of albumin present in the medium (10, 14, 26). In addition, affinity of albumin for FFA decreases exponentially as the binding sites are occupied (31, 36). In the present study, the albumin concentration in the incubation medium was 0.6 mM (4%) and, thus, total hydrolysis could be expected if the triglyceride concentration did not exceed 1.4 mM. About 25% of the triglyceride was hydrolyzed during the preparation of chylomicrons-LPL and another 6–36% was hydrolyzed during 30 min of incubation (Table I). The percentage of triglyceride hydrolyzed was inversely related to the triglyceride concentration, during both preparation and incubation, and limited, at least in part, by the availability of FFA binding sites on albumin. The molar ratio of FFA to albumin was 5–7 in the infranatant layer after the centrifugation of the preparative procedure and 5–7 in the incubation mixture after 30 min in most experiments (Table I). In one experiment, however, the final ratio was 11 when chylomicrons-LPL at a triglyceride concentration of 4 mM were incubated for 85 min (Figs. 25–27). Although small amounts of diglyceride and monoglyceride accumulated during incubation, FFA and glycerol were the main products of hydrolysis (Table II).

Chylomicrons-LPL assumed many different shapes, depending on the conditions of incubation and the methods used for visualization with the electron microscope. There were multiple indentations of the osmiophilic core in sections of chylomicrons-LPL fixed with OsO4 (Figs. 8 and 13). In some preparations a zone of decreased electron opacity either filled or lined the indentations (Figs. 5, 16, and 17), whereas in others the zone completely surrounded the indented cores (Figs. 4, 6, and 14). In most preparations, however, only the indented core was preserved (Figs. 8 and 13). It was found in an earlier study (3) that FFA, but not triglyceride, were extracted by acetone from chylomicrons that had been incubated with lipoprotein lipase, treated with Pb++, and fixed with OsO4. Thus, the production of FFA from triglyceride during incubation was evidenced by loss of electron opacity as well as by deposition of electron-opaque lead fatty acid precipitates in chylomicrons (3). It is possible that the core indentations and the peripheral zones of decreased electron opacity seen in chylomicrons-LPL fixed with OsO4, in the present study, were also produced by acetone extraction of FFA from the chylomicron core. This conclusion is supported by the relationship observed between the size of core indentations and the molar ratio of FFA to albumin in the incubation mixture at the time chylomicrons were fixed with OsO4. Small indentations were seen when the molar ratio was between 1.4 and 3.0 (Figs. 4, 6, and 10), and large indentations were seen when the ratio exceeded 6.3 (Figs. 13, 16, and 17). The relatively high FFA-albumin molar ratios found at the start of the incubation (Table I) were probably due in part to transfer to the incubation medium of FFA present within the chylomicrons-LPL at the end of the second preparative centrifugation. At that time the molar ratio in the infranatant solution was 5–7 and, accordingly, the FFA content of the chylomicrons-LPL was higher than that of control chylomicrons, 2–4 vs. 1% of total fatty acids.

Changes in shape of chylomicrons-LPL were confirmed in preparations negatively stained with sodium phosphotungstate and examined as whole mounts. (With this technique core lipid appeared electron-lucent.) Preparations of chylomicrons-LPL incubated 85 min contained (electron-lucent) chylomicrons with small (electron-opaque) protrusions (Figs. 21 and 26), dumbbell-shaped chylomicrons in various stages of core-lipid depletion (Figs. 21–23), and (electron-opaque) disks free of core lipid (Figs. 21 and 25). The dumbbell-shaped chylomicrons may be core-depleted particles that were twisted by the shaking action of the incubator. Abnormally shaped chylomicrons-LPL were also seen in sections of specimens fixed with OsO4 and mounted in gelatin (Fig. 18). The profile of core-depleted chylomicrons was usually smooth, although not circular, when examined in whole mounts (Figs. 21, 25, and 26) and in sections of specimens mounted in gelatin blocks (Figs. 16 and 17).
Collapsed chylomicrons depleted of core lipid were seen also in venous blood of lactating rat mammary tissue perfused 2–3 min with chylomicrons (33). These particles, produced by lipolysis within the mammary capillary bed (21, 33), are undoubtedly similar to the chylomicron "remnants" and "skeleton" proposed by others (1, 23). Such particles are probably present in both very low density lipoprotein and chylomicron fractions separated from blood (16, 23).

Analyses of surface and core lipid fractions obtained from chylomicrons, ruptured by freezing and thawing, showed that phospholipid of chylomicrons was present only in the surface fraction (40, 41). Although as much as 9% of the lipid in chylomicrons may be phospholipid (40–42), the amount present is only sufficient to cover as a monomolecular layer 80–100% of the chylomicron surface (7, 40). More than half of the phospholipid in chylomicrons is lecithin (34, 39, 40). The surface lipid fraction also contained free cholesterol and protein, with traces of triglycerides containing only saturated fatty acid (40, 41). The molar ratio of free cholesterol to phospholipid in the surface fraction was 0.1 in rat and 0.2 in dog chylomicrons (40). It has been estimated that chylomicron protein could cover only 10–20% of the chylomicron surface (20, 40). On the basis of these findings it has been proposed that the chylomicron core is surrounded by a monomolecular layer of phospholipid containing small amounts of protein, cholesterol, and saturated triglyceride (7, 40–42). X-ray studies of bilayers of lecithin (18, 19, 35) and of lecithin and cholesterol (18, 19) in water showed that the lipid bilayer is 30–42 Å thick. Thus, the monolayer surface film of chylomicrons should be 15–21 Å thick. In a study of very low density lipoproteins, which are smaller but similar in structure to chylomicrons, the relationship between diameter and chemical composition of particles of different sizes suggested that the surface film, also composed of phospholipid, cholesterol, and protein, was 21.5 Å thick (28).

Several electron microscopic studies have described a discontinuous electron-opaque structure at the periphery of chylomicrons but the widths reported were between 25 and 100 Å (6, 13, 27, 29). In the present study, osmiophilic granules, sometimes arranged concentrically, were seen at the periphery of the core in sections of chylomicrons isolated from rat lymph and suspended twice in 4% albumin-Tris solution (Figs. 1 and 2). The granules were not found, however, in chylomicrons which had been mixed for a short time with normal plasma, and then isolated and suspended twice in albumin solution (Fig. 3). Loss of the osmiophilic granules from the periphery of the core did not affect stability of the chylomicrons.

A peripheral thin discontinuous electron-opaque line, 25–30 Å wide, was seen in sections of chylomicrons-LPL where the periphery of the electron-opaque core was replaced by zones of decreased electron opacity (Figs. 4 and 5). In most sections, however, this fine structure was not preserved, especially when the chylomicron core was deeply indented (Figs. 6, 8, and 13). Perhaps, it was lost at the time FFA were extracted from the core by acetone during dehydration (3). In some sections, thin double electron-opaque lines, 55 Å wide, were seen extending outward from the chylomicron core (Figs. 10–12 and 35). The single lines seen bordering the peripheral zones of decreased electron opacity (Figs. 4 and 5) undoubtedly represent the chylomicron surface film, and the double lines (Figs. 10–12), folds of surface film in cross section.

**Figures 20 and 21** Chylomicron suspensions stained with sodium phosphotungstate and examined as whole mounts.

**Figure 20** Control chylomicrons incubated 60 min. The chylomicrons appear as spheres with smooth contours and electron-lucent cores (C). X 20,000.

**Figure 21** Chylomicrons-LPL incubated 100 min. The supernate obtained by ultracentrifugation was stained with sodium phosphotungstate and examined as a whole mount. Note spherical and irregularly shaped electron-lucent chylomicrons, dumbbell-shaped chylomicrons (D), and electron-opaque disks (SF). The disks are free in suspension or associated with electron-lucent lipid globules (arrows). The encircled disk is 0.25 μm in diameter. FFA/albumin was not measured. X 25,000.
The findings in negatively stained preparations of incubated chylomicrons-LPL showed that chylomicrons became disk-like structures when completely depleted of core lipid (Figs. 21 and 28). When the discs were fixed with OsO₄ and sectioned, they appeared as collapsed osmiophilic vesicles (Figs. 32 and 33). They were seen in cross section (Figs. 29-31 and 34) either as two lines separated by a very thin space or as a single dense line, 55 Å thick, twice the expected width of the chylomicron-surface film. The findings indicate that total removal of core triglyceride from chylomicrons results in empty sacks of surface film.

Durability of the surface film, as well as the concaved circular shape of the empty sacks, is probably due to the protein present in the surface film. Analyses of human chylomicrons isolated from thoracic duct lymph showed that the protein fraction of chylomicrons consists of 65-70% apolipoprotein C (very low density lipoprotein), 19-23% apolipoprotein B (low density lipoprotein), and 11-12% apolipoprotein A (high density lipoprotein) (16). The apolipoprotein C component, which comprises three polypeptides ranging in molecular weight from 7,000 to 10,000 (16), has been reported to be the serum factor which activates triglyceride in artificial emulsion-to make the triglyceride accessible to the lipolytic action of lipoprotein lipase (2, 12, 17). These peptides may also have a role in complexing chylomicrons with lipoprotein lipase, whether the enzyme is present in postheparin plasma or associated with the luminal plasma membrane of capillary endothelium (3).

The earliest sign of lipolysis in sections of chylomicrons-LPL is probably the appearance of areas of decreased electron opacity between the surface film and core (Figs. 5 and 17). As lipolysis proceeded, core indentations were

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**Figures 22–26** Chylomicron suspensions stained with sodium phosphotungstate and examined as whole mounts.

**Figure 22** Chylomicrons-LPL incubated 100 min. Enlargement of a dumbbell-shaped chylomicron, shown in Fig. 20, which consists of two electron-lucent parts connected by an electron-lucent line. Note that the border of the lower part is not as distinct as that of the upper part. FFA/albumin was not measured. X 210,000.

**Figure 23** Chylomicrons-LPL incubated 40 min. The upper part of this dumbbell-shaped particle is filled with lipid (electron-lucent) while the lower part is relatively free of lipid, thus allowing visualization of the electron-opaque chylomicron surface film (SF). The surface film is similar in appearance to the disks (SF) seen in Fig. 20. Laminate structures, seen as light and dark bands, are present with the surface film (arrowhead). FFA/albumin was not measured. X 175,000.

**Figure 24** Chylomicrons-LPL unincubated. An electron-lucent aspherical globule is attached to a narrow electron-opaque surface film (SF). Compare with Fig. 11. FFA/albumin was not measured. X 140,000.

**Figure 25** Chylomicrons-LPL incubated 85 min. The chylomicron on the left is filled with electron-lucent core lipid (C). The chylomicron on the right consists mostly of electron-opaque surface film (SF) and small patches of electron-lucent core lipid (L) at the periphery. FFA/albumin = 11.0. X 150,000.

**Figure 26** Chylomicrons-LPL incubated for 85 min. A surface film (SF) is seen among electron-lucent chylomicrons. Note the chylomicron with a protrusion (arrow) of the same electron opacity as that of surface film. FFA/albumin = 11.0. X 100,000.

**Figure 27** Chylomicrons-LPL incubated 85 min, fixed with OsO₄, and placed on specimen grid for visualization as a whole mount. Chylomicrons with electron-opaque cores (C) are intermixed with surface film (SF) of chylomicrons depleted of electron-opaque lipid FFA/albumin = 11.0. X 70,000.
found and they were lined by peripheral zones of decreased electron opacity (Figs. 6, 13, and 16). Although the surface film was usually seen in direct contact with either the peripheral zone or core (Figs. 4 and 5), an electron-lucent zone was sometimes present between the surface film and peripheral zone (Fig. 5). Therefore, it is proposed that hydrolysis of core triglyceride by lipoprotein lipase occurs at the periphery of the core underneath the surface film. Since FFA accumulate within the limits of the surface film when FFA binding sites on albumin are not available, it seems likely that albumin may have a special role in the transfer of FFA across the surface film. The fact that FFA accumulated in localized areas, near the periphery (Figs. 8, 10, 13, 16, and 17), instead of diffusing throughout the core triglyceride, strongly suggests that they were present as soaps within the chylomicrons.

Our findings demonstrate that chylomicrons undergo striking changes in size, shape, and appearance as they are depleted of triglyceride by lipoprotein lipase (Figs. 8 and 21), and that the enzyme reduces the triglyceride core without disrupting the surface film. They also show that chylomicrons consist of a liquid triglyceride core enveloped by a pliable and durable monolayer surface film. These conclusions are based on the use of different electron microscope techniques in combination with biochemical analyses. This multiple approach was necessary because of the chemical nature of chylomicrons and the products of lipolysis, dependence of FFA release on availability of FFA binding sites on albumin in the incubation medium, and, finally, the difficulty of visualizing the monolayer surface film.

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**Figure 28** Chylomicrons-LPL incubated 100 min. The sediment obtained by ultracentrifugation was stained with sodium phosphotungstate and examined as a whole mount. The sediment contains numerous disk-like structures similar to those shown in Fig. 20. The marked disks (SF) are 0.20-0.25 μm in diameter. These structures are empty sacks of surface film, the remnants of chylomicrons depleted of triglyceride. A few of them still contain traces of residual electron-lucent core lipid (L). FFA/albumin was not measured. × 40,000.

**Figure 29** Chylomicrons-LPL incubated 100 min. The sediment obtained by ultracentrifugation was fixed with OsO₄ and sectioned. The collapsed sacks of surface film, shown in Fig. 28, appear in cross section as connected slightly curved electron-opaque lines. Note the points of contact between adjacent sacks (arrows). The encircled curved linear segment is about 0.2 μm in length. FFA/albumin was not measured. × 40,000.

**Figure 30** Same preparation as Fig. 29. The sacks of surface film are seen in oblique section as diffuse osmiophilic areas (SF), and in cross section as electron-opaque lines. × 55,000.

**Figure 31** Same preparation as Fig. 29. Collapsed sacks of surface film appear here in cross section either as a single electron-opaque line (D), or as 2 separate less opaque lines (SF). (See Fig. 34 for enlargement of this micrograph.) FFA/albumin was not measured. × 57,000.

**Figures 32 and 33** Same preparation as Fig. 31. The sacks of surface film appear in these sections as electron-opaque rings (R), 0.2-0.4 μm in diameter, and as circular structures (arrow), less than 0.2 μm in diameter. × 140,000.
FIGURE 34 Enlargement of Fig. 31. This cross section shows that collapsed empty sacks of surface film (D) are 55 Å thick and that the surface film (SF) is about 25 Å thick. X 350,000.

FIGURE 35 Enlargement of Fig. 11. This cross-section shows that the double electron-opaque line (D) extending from the core (C) of chylomicrons-LPL (Figs. 10–12) is 55 Å thick and that each linear density (SF) is about 25 Å thick. The projection represents a fold of the surface film of a partially depleted chylomicron. X 900,000.

REFERENCES


