The Removal and Metabolism of Chylomicrons by Adipose Tissue in Vitro

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Alimentary triglycerides are transported in the blood primarily in the form of emulsified particles called chylomicrons, which consist predominantly of triglycerides, but also contain a small proportion of cholesterol, phospholipid, and protein (1). Recently, Rodbell and Fredrickson (2) have found that one of the proteins associated with chylomicrons appears to be the same as the protein isolated from the plasma alpha or high density lipoprotein. This finding, plus the observation by Korn (3) that alpha lipoprotein “activates” or increases the rate of hydrolysis of triglycerides by lipoprotein lipase has enhanced the possibility that the metabolism of chylomicrons is mediated through the action of lipoprotein lipase as initially proposed by Korn (3).

Although there has been some evidence for hydrolysis of chylomicron triglycerides intravascularly by lipoprotein lipase (4), the recent findings by Bragdon and Gordon (5) indicate that the action of lipoprotein lipase as initially proposed by Korn (3) is necessary for their removal from blood, at least in the rat, is independent of any significant hydrolysis of triglycerides during their brief circulating time. Accordingly, if hydrolysis of the chylomicrons is necessary for their removal from blood, it would appear more likely that the enzyme is acting in the tissues responsible for their removal. One very active site for triglyceride metabolism is adipose tissue which has been found to take up triglycerides selectively in vitro (7) and is very rich in lipoprotein lipase (8) (for a comprehensive review of chylomicron metabolism, see Fredrickson and Gordon (6)).

The present investigation was undertaken to determine the role, if any, that chylomicron proteins and lipoprotein lipase play in the removal and metabolism of triglycerides by adipose tissue in a system in vitro.

Evidence will be presented that triglycerides in the form of artificial emulsions can be removed intact and metabolized by adipose tissue as efficiently as chylomicrons. Lipolysis appears to be obligatory for the metabolism of triglycerides but not for their removal by adipose tissue.

EXPERIMENTAL

Male Sprague-Dawley rats were used throughout this investigation. Animals of the same weight (200 to 250 g) were used for each set of experiments and were maintained in separate cages on a Purina chow diet.

Radioactive Substrates—C14-carboxyl-labeled tripalmitin, linoleic acid, palmitic acid, and uniformly labeled glucose were obtained from the Nuclear Instrument and Chemical Company, Chicago, Illinois. Cottonseed oil emulsions (Lipomul) labeled with C14-tripalmitin were kindly supplied by the Upjohn Company, Kalamazoo, Michigan.

Preparation of Triglyceride Substrates—Three types of triglyceride substrates were used in this study and are referred to as (a) C14-chylomicrons, (b) activated C14-Lipomul, and (c) washed C14-Lipomul.

(a) C14-chylomicrons were obtained from chyle after feeding C14-palmitic or linoleic acids along with olive oil to thoracic duct cannulated rats. The chylomicrons were isolated and washed by previously described procedures (2).

(b) Activated C14-Lipomul refers to C14-tripalmitin-labeled cottonseed oil emulsions (Lipomul) which were incubated with human plasma in the following manner. A 15% suspension of the labeled Lipomul, 10 ml, was added to 250 ml of human plasma obtained from the NIH blood bank. Only plasma samples were used which had been obtained within 2 weeks after withdrawal from the subjects. The mixture was incubated with gentle stirring for 1 hour at room temperature. The fat emulsion was resolated and washed 3 to 5 times by the same procedures used for the rat chylomicrons. Analyses of the “activated” triglyceride preparations were performed for total cholesterol (9) and phospholipid, protein and triglycerides by previously described methods (10). Based on the total weight of the samples, cholesterol values varied from 4 to 9%; phospholipid, 3 to 5%; protein, 1.5 to 2%; and triglycerides, 88 to 90%. No free fatty acids could be detected in any preparation. Since the nontriglyceride lipid fraction represented a small percentage of the total ester in the chylomicrons and “activated” triglyceride preparations, specific activities of the esterified fatty acids were calculated on the basis of their total ester content.

(c) Washed C14-Lipomul refers to the Lipomul emulsions which were not exposed to plasma but were simply washed in the same manner as the C14-chylomicron and C14-activated Lipomul preparations.

Preparation of Adipose Tissue and Incubation Procedures—The animals were killed by decapitation and the epididymal fat pads rapidly removed by the techniques described by Winegrad and Renold (11). Tissues of approximately equal size were immediately washed in two 25-ml volumes of Krebs-Ringer phosphate buffer, pH 7.4 at room temperature and placed in 25-ml Erlenmeyer flasks containing Krebs-Ringer phosphate buffer, pH 7.4 and glucose at a concentration of 0.5 g per liter (unless otherwise specified) and the experimental materials in a final volume of 2 ml. The flasks were shaken at 45 cycles per minute in a Dubnoff shaker at 38°.

After the incubation period, the tissues were carefully lifted from the medium with forceps and drawn along the side of the flask in order to express as much of the adherent medium as
than 1% of the radioactivity taken up could be detected as fatty acids found in the medium and is expressed as microequivalents per gram of tissue. Since most of the experiments were performed in order to rule out contamination of the tissues by simple adsorption of the radioactive triglycerides to the tissue. Paired fat pads were incubated for 2 hours with 15 μmoles of C14-chylomicron triglycerides per ml of medium. One tissue was washed 3 times (2 minutes each wash) with 3 ml of medium containing 45 μmoles of unlabeled triglyceride per ml. Only the first wash contained radioactive activity. The second tissue was washed with two 100-ml volumes of Krebs-Ringer medium. The tissues were reincubated for 2 hours in 3 ml of Krebs-Ringer solution containing no added triglyceride. No radioactivity appeared in the medium during the reincubation. The tissues were washed again with unlabeled triglycerides. No radioactivity appeared in the wash media. Both tissues contained the same quantity of radioactivity. Therefore, the results subsequently described represent the quantity of C14-triglycerides and fatty acids which were neither exchangeable with unlabeled triglycerides in the medium nor removed by the washing procedure.

The term “uptake or removal of radioactivity” refers to the total radioactivity found in the tissues plus the radioactive fatty acids found in the medium and is expressed as microequivalents per gram of tissue. Since most of the experiments were performed in the absence of albumin in the medium, usually less than 1% of the radioactivity taken up could be detected as free fatty acids in the medium (12).

Procedures for Extraction and Counting Triglycerides and Fatty Acids from the Tissues and Media—The tissues were homogenized at room temperature in a Teflon Potter Elvehjem type homogenizer containing 7 ml of Dole’s extraction mixture (13). The homogenate was decanted and the homogenizing vessel was washed with 3 ml of Dole’s mixture and finally with 5 ml of normal heptane (Eastman). The extracts were combined in a separatory funnel and 3 ml of water were added to break the phases. The upper heptane phase was found to contain all the radioactivity of the original tissue homogenate. After determining the volume of the heptane phase, an aliquot was taken for counting and another aliquot for separation of the free fatty acids from esterified fatty acids by the procedure described by Borgström (14). The titrimetric procedure of Gordon and Cherkes (15) was used for fatty acid titration. Each fraction (original, free fatty acid, and esterified fatty acids) was evaporated to dryness in counting vials under a stream of air at room temperature and the residue dissolved in 15 ml of a 0.4% solution of 2,5-diphenyloxazole in toluene and counted in a Packard Tri-Carb scintillation counter. Complete recovery and separation of added C14-palmitic, linoleic, and tripalmitin were obtained when they were added to adipose tissue and carried through the described procedures.

When the lipoprotein lipase content of the tissue was also measured, the triglycerides and fatty acids were isolated as follows. The fat pad was homogenized with 10 volumes of acetone at 5°. The homogenate was centrifuged in the cold to remove the insoluble residue and the acetone extract decanted. The residue was re-extracted with 40 ml of cold ether and the combined either and acetone extracts evaporated to dryness at 50° under a stream of air, the oily residue dissolved in Dole’s mixture, and carried through the same procedure as described above. Both homogenization methods gave the same results.

Essentially the same method as described by Dole (13) for extracting fatty acids from serum was used for extracting both the neutral fats and free fatty acids from the medium. The procedures described above were used for the separation of the labeled fatty acids.

The ester content of the medium and tissues was determined by the method of Rapport and Alonzo (16) as described for phospholipids. This method was found to be excellent for triglyceride determination provided that the lipids were removed from salts by extraction into the heptane phase according to the previously described procedures. A molar extinction coefficient of 2970 at 530 mμ was obtained for tripalmitin and as little as 0.1 μmole of triglyceride could be determined by using 3 ml of the mire perecholrate reagent.

Separation of Di- and Tri-saturated Triglycerides from Triglycerides Containing Mixed Saturated and Unsaturated Fatty Acids—A modification of the technique described by Reiser et al. (17) was used for separating saturated from unsaturated triglycerides. In a typical experiment, the triglyceride fraction (in heptane) obtained from tissues incubated with the activated C14-Lipomul, was taken to dryness at 55° under nitrogen and the residue dissolved in 100 ml of boiling alcohol-acetone (85:15) per mm of ester. After the fat was completely dissolved, the solution was stored overnight at 5°. The insoluble fraction was collected on a medium porosity sintered glass filter and washed with cold alcohol. The insoluble fraction was then dissolved in 2,5-diphenyloxazole-toluene at room temperature and counted. The alcohol-acetone soluble filtrate was evaporated to dryness at 55° under nitrogen, the residue dissolved in 2,5-diphenyloxazole-toluene, and counted.

As an example of the efficacy of the procedure for the isolation of C14-tripalmitin from adipose tissue, a typical experiment is described in Table I. The C14-Lipomul was added to the lipids extracted from a section of adipose tissue used as a zero time control. The separation of the saturated triglycerides from the mixture of saturated and unsaturated triglycerides was car-

<table>
<thead>
<tr>
<th>Specific activity of triglycerides</th>
<th>Total triglycerides</th>
<th>Counts in fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.p.m./μmole</td>
<td>μmole</td>
<td>%</td>
</tr>
<tr>
<td>Original triglycerides</td>
<td>14.80</td>
<td>355</td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>0.74</td>
<td>340</td>
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<tr>
<td>Insoluble fraction</td>
<td>411</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Table I

Isolation and recovery of C14-tripalmitin and other saturated triglycerides from adipose tissue

C14-Lipomul triglycerides, 2.3 μmoles, containing 5354 c.p.m. were added to 353 μmoles of triglycerides isolated from adipose tissue. The triglycerides were separated into an insoluble fraction (saturated triglycerides) and a soluble fraction (mixed triglycerides) by the procedure described in "Experimental."
**TABLE II**

Metabolism of C14-chylomicrons, activated C14-Lipomul, and washed C14-Lipomul

| Substrate          | (1) C14-triglyceride disappearing from medium | (2) Total C14 uptake | (3) C14 fatty acid in tissue | (4) Total fatty acid in tissue | (5) Specific activities of triglycerides in medium | (6) C14O2
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat chylomicrons</td>
<td>14.0</td>
<td>11.4</td>
<td>10.3</td>
<td>1.14</td>
<td>8.1</td>
<td>18,361</td>
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<tr>
<td>16.5</td>
<td>12.4</td>
<td>11.8</td>
<td>0.58</td>
<td>4.4</td>
<td>18,361</td>
<td>17,950</td>
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<tr>
<td>17.9</td>
<td>11.9</td>
<td>11.3</td>
<td>0.65</td>
<td>4.9</td>
<td>18,361</td>
<td>18,451</td>
</tr>
<tr>
<td>Activated Lipomul</td>
<td>16.0</td>
<td>11.1</td>
<td>9.7</td>
<td>1.36</td>
<td>9.6</td>
<td>1,900</td>
</tr>
<tr>
<td>16.5</td>
<td>10.0</td>
<td>9.6</td>
<td>0.65</td>
<td>4.3</td>
<td>1,900</td>
<td>1,910</td>
</tr>
<tr>
<td>22.0</td>
<td>10.5</td>
<td>9.7</td>
<td>0.76</td>
<td>5.9</td>
<td>1,900</td>
<td>1,895</td>
</tr>
<tr>
<td>Washed Lipomul</td>
<td>21.0</td>
<td>10.4</td>
<td>8.5</td>
<td>1.90</td>
<td>10.0</td>
<td>3,400</td>
</tr>
<tr>
<td>17.0</td>
<td>9.7</td>
<td>8.8</td>
<td>0.86</td>
<td>5.8</td>
<td>3,400</td>
<td>3,450</td>
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<tr>
<td>15.0</td>
<td>11.5</td>
<td>11.0</td>
<td>0.55</td>
<td>4.9</td>
<td>3,400</td>
<td>3,100</td>
</tr>
</tbody>
</table>

**TABLE III**

Comparison of specific activities of tissue C14-fatty acids and esters derived from chylomicrons

<table>
<thead>
<tr>
<th>Tissue fatty acids</th>
<th>Tissue esters</th>
<th>C14O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/g tissue</td>
<td>c.p.m./µg</td>
<td>µg/g tissue</td>
</tr>
<tr>
<td>Total Specific activity</td>
<td>Total Specific activity</td>
<td>µg/g tissue</td>
</tr>
<tr>
<td>4.8</td>
<td>2387</td>
<td>2100</td>
</tr>
<tr>
<td>4.9</td>
<td>2430</td>
<td>2300</td>
</tr>
</tbody>
</table>

* Values obtained from the paired fat pads from the same animal.

Fig. 1. Uptake of C14-chylomicrons versus tissue weight. Paired fat pads were sectioned into four pieces and incubated in separate flasks for 3 hours at 38° in 2.0 ml of the Krebs-Ringer-glucose medium containing 22 µmoles of C14-chylomicron triglycerides per ml.

Ried out as described above. It can be seen that 95% of the radioactivity could be recovered as tripalmitin in the insoluble fraction. It should be noticed that approximately 3% of the triglycerides were precipitated under the conditions described above. The percentage of insoluble triglycerides varied between 2 and 5% of the total triglycerides isolated from the adipose tissue of the animals used in this study.

The insoluble triglycerides are referred to as C14-tripalmitin, although the precipitate probably contains mixtures of di- and tri-saturated fatty acids. The alcohol-acetone soluble triglycerides are referred to as "soluble" triglycerides. In the experiments to be described, the C14-Lipomul substrate was added to a portion of the adipose tissue at zero time and immediately carried through the processes described above. This represented the zero time distribution of the C14-tripalmitin in the insoluble and soluble fractions. The amount of soluble radioactivity noted at zero time was subtracted from the amount found in the soluble fractions after the incubation of the tissues.

Lipoprotein Lipase Activity—Lipoprotein lipase activities of the residues obtained from the acetone ether extracted tissues were determined by the method described by M. Rodbell (20) except that "activated" cottonseed oil emulsions rather than coconut oil emulsions were used as substrate. Activities are expressed as microequivalents of fatty acids produced per hour per milligram of protein. Protein was determined by the method of Lowry et al. (19). C14O2 was determined by counting the center well KOH solutions directly in the scintillation counter according to the anthracene method described by Steinberg (20).

**RESULTS**

The uptake of isotope labeled triglycerides was found to be proportional to the wet weight of the adipose tissues (Fig. 1).

**Substrate Specificity and Metabolism of Triglycerides**—In Table II are shown some representative results obtained from experiments comparing the ability of adipose tissue to metabolize equal concentrations of rat chylomicrons, activated C14-Lipomul, and washed C14-Lipomul. It can be seen that the total lipid radioactivity in the tissues was essentially the same for the three substrates (Table II, Column 8). The quantity of labeled triglycerides disappearing from the medium did not correspond to the total radioactivity recovered in the tissues (Table II, Column 1). It was also observed that values for disappearance were quite variable in contrast to the values for the tissue radioactivity. These discrepancies were probably due to the sampling errors introduced by the variable breakdown of the emulsions to large oil droplets.

The specific activities of the substrates in the medium did not change during the incubation period (Column 6), indicating that no significant quantities of unlabeled endogenous triglycerides were entering the medium from the tissues. Furthermore, since the specific activities of the triglycerides in the medium did not rise, it appeared that the adipose tissue can metabolize tripalmitin as well as the other triglycerides present in the emulsion.

It should be noted that there is a correspondence between the increase in the quantity of labeled free fatty acids and the total fatty acids in the tissue (Columns 4 and 5). This might indicate that the fatty acids produced by the hydrolysis of the exogenous triglycerides entered a common pool of fatty acids formed from the endogenous sources of fatty acids. Alternatively, it is possible that the labeled triglycerides were mixed with the endogenous pool of triglycerides and subsequently metabolized. If
this were the case the resultant fatty acids should have a specific activity comparable to that of the total esterified fatty acids in the tissue. However, as shown in Table III, the specific activity of the free fatty acids is over 200 times greater than the specific activity of the esterified fatty acids.

No significant hydrolysis of exogenous triglycerides was detected in the medium at any time during the incubation period. This was examined by removing the tissues at various time intervals, adding albumin to ensure a maximal rate of hydrolysis (18) and further incubating the medium for 2 hours. No increase in fatty acid concentration was observed. Accordingly, this would indicate that the hydrolysis of exogenous triglycerides occurred at some tissue site and not in the medium.

Rate of Uptake and Metabolism—The free fatty acid content of the tissue rose rapidly for the first 60 minutes and then leveled off, whereas the C14 activity of the ester fraction continued to rise as shown in Fig. 2. Since the total amount of labeled triglycerides increased with time, the free fatty acid concentration probably represents the steady state concentration resulting from the hydrolysis of exogenous triglycerides and the fatty acids formed from endogenous sources. From the previous results (Table III) the steady state concentration of fatty acids derived from chylomicron triglycerides would vary according to the quantity of endogenous fatty acids.

\[
\text{Rate of Uptake of Triglycerides} = \text{Rate of Uptake of Free Fatty Acids} - \text{Rate of Metabolism}
\]

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Effects of Metabolic Inhibitors on Metabolism and Removal of Chylomicrons—Cyanide (2 \( \times 10^{-4} \) M), dinitrophenol (5 \( \times 10^{-4} \) M), and epinephrine (0.5 \( \mu \)g per ml) did not alter the rate of uptake of triglycerides (Table IV). However, their addition caused a marked increase in the quantity of fatty acids produced both from the chylomicrons of the medium and from endogenous triglycerides.

Since dinitrophenol and cyanide are inhibitors of energy utilization, the apparent stimulation of fatty acid production by these inhibitors was ascribed to an increased rate of hydrolysis of endogenous triglycerides.

Initial uptake fell off rapidly. Thus, an 8-fold increase in concentration resulted in a 3-fold increase in uptake. On the other hand, after 4 hours incubation, the total uptake was directly related to concentration of up to 25 \( \mu \)moles of triglyceride per ml. These results could be explained if the initial uptake represented the adsorption of triglycerides to sites of limited capacity, whereas the later phase of uptake represented the relatively higher capacity of the enzyme systems affecting the metabolism and deposition of the triglycerides.

Effects of Metabolic Inhibitors on Metabolism and Removal of Chylomicrons—Cyanide (2 \( \times 10^{-4} \) M), dinitrophenol (5 \( \times 10^{-4} \) M), and epinephrine (0.5 \( \mu \)g per ml) did not alter the rate of uptake of triglycerides (Table IV). However, their addition caused a marked increase in the quantity of fatty acids produced both from the chylomicrons of the medium and from endogenous triglycerides.

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these compounds might be due to their inhibition of some energy-requiring step necessary for the esterification of fatty acids. This could result in an increase in fatty acid production without altering the rate of hydrolysis of the exogenous triglycerides by the tissue lipase. Indeed, it should be noted that the content of lipoprotein lipase in the tissues was not affected by cyanide, dinitrophenol, or epinephrine. It is possible, of course, that a localized stimulation of the tissue lipase could occur without altering the apparent content in the tissue.

Several investigators (15, 21, 22) have found that epinephrine elicits an apparent increase in production of fatty acids from adipose tissue both in vivo and in vitro. It should be noted that epinephrine increased the production of fatty acids only in the absence of glucose in the medium (23), indicating that the mode of action of epinephrine is probably not the same as cyanide and dinitrophenol, since the latter caused an elevation in the quantity of fatty acids in the presence of glucose.

**Conversion of C^{14}-Tripalmitin to C^{14}-Triglycerides Containing Mixtures of C^{14}-Palmitic and Unsatuated Fatty Acids**—The results of the previous experiments suggested that exogenous triglycerides were hydrolyzed in the cytoplasm to fatty acids which were subsequently re-esterified. In order to measure the extent of re-esterification of the exogenous triglycerides, it was necessary to distinguish between the quantity of triglycerides that may have been removed intact from the medium and those formed by re-esterification of fatty acids produced from the exogenous triglycerides. The experiments presented in Figs. 4 to 6 were designed to test this question. C^{14}-tripalmitin, representing exogenous triglycerides, was readily separated from triglycerides containing mixtures of C^{14}-palmitic acid and unsaturated fatty acids by the alcohol-acetone precipitation method described in the text under “Experimental.”

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**Fig. 4.** Effect of DFP on uptake and distribution of activated C^{14}-Lipomul between “soluble” and saturated di- and triglycerides (C^{14}-tripalmitin). Adipose tissues from pair-fed rats were sectioned into eight segments of approximately equal size. Four of the segments were incubated separately in the Krebs-Ringer-glucose medium containing 10 μmoles of activated C^{14}-Lipomul triglyceride per ml. Four of the segments were incubated under the same conditions without the addition of DFP. C^{14}-tripalmitin refers to the insoluble C^{14}-triglycerides precipitated with alcohol-acetone (85:15) at 5° as described in the text under “Experimental.”

**Fig. 5.** Effect of temperature on uptake and distribution of activated C^{14}-Lipomul between “soluble” and saturated di- and triglycerides (C^{14}-tripalmitin). Adipose tissues from pair-fed rats were cut into eight sections of approximately equal weight and were incubated in separate flasks in 2.0 ml of Krebs-Ringer-glucose medium containing 10 μmoles of activated C^{14}-Lipomul triglyceride per ml. Four of the flasks were incubated at 20° and the remaining four at 38°. The tissue triglycerides were separated into “soluble” triglycerides and saturated triglycerides (C^{14}-tripalmitin) by the alcohol-acetone precipitation method described under “Experimental.”

**Fig. 6.** Effect of albumin on uptake and distribution of C^{14}-Lipomul between soluble and saturated di- and triglycerides. Adipose tissues from pair fed rats were cut into eight sections of approximately equal weight and were incubated in 2.0 ml of the Krebs-Ringer-glucose medium containing 10 μmoles of activated C^{14}-Lipomul triglycerides per ml. Four of the flasks contained albumin (Armour’s bovine) at a concentration of 50 mg per ml of medium. After removing the tissues at the indicated time periods, the tissue triglycerides were separated into “soluble” triglycerides and saturated triglycerides (C^{14}-tripalmitin) by the alcohol-acetone precipitation method described under “Experimental.”
acids by precipitating the saturated triglycerides from an alcohol-acetone (85:15) solution at 5°C (cf. "Experimental"). Assuming that the C⁴-triglycerides not precipitated by alcohol-acetone ("soluble" triglycerides) were formed by the re-esterification of C⁴-fatty acids produced from the labeled exogenous substrate, a reasonable estimate could be made of the rate of conversion of exogenous triglycerides to re-esterified fatty acids. The validity of this experiment rests on the small probability of the re-esterification of C⁴-palmitic acid to di- and trisaturated triglycerides. Since Lipomul (a cottonseed oil emulsion) contains unsaturated fatty acids primarily, it was assumed that the trace amounts of C⁴-palmitic acid derived from the tripalmitin would be mixed with fatty acids recovered in the heptane soluble phase from the incubation of adipose tissue with C⁴-Lipomul, were saponified and the resultant fatty acids oxidized with periodate-permanganate (24). Accordingly, the C⁴-"soluble" triglycerides, isolated after the incubation of adipose tissue with C⁴-Lipomul, were saponified and the resultant fatty acids oxidized with periodate-permanganate (25). This method oxidizes unsaturated fatty acids to mono- and dicarboxylic acids which could be readily separated from saturated or nonoxidizable acids by partitioning the fatty acids between heptane and 70% acetic acid. As shown in Table V, 90% of the radioactivity was recovered in the saturated fatty acid fraction (heptane soluble fraction), whereas the acetic acid phase, representing the oxidized fatty acids, contained only 10% of the radioactivity. This would appear to rule out any significant desaturation of C⁴-tripalmitin or palmitic acid.

Effect of DFP on Triglyceride Uptake and Metabolism—DFP⁶ has been shown to be a potent inhibitor of lipoprotein lipase (26). The addition of DFP to the medium did not appreciably alter the total uptake of triglycerides by adipose tissue, although the lipoprotein lipase activity was reduced to approximately one-sixth the control tissue (Table VI). However, the recovery of C⁴-fatty acids in the DFP-treated tissue was one-third that found in the control tissue, which is consistent with the observed inhibition of the lipase by DFP. It could not be determined, under these experimental conditions, whether DFP inhibited the hydrolysis of endogenous triglycerides.

The rate of uptake of triglycerides in the presence of DFP was not substantially different from the control tissue as shown in Fig. 4. However, DFP inhibited the conversion of C⁴-tripalmitin to C⁴-"soluble" triglycerides as expected, if the conversion required the prior formation of free fatty acids (cf. Table III). These results demonstrate, therefore, that adipose tissue can remove exogenous triglycerides from the medium without their prior hydrolysis.

Temperature Effects—A marked decrease in the rate of uptake and subsequent metabolism of exogenous triglycerides was observed at 20°C as compared to 38°C (Fig. 5). The recovery of 1.2 μeq of exogenous C⁴-ester from the tissues incubated for 30 minutes at 38°C, compared to less than 0.2 μeq at 20°C for the same time interval, indicates that the removal of exogenous triglycerides is temperature dependent. However, in contrast to the 3.5-fold increase in the removal rate, there was a 10-fold increase in the rate of re-esterification of the triglycerides at 38°C for a 30-minute incubation, which suggests that the process of initial uptake of the triglycerides is less temperature dependent than their subsequent metabolism.

Stimulation of Triglyceride Uptake by Albumin—The addition of albumin (50 mg per ml) caused over a 2-fold increase in the total uptake of "activated" C⁴-Lipomul and some increase in the metabolism of the triglycerides (Fig. 6). Approximately 70% of the exogenous triglycerides taken up by the tissue were converted to the "soluble" form within 30 minutes in the presen...
TABLE VII

Effect of nutritional state of animal on metabolic patterns and uptake of triglycerides by adipose tissue

Same conditions as described in Table II, 25.0 μmoles of "activated" C¹⁴-Lipomul triglycerides per ml of medium. The probability values given refer to differences between fed and fasting animals (24-hr fasted rats). The mean and standard error of the mean is given in each case.

<table>
<thead>
<tr>
<th>Nutritional condition</th>
<th>No. of experiments</th>
<th>Total uptake C¹⁴</th>
<th>C¹⁴-Fatty acids</th>
<th>Conversion of C¹⁴-tripalmitin to &quot;soluble&quot; triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μg/100 g tissue</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Fasting rats</td>
<td>6</td>
<td>9.23 ± 2.21</td>
<td>33 ± 11</td>
<td></td>
</tr>
<tr>
<td>Fed rats</td>
<td>6</td>
<td>8.90 ± 1.92</td>
<td>72 ± 15</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.9</td>
<td>&lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Relationship Between Serum Lipoproteins and Tissue Lipase to Equally important is the observation that, once removed, the triglycerides of the medium to the "soluble" form as the tissues from fasting animals (Column 3). Previous results (cf. Table III) suggested that the triglycerides of the medium do not mix, during their metabolism, with the main pool of triglycerides in the tissue. This might indicate that the storage or net uptake of triglycerides by adipose tissue triglycerides and that albumin exerted its effect simply by causing a release of the tissue fatty acids into the medium.

"Nutritional" State of Adipose Tissue and Metabolism of Triglycerides—An approximately 2-fold increase in fatty acids produced from "activated" C¹⁴-Lipomul was observed in the tissues from fasting animals, as compared to tissues from rats maintained on a normal chow diet (Table VII, Column 2). Whereas the total uptake of labeled triglyceride was approximately the same in the fasting and fed states (Column 1), the adipose tissues from the fed animals converted over twice as much of the C¹⁴-Lipomul in the medium to the "soluble" form as the tissues from the fasting animals (Column 3). Previous results (cf. Table III) suggested that the triglycerides of the medium do not mix, during their metabolism, with the main pool of triglycerides in the tissue. This might indicate that the storage or net uptake of triglycerides in the medium triglyceride pool requires prior hydrolysis and re-esterification of the exogenous triglycerides. Accordingly, the results described in Table VII indicate that the adipose tissue from the fed animals effected a net increase in the storage form of the exogenous triglycerides of 6 to 7 μeq of ester per g of tissue, in contrast to 2.5 to 4 μeq per g of tissue in the fasting state. The remainder of the radioactive fat in the tissue represents that amount of exogenous triglyceride which had not been hydrolyzed but is still associated with some cellular compartment other than the fat globule.

Discussion

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that exogenous triglycerides can be removed from the medium by adipose tissue without their prior hydrolysis, as evidenced by the finding that inhibition of the tissue lipase with DFP did not appreciably inhibit the removal of triglycerides and that intact exogenous triglycerides could be isolated from the tissues. Serum or chylomicron proteins are apparently not required for this process. This is in contrast to the observation made by Shapiro et al. (27) that fat uptake by mesenteric adipose tissue was found to be dependent upon the presence of serum in the medium. This disparity, however, may well be due to the particle size and stability of the fat emulsions used in their studies. In this respect, albumin appeared to cause a greater dispersion of the fat emulsion and could account for the observed increase in fat uptake when albumin was added to the medium.

Although the action of a tissue lipase is not obligatory for the removal of triglycerides by the adipose tissue, it is obvious that the metabolism of triglycerides is dependent upon their hydrolysis to fatty acids by the tissue lipase. It can be inferred from the finding that inhibitors of energy utilization such as dinitrophenol and cyanide caused an increase in free fatty acids produced from both endogenous and exogenous triglyceride, that the energy state of and, perhaps, availability of glycerol precursors determines the extent of the metabolic distribution of the exogenous and endogenous fat between triglycerides and fatty acids. This is also suggested by the results demonstrating that the net uptake of triglycerides in the tissues is increased in the adipose tissues obtained from fed animals as compared to the tissues from fasting animals. Similar results have been found for the uptake in vitro of labeled fatty acids by adipose tissue (28).

Mechanism of Removal of Chylomicrons by Adipose Tissue—One of the most interesting aspects of chylomicron transport and metabolism is the manner in which the triglycerides are removed from the blood. It should be emphasized that the results of this investigation are not pertinent to the problem of fat traversing the capillary endothelium, since this study pertains to the transfer of fat across the peritoneal membrane surrounding the fat cells. However, with this limitation, there were some interesting relationships to the observations made in vivo.

The initial rate of removal is quite rapid; under conditions where maximal removal rates were observed (cf. Fig. 6), between 0.5 and 1.0 μ mole of triglyceride per g of tissue was taken up in 30 minutes. Extrapolating to a 200-g rat (13% fat (30)), approximately 10 to 20 mg of triglyceride would be removed in 30 minutes, as compared to the observations in vivo that between 10 and 15 mg of fat disappear from the blood stream 10 minutes after the intravenous injection of 20 to 30 mg of chylomicron triglycerides (31). Since about 25% of the chylomicron triglycerides appear in adipose tissue under optimal conditions (5), the in vitro values are remarkably close to the findings in vivo.

Equally important is the observation that, once removed, the C¹⁴-triglycerides are neither exchangeable with unlabeled triglycerides, by the observation that the triglyceride emulsions were noticeably less turbid in the presence of 5% albumin, which suggests that the albumin increased the rate of uptake considerably as compared to 50% in the control. It would appear that the albumin increased the rate of uptake considerably more than the rate of re-esterification. This might be explained by the observation that the triglyceride emulsions were noticeably less turbid in the presence of 5% albumin, which possibly indicates that the particles were more dispersed or stabilized.

The addition of albumin elicited the appearance of 65 to 70% of the total free fatty acids in the medium as compared to less than 1% in the control flask, as has been reported by Reshef et al. (12). In the experiment described in Fig. 6 the C¹⁴-fatty acid content of the medium plus tissue at the end of 3 hours of incubation was essentially the same (0.53 and 0.75 μeq per g of tissue in the nonalbumin- and albumin-treated tissues, respectively). These results suggest that the presence of albumin had little effect on the hydrolysis of adipose tissue triglycerides and that albumin exerted its effect simply by causing a release of the tissue fatty acids into the medium.

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This is also suggested by the results demonstrating that the net uptake of triglycerides in the tissues is increased in the adipose tissues obtained from fed animals as compared to the tissues from fasting animals. Similar results have been found for the uptake in vitro of labeled fatty acids by adipose tissue (28). Bragdon and Gordon (5) have observed that the nutritional state of the animal determines the tissue distribution of labeled fat shortly after the injection of chylomicrons labeled with C¹⁴-palmitic acid. Carbohydrate feeding caused a dramatic increase in the appearance of C¹⁴-triglycerides in the adipose tissue compared to the fasting animals. Thus, it would appear from the findings in vitro and in vivo that the storage of exogenous fat by the adipose tissue is dependent upon its hydrolysis and the subsequent re-esterification of the fatty acids to triglycerides, as has been previously proposed by Shapiro (29).
erides nor reversibly removed by washing. Furthermore, the rate of fat uptake appears to reach a maximum and then rapidly fall off in spite of the fact that metabolism of the fat continues. It would appear from these results that the initial uptake represents a compartmentalization of the fat at sites which rapidly become saturated and thus limit further uptake. Similarly, it has been found in vivo that the metabolism of the chylomicron triglycerides takes place in a separate compartment from the total body pool of fat for as long as 90 minutes after their disappearance from the blood (32).

Since there appears to be some similarity between the findings in vitro and in vivo, it is pertinent to point out that the removal of chylomicrons in vitro resembles, in some respects, the behavior of particles which are known to be taken up by phagocytosis. Chylomicrons and carbon particles, for example, are removed from the blood at a rate inversely proportional to their initial concentration (31) (cf. Fig. 3). This is said to be characteristic of phagocytic activity (33). In this respect, it is of great interest that pinocytosis (a cellular process which involves the plasma membrane and which resembles phagocytosis) has recently been shown by Palay and Karlin (34) to be involved in the absorption of fat by the intestine. In addition, there has been a recent report (35) that adipose tissue displays cellular processes characteristic of pinocytosis. The data presented in this paper are compatible with an absorption process such as pinocytosis, and the mechanism of fat uptake by adipose tissue, as possibly related to this process, is presently under investigation.

SUMMARY

1. Rat chylomicrons and a synthetic fat emulsion were found to be taken up and metabolized to an equivalent extent by rat epididymal adipose tissue, suggesting that the chylomicron proteins are not essential for fat uptake or metabolism by this tissue.

2. Inhibition of the tissue lipase did not substantially reduce the rate of uptake of triglycerides by adipose tissue, although there was a marked reduction in the production of fatty acids derived from chylomicron triglycerides or synthetic fat emulsions.

3. Chylomicron triglycerides and synthetic triglyceride emulsions were found to be taken up intact, before their metabolism, into a tissue compartment in which the triglycerides were no longer exchangeable with triglycerides of the medium and were inaccessible to removal by washing. It is suggested that the tissue compartment represents the rim of cytoplasm or cytoplasmic membrane surrounding the fat globule in the adipose cell.

4. The effects of metabolic inhibitors, such as cyanide and dinitrophenol, and the nutritional requirements found to be necessary for maximal net incorporation of exogenous triglycerides suggest that storage of chylomicron triglycerides in adipose tissue occurs subsequent to hydrolysis and re-esterification.

5. The possibility that chylomicron triglycerides are taken up intact through a process resembling pinocytosis is discussed.

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REFERENCES

27. Shapiro, B., Weismann, D., BENTOR, V., and Wertheimer, E., Metabolism, 1, 206 (1952).