Mechanism of triglyceride accumulation in rat preadipocyte cultures exposed to very low density lipoprotein

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Abstract Cultured preadipocytes derived from the stromal-vascular fraction of adipose tissue have been shown to accumulate sufficient triglycerides to assume adipocyte morphology when exposed to high concentrations of very low density lipoprotein. Since these cells synthesize and secrete lipoprotein lipase it was of interest to determine whether the accumulation of intracellular triglyceride originated from the uptake of products of the lipase reaction or whether the cells were utilizing intact lipoprotein particles. Upon incubation of preadipocytes with very low density lipoprotein, the triglyceride disappeared from the medium and accumulated in the cells. This response was accentuated by the addition of heparin to the culture medium. Balance studies conducted in the presence of heparin demonstrated that the loss of medium triglyceride could account for the increase in cell stores. Quantitative studies demonstrated that the increase in cellular triglyceride was a result of the cellular uptake and reesterification of the fatty acids liberated from very low density lipoprotein triglycerides by the action of cellular lipoprotein lipase. The magnitude of the cellular response was dependent on the concentration of fetal bovine serum in the incubation medium and increased as the serum level decreased. Likewise, when albumin was substituted for the serum, increasing amounts of albumin decreased the cellular triglyceride accumulation. It was concluded that the presence of albumin in the culture medium modulated cellular triglyceride accumulation by *1*) influencing the extent of triglyceride lipolysis and *2*) by regulating the uptake of liberated fatty acid into the cells. Experiments using very low density lipoprotein containing radiolabeled triglyceride and cholesteryl esters demonstrated that the uptake of the very low density lipoprotein or the remnant particle produced by the action of lipase did not significantly contribute to the accumulation of triglyceride in the cells.—**de la Llera, M., J. M. Glick, and G. Rothblat.** Mechanism of triglyceride accumulation in rat preadipocyte cultures exposed to very low density lipoprotein. *J. Lipid Res.* 1981. **22:** 245–253.

Supplementary key words lipoprotein lipase · fatty acids · albumin

It has been demonstrated that cells in culture will accumulate intracellular triglyceride stores when exposed to high concentrations of lipoproteins (1, 2). In the case of fibroblasts and endothelial cells, the accumulation of triglyceride has been shown to be related to the uptake of intact triglyceride molecules (3, 4). The actual mass of triglyceride stored in these cells is rather modest when compared to that observed for cultured cells derived from the stromal-vascular fraction of adipose tissue (5). The adipose-derived cells, which have been called preadipocytes, accumulate sufficient triglyceride to assume adipocyte morphology when exposed to high concentrations of very low density lipoprotein (VLDL) (5) or Intralipid (6). Since the adipose-derived cells synthesize and secrete lipoprotein lipase (7, 8), it was of interest to determine whether the accumulation of intracellular triglyceride was a result of uptake of products of the lipase reaction or whether the cells were utilizing intact lipoprotein particles.

MATERIALS

All solvents for lipid extraction were obtained from Arthur H. Thomas Company, Philadelphia, PA and were analytical reagent grade. All chemicals used were reagent grade. Williams medium E was obtained from Flow Laboratories, Inc., McLean, VA, antibiotics from Microbiological Associates, Bethesda, MD, and fetal bovine serum from Gibco, Grand Island, NY. Flasks and plates for tissue culture were bought from Lux Scientific Corporation, McLean, VA. Bovine albumin preparations used were either Fraction V (essentially fatty acid-free) from Sigma Chemical Company, St. Louis, MO, or Fraction V from Miles Laboratories, Elkhart, IN. Heparin was bought from Calbiochem Co., LaJolla, CA. HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid) was obtained from Sigma Chemical

Abbreviations: VLDL, very low density lipoprotein; FBS, fetal bovine serum.

Co., St. Louis, MO. [4-^{14}C]Cholesterol was obtained from New England Nuclear, Boston, MA. Tri [9,10-(n)-^{3}H]oleoylglycerol, trioleoyl[2(n)-^{3}H]glycerol, tri[1-^{14}C]oleoylglycerol, and [4-^{14}C]cholesteryl linoleate were obtained from Amersham Searle, Amersham, England. Radiolabeled lipids were purified before use by thin-layer chromatography on silica gel G plates as described below for cellular lipids. Precoated silica plates were obtained from Analtech, Inc., Newark, DE. ScintiVerse (Fisher Scientific Co., NJ) was used to measure radioactivity.

METHODS

Cells

Cultures of rat preadipocytes were grown from collagenase digests of epididymal fat pads obtained from mature Fischer 344 rats as previously described (7, 8). These cells were grown in Williams medium E buffered with sodium bicarbonate and supplemented with 10% fetal bovine serum (FBS) and antibiotics (50 μg/ml penicillin, 50 μg/ml streptomycin). Cells used for experiments were between the second and fourth passage. Each passage is approximately equivalent to one population-doubling of a cell line obtained from a primary culture. Cells were grown to confluent monolayers in either petri plates (35 mm and 60 mm) or flasks (75 cm^2) and were kept at 37°C in 6% CO_2–94% air. Cultures were fed twice a week and used 2 to 3 weeks after plating. Confluent monolayers of these cells released lipoprotein lipase to the culture medium when treated with heparin (7, 8).

Preparation of isolated lipoprotein fractions

Human very low density lipoprotein (VLDL) was prepared from citrate-buffered plasma using outdated blood obtained from the blood bank at the Medical College of Pennsylvania in a Beckman L3-50 ultracentrifuge using standard density flotation procedures as modified by Marsh (9). Chylomicrons were removed by centrifugation for 30 min at 10,000 rpm in a Sorvall RC2B centrifuge. VLDL was isolated at d 1.006 g/ml, sterilized by filtration through a 0.45 μm Millipore filter and stored at 4°C.

Cell incubations

To start an experiment, confluent monolayers of preadipocytes were refed with medium containing the desired amount of VLDL triglyceride. Incubations were always done in the presence of either fetal bovine serum (FBS) or fatty acid-free bovine serum albumin. The minimum amount of FBS used was 2% or its equivalent albumin content (0.4 mg/ml). Heparin was used at 10 IU/ml. Cells were maintained at 37°C in 6% CO_2–94% air.

To end an experiment the medium was decanted, the cells were rinsed five times with cold phosphate-buffered saline, and the drained monolayers were immediately frozen. When medium triglyceride levels were measured, aliquots of the medium were either collected directly in methanol or immediately frozen. In each experiment cell incubations and medium triglyceride assays were done in duplicate.

Analysis of lipoproteins and cells

Total triglyceride in cells and lipoproteins was determined as previously described (2). Briefly, total lipids were extracted by the procedure of Bligh and Dyer (10) and the triglycerides were isolated by thin-layer chromatography on precoated silica plates in a solvent system of light petroleum–diethyl ether–acetic acid 74:25:1 (v/v). A known amount of [^{14}C]cholesterol was added to each sample immediately before lipid extraction to monitor recovery and the triglycerides were quantified by H_2SO_4 charring (11). Cell protein was measured by the method of Lowry et al. (12).

Lipoprotein lipase release and lipase treatment of VLDL

To release lipoprotein lipase from cells, confluent monolayers in 75 cm^2 tissue culture flasks were washed with serum-free medium buffered at pH 7.4 with HEPES. The same medium containing 10 IU/ml of heparin was then added and the cells were incubated for 4 hr at 37°C. The medium was then decanted and stored at −70°C. Lipoprotein lipase activity was measured using a modification of the method of Nilsson-Ehle and Schotz (13). To measure lipolysis of VLDL in the absence of cells, lipoprotein lipase was added to 300 μg of VLDL in 1 ml of Williams medium E buffered with HEPES at pH 7.4 and containing 10 mg/ml of bovine albumin. Incubations were done in duplicate at 37°C for varying lengths of time and terminated by adding 2 ml of methanol to each tube. Following lipid extraction, triglyceride per tube was determined as previously described.

Isotope addition to VLDL

VLDL was prepared as described and then washed once by reflotation at d 1.006 g/ml. Radioactive lipids were dissolved in dimethyl sulfoxide and introduced into the VLDL following the procedure of Fielding (14). The labeled VLDL was reisolated by centrifugation. A 1/20 volume of 40% sucrose was

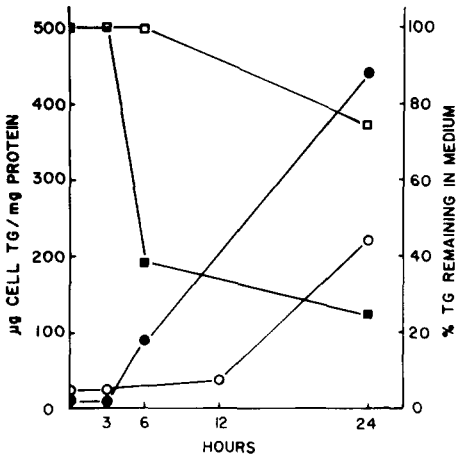

Fig. 1. Time course of VLDL triglyceride utilization by preadipose cells with and without heparin. Preadipose monolayers, grown in 35-mm petri plates, were incubated with medium containing 0.4 mg/ml albumin and 100 μg VLDL triglyceride/ml at 37°C. At the indicated times, duplicate plates were harvested and the medium and cell triglyceride were measured. Closed symbols represent cultures incubated in the presence of heparin (10 IU/ml). ● —— ●, ○ —— ○, cell triglyceride; ■ —— ■, □ —— □, % triglyceride remaining in medium.

added to the VLDL. This VLDL preparation was then sequentially centrifuged: 10 min at 12,000 rpm, 30 min at 17,500 rpm, and finally 24 hr at 38,000 rpm. All centrifugations were in a Beckman ultracentrifuge using a 40 rotor. Prior to each centrifugation the VLDL preparation was overlaid with saline (d 1.006 g/ml). The top 1-ml of supernatant was removed following each centrifugation. Using this procedure, 70% of the starting radioactivity was recovered in the VLDL preparation collected after the final 38,000 rpm centrifugation. The following radiolabeled lipoproteins were prepared: *1*) VLDL singly labeled with tri[9,10(n)-^{3}H]oleoylglycerol (596 mCi/mmol); *2*) VLDL doubly labeled with the same [^{3}H]triolein plus [4-^{14}C]cholesteryl linoleate (20.9 mCi/mmol); and *3*) VLDL doubly labeled with trioleoyl[2(n)-^{3}H]glycerol (478 mCi/mmol) and tri[1-^{14}C]oleoylglycerol (55 mCi/mmol). Radioactivity was measured in a Packard Tri-Carb Liquid Scintillation Spectrometer.

RESULTS

We have proposed that the cell triglyceride accumulation observed when rat preadipocytes were treated with VLDL and heparin was due to release of lipoprotein lipase by these cells with the subsequent uptake and reesterification of a product of VLDL lipolysis (5). To characterize further the cellular response to lipoprotein lipase degradation of VLDL, both the time course of VLDL triglyceride disappearance from the medium and the concomitant cellular triglyceride increase were followed in the presence and absence of heparin. The results of this experiment are shown in **Fig. 1.** It can be seen that while the disappearance of VLDL triglyceride from the heparin-containing medium is initially very rapid, the increase in cell triglyceride lags behind the rapid changes in the medium. In the absence of heparin, the change in medium VLDL triglyceride occurs at a slower rate; less triglyceride is hydrolyzed and, in turn, the cells do not accumulate as much lipid. In a number of experiments we found that the increase in cell triglyceride stores could be accounted for by the loss of triglyceride from the culture medium (**Table 1**), thus suggesting that the source of cell triglyceride is one of the products liberated during VLDL lipolysis.

During the course of these experiments it was consistently observed that from 30% to 50% of the starting triglyceride remained intact. Since released lipase activity has a short half life, the following experiment was designed to determine if the incomplete VLDL lipolysis was due to inactivation of the enzyme. Medium was supplemented with 10%

TABLE 1. Changes in medium and cellular triglyceride upon exposure of rat preadipocyte cells to VLDL

Experiment	Treatment	Total Medium Triglyceride		Triglyceride Lost from Medium	Cellular Triglyceride[a]
		Start	End		
				μg	
1	1 Day + Heparin	228	60	168	90
	1 Day − Heparin	228	174	54	43
2	1 Day + Heparin	1500	910	590	61
	4 Days + Heparin	1500	820	680	296
	7 Days + Heparin	1500	820	680	512

[a] The triglyceride content of the cells at the start of Experiment 1 was less than 5 μg (35-mm plates). This value was 28 μg in Experiment 2 (60-mm plates). Both have been subtracted to indicate the net increase in cell triglyceride.

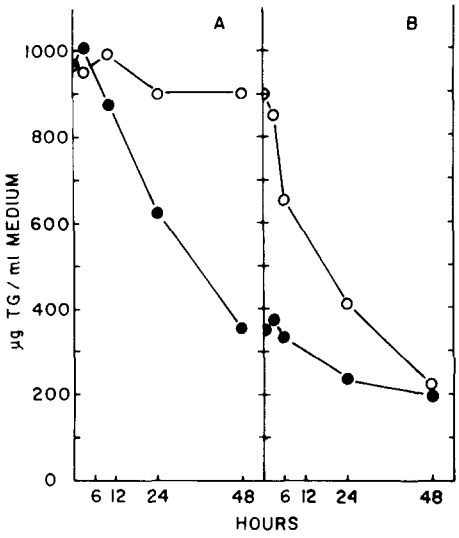

Fig. 2. Effect on triglyceride lipolysis of repeated exposure of VLDL to preadipocytes. Medium containing 100 μg VLDL triglyceride/ml, 10 IU/ml heparin, and 10% FBS was divided in half and incubated at 37°C for 48 hr in tissue culture flasks (75 cm²) both in the presence (● —— ●) and absence (○ —— ○) of preadipocytes (2A). Both halves were then transferred to new flasks containing fresh cells and the medium was further incubated for an additional 48 hr at 37°C (2B). Aliquots of medium from all flasks were sampled and the triglyceride content of each was measured.

FBS, VLDL, and heparin. One-half of this solution was incubated with preadipocytes for 2 days and the other half was kept at 37°C. After the initial incubation, both VLDL solutions were further incubated for an additional 2 days with replicate preadipocyte cultures that had not been previously exposed to VLDL or heparin. Aliquots of medium were taken throughout. The results of this experiment are shown in **Fig. 2.** VLDL triglyceride decreased during the first incubation with preadipocytes to 38% of the original value (Fig. 2A). When this treated VLDL was incubated further with fresh cells for an additional 48 hr, the triglyceride content of the medium was only slightly decreased and 20% of the starting triglyceride remained at the end of the second incubation (Fig. 2B). Incubation of the other half of the original VLDL solution at 37°C for 2 days caused no change in its triglyceride content, but addition of this VLDL to preadipocytes resulted in a disappearance curve almost identical to that observed during the first incubation with cells (Fig. 2B). Similar results were obtained when VLDL was incubated with lipoprotein lipase released by heparin from cultured preadipocytes. When VLDL was incubated at 37°C with lipase and the time course of triglyceride disappearance was monitored for several hours, the reaction rate decreased with time and approximately 25% of the starting triglyceride remained intact at the end of 6 hr (**Fig. 3**). To determine if the decreasing rate of lipolysis was due to inactivation of the lipoprotein lipase, a similar experiment was performed, except that fresh enzyme was added after 4 hr and the incubation was continued for an additional 2 hr. The amount of triglyceride remaining in these tubes was identical to that found in parallel samples incubated for 6 hr without the addition of fresh enzyme. The results from these experiments are consistent with the concept that the lipase produced by cultured preadipose cells behaves like other lipoprotein lipases and forms remnant particles which are poor enzyme substrates (15).

The experiments described above suggest that a product released during lipoprotein lipase degradation of VLDL is stored as triglyceride by preadipocytes in culture. Experiments were next done to determine if the fatty acid released during the lipase reaction was being utilized by the cells. Spector, Steinberg, and Tanaka (16) have demonstrated that the important determinant of cellular fatty acid utilization is not the absolute concentration of fatty acid, but rather the fatty acid to albumin molar ratio of the fatty acid-albumin complex formed in solution. It was of interest, therefore, to examine the effects of changes in the concentration of albumin present in the incubation medium on VLDL lipolysis and cell triglyceride accumulation. Because fetal bovine serum is an important component of tissue culture media, all our studies on preadipocyte triglyceride accumulation were initially done in medium supplemented with 10% FBS. When the concentration of FBS used to supplement the cellular incubations with VLDL and

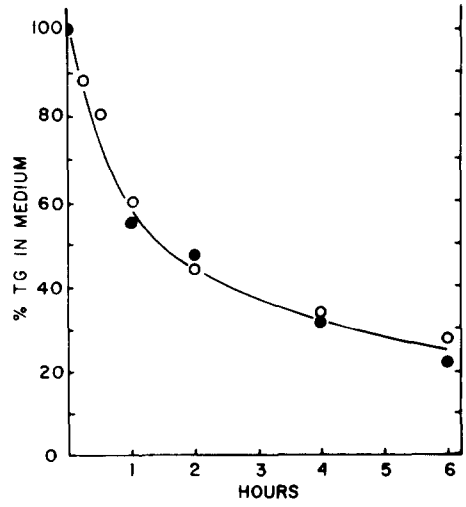

Fig. 3. VLDL triglyceride lipolysis by preadipose lipase in the absence of cells. VLDL (300 μg/ml) was incubated with lipase previously released from rat preadipocytes as described in Methods. The triglyceride was measured at indicated times. Opened and closed symbols represent separate experiments.

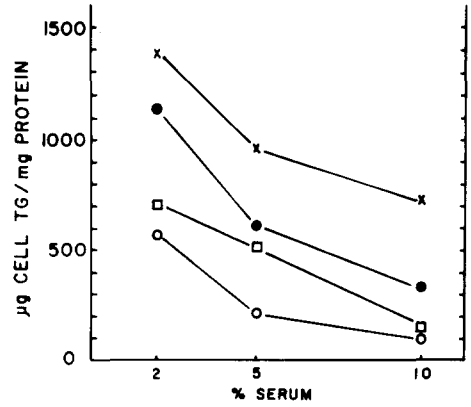

Fig. 4. Effect of serum concentration on triglyceride accumulation in preadipose cells. Replicate monolayers of rat preadipocytes were incubated with medium containing various concentrations of VLDL plus heparin (10 IU/ml) together with either 2%, 5%, or 10% fetal bovine serum. The cell triglyceride content was measured after 4 days at 37°C. X —— X, 800 μg of VLDL triglyceride/ml; ● —— ●, 300 μg of VLDL triglyceride/ml; □ —— □, 268 μg of VLDL triglyceride/ml; ○ —— ○, 100 μg VLDL triglyceride/ml.

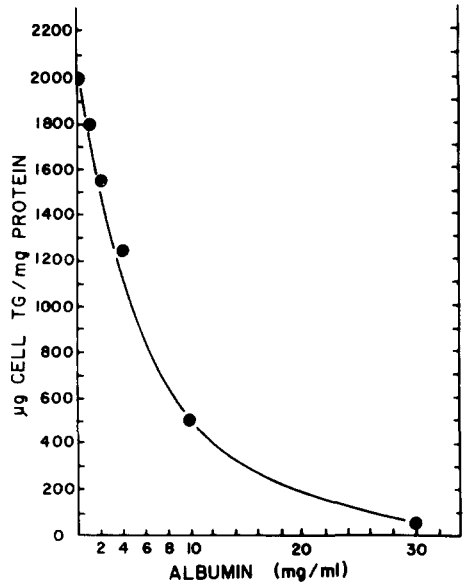

Fig. 5. Effect of albumin concentration on triglyceride accumulation in preadipose cells. Preadipocyte monolayers were incubated with VLDL (400 μg of triglyceride/ml), heparin (10 IU/ml), 2% fetal bovine serum, and the indicated concentrations of fatty acid-free bovine albumin. Cell triglyceride was measured after 4 days at 37°C.

heparin was varied, it was found that at any given concentration of VLDL triglyceride in the medium, the cellular response increased as the concentration of serum decreased (**Fig. 4**). Moreover, if media containing VLDL, heparin, 2% FBS, and increasing amounts of fatty acid-free albumin were incubated with preadipocytes, the cellular response decreased as the medium albumin concentration increased (**Fig. 5**). Both these results strongly suggest that the molar ratio of the fatty acid to albumin mediates the preadipocyte response.

To characterize further the VLDL lipolysis and cell triglyceride accumulation by preadipocytes in culture, isolated VLDL was labeled with radioactive lipids. When [^{3}H]triolein-labeled VLDL was incubated with cells in the presence of heparin, the kinetics of triglyceride disappearance were similar to that of native lipoprotein. The specific activity of the ^{3}H-labeled VLDL did not change during the incubation, indicating that there was no selective utilization of the isotope. No partial glycerides accumulated in either the medium or the cells during the incubation, but there was a time-dependent increase in ^{3}H-labeled cell triglyceride. When lipolysis of ^{3}H-labeled VLDL was monitored in medium supplemented with high and low concentrations of fatty acid-free albumin, the results shown in **Fig. 6** were obtained. As can be seen in Fig. 6A, when the medium albumin concentration was high (40 mg/ml), the fatty acid concentration in the medium increased with time. Much less triglyceride was hydrolyzed at the low albumin concentration (Fig. 6B). As predicted from our experiments with native VLDL, the cellular uptake and re-esterification of ^{3}H-labeled fatty acids was greater when the medium albumin concentration was 4 mg/ml. At an albumin concentration of 40 mg/ml, 79% of the VLDL triglyceride was hydrolyzed in 7 hr and 90% of the lipolytic products remained in the

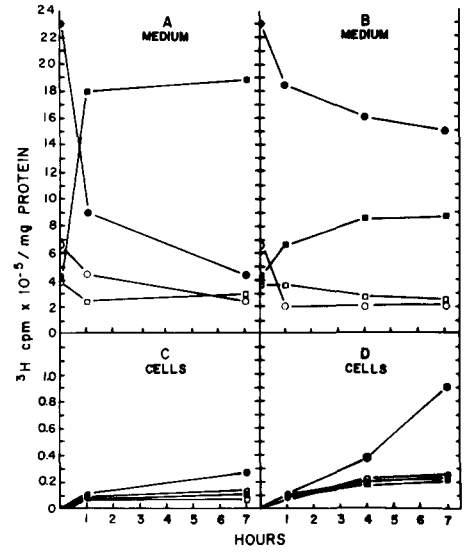

Fig. 6. Metabolism of radio-labeled triglyceride by preadipose cells. Preadipocytes were incubated with medium containing VLDL labeled with tri[9,10(n)-^{3}H]oleoylglycerol (400 μg VLDL triglyceride/ml; 930 cpm/μg triglyceride). The medium also contained heparin (10 IU/ml) and either a high (40 mg/ml, panel A and C) or low (4 mg/ml, panel B and D) concentration of fatty acid-free bovine albumin. The cells were incubated for 6 hr at 37°C and the radioactivity in both the medium and cell lipids was measured at various times. Triglyceride ● —— ●; free fatty acid ■ —— ■; diglyceride □ —— □; monoglyceride ○ —— ○.

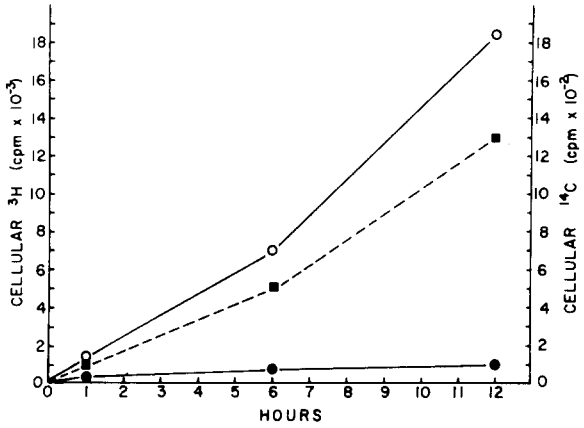

Fig. 7. Cellular incorporation of triglyceride fatty acid and cholesteryl esters by preadipose cells exposed to doubly labeled VLDL. Preadipocytes were incubated with VLDL doubly labeled with tri[9,10(n)-^{3}H]oleoylglycerol and [4-^{14}C]cholesteryl linoleate. The medium contained 200 μg VLDL triglyceride/ml (24×10^4 ^{3}H cpm/ml, 0.17×10^3 ^{14}C cpm/ml), heparin (10 IU/ml), and albumin (0.4 mg/ml). Cells were incubated with this medium at 37°C for 12 hr and both the ^{3}H and ^{14}C radioactivity of the cell lipids were measured at the indicated times. ○ —— ○, ^{3}H cpm in cell total lipids; ● —— ●, ^{14}C cpm in cell total lipid; ■ —— ■ ^{14}C cpm expected in cell total lipid from the ^{3}H/^{14}C ratio of the VLDL triglyceride.

culture medium. At an albumin concentration of 4 mg/ml, less VLDL triglyceride was hydrolyzed (32%) in 7 hr, but only 60% of the liberated fatty acids remained in the medium. At the higher albumin concentration, the fatty acid to albumin molar ratio was 2.5 after 7 hr incubation. At the lower albumin concentration, this molar ratio was 4 after 1 hr and rose to 7.5 after 7 hr incubation.

To eliminate the possibility that cellular internalization of entire VLDL or remnant particles could contribute significantly to the increase in cell triglyceride the following experiment was done. Doubly labeled VLDL was prepared containing [^{14}C]cholesteryl linoleate and tri[9,10(n)-^{3}H]oleoylglycerol. This preparation was then incubated with preadipocytes in medium containing low concentrations of fatty acid-free albumin and the ^{3}H:^{14}C ratio in the medium and cell lipids was measured. Since cholesteryl esters do not readily exchange with cellular lipids, any ^{14}C in the cell would be derived from the internalization of whole lipoprotein particles. **Fig. 7** shows the isotope distribution in cell total lipids. As can be seen, only the ^{3}H entered the cells. The ^{3}H:^{14}C ratio of the cell lipid increased with time and was 10-fold higher than that of the medium lipids after 12 hr incubation. The amount of ^{14}C expected in cell lipids if the ^{3}H:^{14}C ratio of VLDL had been conserved is also shown in Fig. 7.

Finally, in order to demonstrate further that fatty acids released from VLDL triglycerides were the primary source of cellular triglyceride, a VLDL preparation was doubly labeled with a mixture of triolein containing ^{3}H in the glycerol and ^{14}C in the fatty acids and added to medium containing an excess of unlabeled glycerol. When this medium was incubated with preadipocytes and the incorporation of the glycerol and fatty acid moiety was measured, a time dependent increase in the incorporation of both ^{14}C and ^{3}H into cell lipid was observed (**Fig. 8**). Using the measured uptake of [^{3}H]glycerol, a predicted value for the uptake of ^{14}C-labeled fatty acid could be calculated assuming that the intact triglyceride molecule was internalized. As is shown in Fig. 8, the ^{14}C-labeled fatty acid incorporated far exceeded the predicted value.

DISCUSSION

The metabolism of chylomicrons and VLDL has been extensively investigated in recent years. We now have a basic understanding of many of the steps involved in the degradation of these lipoproteins and the subsequent utilization of the triglyceride moiety by peripheral cells. It is well established that lipoprotein lipase, an enzyme abundant in heart, muscle, and

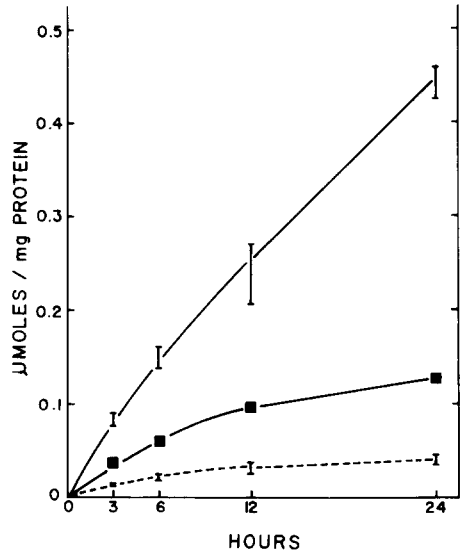

Fig. 8. Cellular incorporation of triglyceride fatty acid and glycerol by preadipose cells exposed to doubly labeled VLDL. Preadipocyte monolayers grown in 60-mm plates were incubated with medium containing VLDL (200 μg triglyceride/ml), doubly labeled with trioleoyl [2(n)-^{3}H]glycerol and tri[1-^{14}C]oleoylglycerol with ^{3}H/^{14}C = 3.85. Heparin (10 IU/ml), fatty acid-free albumin (0.4 mg/ml), and a 100-fold excess of unlabeled glycerol were also present in the incubation medium. The μmoles of both fatty acid and glycerol incorporated into cell lipids were calculated from the specific activities of the isotopes. I —— I, incorporation of fatty acid. Bars represent the range of three independent determinations. I-----I, incorporation of glyceride glycerol. Bars represent the range of three independent determinations. ■ —— ■, predicted fatty acid incorporation calculated on the basis of glycerol incorporation if entire triglyceride molecule was internalized.

adipose tissue, is a key enzyme in this process (17). Current models for triglyceride utilization propose that the site of action of lipoprotein lipase is the surface of capillary endothelial cells (18). Lipolysis of chylomicrons and VLDL triglycerides at the capillary wall results in the production of fatty acids, partial glycerides, free glycerol, and remnant or intermediate lipoproteins. Most of these products are released into the circulation, but the fatty acids and lower glycerides are transported from the circulation to tissues where they are either further metabolized or, in the case of adipose cells, stored as cellular triglyceride. The mechanism of transfer of lipolytic products from the site of lipase activity to cells is currently under intensive investigation and the evidence supporting various models has been recently reviewed by Smith and Scow (19).

A wide variety of experimental systems has been used to obtain information on the individual steps of lipoprotein triglyceride metabolism. Because of experimental limitations, most investigators have studied only isolated steps in this process. However, with the development of techniques for the growth of preadipocytes from the stromal-vascular fraction of adipose tissue, a cell culture system is now available which can both synthesize lipoprotein lipase and store the lipolytic products as triglycerides. As has been shown in this and other laboratories (6–8, 20, 21), preadipocytes contain lipoprotein lipase. The enzyme is synthesized by the cells and released into the culture medium when heparin is added to this medium (7). Work done in this laboratory has demonstrated that the lipase release triggered by heparin is biphasic with a rapid release of surface-bound enzyme followed by a slower release of newly synthesized lipase (8). We have also previously shown that addition of VLDL to cultures of cells actively producing lipase results in the accumulation of large amounts of triglyceride in the preadipocytes (5). While other cells in culture can store triglyceride (1, 22–24), we have shown that rat preadipocytes do this more efficiently and that the presence of lipoprotein lipase in the culture medium can enhance the utilization of VLDL triglyceride by skin fibroblasts co-cultivated with adipose cells (2). Thus, we have chosen the preadipocyte culture system to study further the events that are initiated by the action of lipoprotein lipase on VLDL and that result in a dramatic increase in cell triglyceride stores.

The accumulation of triglyceride by preadipocytes exposed to VLDL in culture differs markedly from the in vivo situation, wherein the preadipocytes are separated from circulating lipoproteins by an endothelial barrier. Present models propose that VLDL and chylomicron lipolysis takes place on the endothelial surface, and that lipolytic products are subsequently transported to the adipose cells. Our studies on triglyceride accumulation by cultured preadipocyte cells exposed to VLDL demonstrated that the addition of heparin promotes a rapid disappearance of triglyceride from the medium and an enhanced accumulation of cellular triglyceride (Fig. 1, Table 1). Earlier studies (8) have shown that exposure of preadipose cells to heparin causes a release of lipoprotein lipase into the culture medium and an increase in the total lipoprotein lipase in the system. Therefore, when heparin is added, VLDL is rapidly lipolyzed by the lipoprotein lipase in the medium, generating lipolytic products at a greater rate than is the case in the absence of heparin. This increased rate of lipolysis and the consequent increased rate of triglyceride accumulation enabled us to examine the interaction of some of the factors involved in these events, namely lipoproteins, lipoprotein lipase, and serum factors such as albumin. Experiments are currently being conducted to assess the mechanism of triglyceride accumulation in preadipose cells under conditions in which heparin is not present in the incubation medium.

In these experiments the lipolysis of VLDL triglyceride was never complete. This occurred when VLDL was incubated with cells and also when lipoprotein was incubated with released lipase in the absence of cells. Therefore, this appeared to be an intrinsic property of the enzymatic reaction (Figs. 2 and 3). Other workers have observed the production of VLDL remnants both in vivo (25) and in vitro (26, 27). It was of interest then to determine if internalization of these remnant particles by cultured preadipocytes could account for a significant amount of the cell triglyceride accumulated. This possibility was tested using VLDL particles that were doubly labeled with [^{3}H]triglyceride and [^{14}C]cholesteryl ester. When cells were incubated with this VLDL there was significant uptake of triglyceride fatty acids with time, but there was no proportional uptake of VLDL cholesteryl ester (Fig. 7). Also, when preadipocytes were incubated with VLDL labeled with triolein molecules that had either ^{3}H in the glycerol or ^{14}C in the fatty acid, we observed a time-dependent increase in the incorporation of fatty acids into cell lipids whereas the incorporation of glycerol was low (Fig. 8). Both of these results indicate that the incorporation of intact triglyceride molecules did not significantly contribute to the increase in cell triglyceride. Uptake of intact triglyceride molecules by cells in culture has been reported (1, 3, 4); however, this uptake was measured in cell systems that do not produce lipase and do not store large quantities of triglyceride.

Our results lead to the conclusions that preadipo-

cytes in culture can assimilate the fatty acid released from VLDL triglyceride by lipoprotein lipase and re-esterify them intracellularly. A major regulatory factor in this process is the concentration of albumin in the medium. It is well known that in vivo the plasma free fatty acids are transported as a complex with albumin. When we varied the concentration of fetal bovine serum present in medium containing a constant amount of VLDL and heparin, we found that preadipocytes accumulated more triglyceride at lower serum concentrations (Fig. 4). In another experiment, medium was prepared containing a constant amount of VLDL, heparin, and 2% fetal bovine serum, but various concentrations of fatty acid-free bovine albumin. Again we found that as we increased the concentration of albumin in the medium we decreased the cell triglyceride accumulated (Fig. 5). These results indicate that as VLDL triglyceride fatty acids are released by lipoprotein lipase, a fatty acid-albumin complex is formed in solution, and as has been proposed by Spector (28), the fatty acid to albumin molar ratio of this complex in turn regulates cellular uptake. In Spector's model it is the small amount of unbound fatty acid which exists in equilibrium with the albumin-fatty acid complex that enters the cells and the magnitude of this fraction is determined by the molar ratio of fatty acid to albumin at any concentration of fatty acid (28). The data presented in Fig. 6 provide additional evidence that this mechanism operates in our culture system. When a constant amount of VLDL labeled with [^{3}H]triolein was incubated with preadipocytes in medium containing low and high concentrations of albumin, the amount of ^{3}H-labeled cellular lipid was 3-fold higher in the cells exposed to the lower albumin concentration. When the medium lipids were analyzed we found that at the lower albumin concentration, 24% of the ^{3}H-labeled fatty acids released from VLDL by lipase was recovered in the cells, whereas at the higher albumin concentration the cells contained only 3% of the ^{3}H-labeled fatty acids released by lipase. The remaining fatty acids could be recovered in the medium. Fig. 6 also shows that the concentration of albumin present in the medium regulates the extent of VLDL triglyceride hydrolysis. This result is consistent with the previous observations which showed reduced lipolysis of chylomicrons at limiting albumin concentrations (29). Thus, in our cell culture system, albumin plays a dual role as a modulator of fatty acid utilization.

In summary, we conclude that the increased triglyceride stores measured in preadipocytes when VLDL and heparin were added to the culture medium resulted from the utilization by these cells of the free fatty acids released from VLDL triglyceride by lipoprotein lipase. Albumin was shown to play a major role by regulating both the extent of the lipase reaction and the cellular uptake of fatty acids. A remnant lipoprotein is probably formed during the course of VLDL lipolysis but it was shown that internalization of this lipoprotein did not contribute significantly to the increased cell triglyceride stores. Thus, the preadipocyte culture system provides a valuable tool for studying lipoprotein triglyceride utilization by cells and the important role that lipoprotein lipase plays in initiating this process.

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