Accumulation and mobilization of triglycerides and cholesteryl esters in Leydig tumor cells

Dale A. Freeman* and Joseph A. Ontko[1,†]

Departments of Internal Medicine* and Biochemistry,† The University of Oklahoma Health Sciences Center,*† the Department of Veterans Affairs Medical Center,* and the Oklahoma Medical Research Foundation†

Abstract Incubating MA-10 Leydig tumor cells with sodium oleate led to the accumulation of triglyceride within the cells. Triglycerides were deposited in a time- and dose-dependent fashion. Cellular triglyceride promoted storage of cholesteryl ester. As much cholesteryl ester was stored in oleate-treated cells as in cells treated with saturating concentrations of low density lipoprotein. Addition of both oleate and low density lipoprotein resulted in additive accumulation of cholesteryl esters. Cholesteryl esters in cells loaded with triglyceride by oleate treatment were mobilized in response to dibutryryl-cAMP to an extent similar to that in cells containing low triglyceride concentrations. Dibutyryl-cAMP stimulated cholesteryl ester mobilization under all conditions, and stimulated triglyceride mobilization when adequate fatty acid acceptors were available. ■ The results indicate that while triglyceride accumulation in MA-10 cells promoted cholesteryl ester deposition, it did not impair cAMP-dependent cholesteryl ester hydrolysis or steroid hormone production.—**Freeman, D. A., and J. A. Ontko.** Accumulation and mobilization of triglycerides and cholesteryl esters in Leydig tumor cells. *J. Lipid Res.* 1992. **33:** 1139–1146.

Supplementary key words oleic acid • cholesterol • lipid droplets • steroidogenesis • cAMP • cholesteryl ester hydrolase

Both cholesteryl esters and triglycerides are stored in discrete lipid droplets within cells. In certain specialized cells cAMP may lead to accelerated mobilization of these esters. Well-known examples of such cAMP sensitivity include glucagon or epinephrine regulation of adipose triglyceride mobilization (1–3), trophic hormone regulation of cholesteryl esters in steroidogenic cells (4–7), cAMP regulation of cholesteryl ester hydrolysis in J774 macrophages (8), or epinephrine or dibutyryl-cAMP regulation of hepatocyte triglyceride concentration (9, 10).

Usually triglyceride and cholesteryl ester mobilization have been studied in completely different tissues or cells. Exceptions are rat (11) and chicken (1) adipose tissue where the cholesteryl ester hydrolase and the hormone-sensitive lipase share many characteristics in common and cannot be physically resolved from one another (1). More recent studies (12–15) have provided further evidence that the hydrolysis of cholesteryl esters and triglycerides in many tissues is catalyzed by the same hormone-sensitive enzyme.

The present studies use the MA-10 Leydig tumor cells (16). These cells are a clonal strain of steroidogenic cells possessing receptors for gonadotropin (17, 18), epidermal growth factor (18), low density lipoprotein (19), and transferrin (20). These cells have been used extensively for studies of cholesterol metabolism (19) and transport (20–22). Cholesteryl ester metabolism in these cells is known to involve cAMP-stimulated cholesteryl ester hydrolysis opposed by cholesterol re-esterification that is regulated largely by the utilization of cholesterol for steroidogenesis (7). Cholesterol released from cholesteryl ester stores is transported and incorporated into the plasma membrane (20–22). Cycling through this membrane seems to be obligatory for the cholesterol to be utilized for steroid hormone synthesis (20).

In the studies reported herein, triglyceride deposition in MA-10 cells was caused by incubation in medium containing sodium oleate. This treatment results in a time-dependent accumulation of triglyceride as well as cholesteryl ester by the cells. Accumulation of cholesteryl esters in the triglyceride-loaded cells allowed comparison of mobilization of cholesteryl esters in cells containing low and high concentrations of triglyceride. Factors influencing mobilization of triglyceride and cholesteryl esters from lipid droplet reservoirs were examined in these cells.

MATERIALS AND METHODS

Materials

[9,10-³H]Oleic acid and [³H]H₂O were from Du Pont New England Nuclear (Boston, MA). Oleic acid and dibutyryl-cAMP were from Sigma (St. Louis, MO). Fatty

---

Abbreviations: LDL, low density lipoprotein.
[1]Present address: Department of Physiology, LSU Medical Center, 1100 Florida Avenue, New Orleans, LA 70119.

acid-free bovine serum albumin No. 82-002 was from ICN Biochemicals (Costa Mesa, CA). All other materials were reagent grade. Human LDL was prepared as described (23).

Cell culture

Culture conditions for the MA-10 cells have been described previously (16, 17). These cells are usually seeded in petri dishes (60 × 15 mm) and grown at 37°C for at least 1 day before fatty acid and/or lipoprotein additions and at least 3 days before biochemical analysis. A semi-confluent monolayer was present at the beginning of the experiment. The dishes of cells used in these experiments contained approximately 1.5 mg of protein and 150 μg DNA per dish. Sodium oleate was added as 20 mM oleate bound to 20% fatty acid-free bovine serum albumin in isotonic saline. The oleate/BSA was added directly to the growth medium. Growth medium consisted of Waymouth MB 752/1 with glutamine, 20 mM HEPES, 1.2 g NaHCO$_3$/1, and 15% horse serum. For mobilization experiments triglyceride-loaded cells were washed twice and placed in warm assay medium. (Waymouth MB 752/1 with glutamine, 20 mM HEPES, 1.2 g NaHCO$_3$/1, and 1 mg/ml BSA, pH 7.4). Additions of dibutryryl-cAMP were in sodium phosphate-buffered saline with 1 mg/ml BSA, pH 7.4. Human LDL was added in 0.9% NaCl buffered with 10 mM HEPES.

Lipid analysis

At the end of experiments the supernatant was removed and the cells were scraped from the dishes and sonicated in a volume of 0.5 ml of phosphate-buffered saline. An aliquot was removed for DNA and, in some instances, protein assay, and the remainder was extracted in chloroform–methanol 2:1 (v/v) for lipid analyses (24, 25). Cellular content of cholesterol, cholesteryl esters, and triglycerides were determined using gas–liquid chromatography (25–27). For this analysis lipid extracts were evaporated to dryness under nitrogen and each residue was immediately dissolved in 4 ml of n-heptane–isopropanol 3:7 (v/v), which contained cholesteryl butyrate as an internal standard, followed by the addition of 5 ml of 0.033 N H$_2$SO$_4$. After mixing for 30 sec the upper phase separated upon standing and was transferred to a 3 ml conical tube. This organic phase was then evaporated to dryness under nitrogen, redissolved in a small volume (50–100 μl) of n-hexane and 2-μl aliquots were injected into the gas chromatograph (25–27). This procedure resolved and quantified free cholesterol, cholesteryl butyrate (internal standard), cholesteryl esters (three peaks corresponding to cholesteryl esters with 16, 18, and 20 carbon chain length fatty acids) and triglycerides (five peaks that contained triglycerides with 48, 50, 52, 54, and 56 carbons in the fatty acid chains). Results are expressed as sum totals of the three molecular species of cholesteryl esters and the

five molecular species of triglycerides. For the measurement of cholesterol synthesis with [³H]H$_2$O, the cells were plated in T-25 culture flasks, grown to near confluency, and then incubated with and without 1 mM sodium oleate for 48 h. Flasks were then washed twice and placed in 3 ml of the assay medium described above containing 4 mCi of [³H]H$_2$O for 4 h at 37°C. The medium was then removed, the cells were washed with phosphate-buffered saline, scraped into this solution, and centrifuged. The rate of [³H]H$_2$O incorporation into cholesterol was determined for digitonin-precipitated sterols as described earlier (28). Thin-layer chromatography was performed as described previously (24, 28).

Other methods

Progesterone and 20α-dihydroprogesterone content of the medium were determined by specific radioimmunoassays (RIA) (20). Assay medium never constituted more than 0.7% of the RIA volume. Each assay was sensitive enough to measure 20 pg steroid.

The binding of LDL to MA-10 cells was performed as described (19) using LDL that was iodinated by use of iodo beads from Pierce Chemical (Rockford, IL). Cellular maximum binding capacity and binding affinity were calculated by linear regression of data from each individual experiment and averaged for presentation in the text. The DNA content of cells was determined using the method of Burton (29).

RESULTS

Incubation of MA-10 cells with sodium oleate resulted in increased intracellular stores of triglyceride and cholesteryl esters

The data of **Fig. 1** show the effects of four different treatments on cellular levels of triglyceride, cholesteryl ester, and free cholesterol. In these experiments cells were incubated with 1 mM oleate, 1 mM oleate + 11 μg/ml LDL, or with 11 μg/ml or 50 μg/ml of LDL alone. The LDL concentration of 11 μg/ml is subsaturating while 50 μg/ml saturates the LDL receptor (19). Treatment of MA-10 cells with oleate alone increased cellular triglyceride from 56 ng/μg DNA to 3053 ng/μg DNA, a 54-fold increase. Associated with this increase was an increase in cellular cholesteryl esters from 35 ng/μg DNA to 180 ng/μg DNA, a 5.1-fold increase. These changes that occurred with oleate treatment increased in a linear fashion with respect to time. Cellular free cholesterol was not changed by the treatment. Incubating the cells with either concentration of LDL resulted in similar qualitative changes. Incubation with 11 μg/ml LDL caused cellular triglyceride levels to increase 1.6-fold at 24 h and 1.8-fold at 48 h. Incubation with 50 μg/ml caused the triglyceride content to increase 2.9-fold at 24 h and 4.0-fold at 48 h.
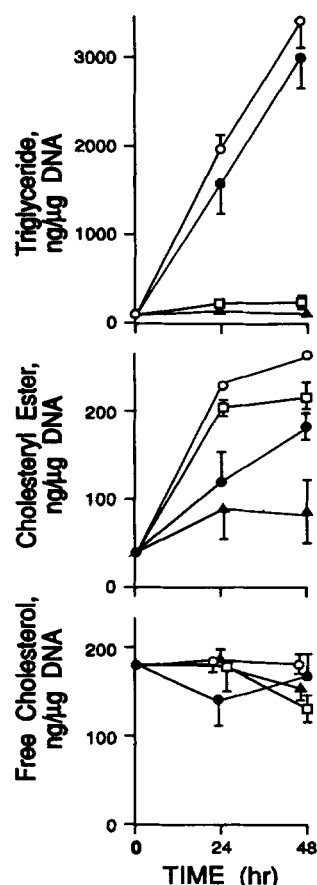
Fig. 1. Triglyceride and cholesteryl ester accumulation in MA-10 cells incubated with oleate. Dishes of MA-10 cells (60 × 15 mm) were put into culture at T = -24 h. At 0 h the medium was changed in all dishes to 4 ml of complete growth medium. To some dishes sodium oleate was added as 200 μl of a 20 mM solution bound to 20% BSA in 0.9% NaCl or LDL was added in 0.9% NaCl. At T = 24 h the medium was again changed. Oleate and/or LDL were added back to the original dishes and to a further group of dishes. At T = 48 h all dishes were harvested, some without any treatment, some with 24 h of treatment with oleate, 11 μg/ml LDL, 50 μg/ml LDL or oleate + 11 μg/ml LDL, and some with 48 h of all treatments. Cell monolayers were scraped from the plates, an aliquot was saved for DNA determination, and the remainder was extracted with chloroform–methanol 2:1 for lipid determinations. Cholesteryl butyrate was used as an internal recovery standard. All data are normalized to the DNA content of individual dishes and are presented as the mean ± SD of three separate experiments: oleate-treated (●-●) or mean ± range of two experiments; 11 μg/ml LDL (▲-▲), 50 μg/ml LDL (□-□) or oleate + 11 μg/ml LDL (○-○).

Cellular cholesteryl ester content of cells incubated with 11 μg/ml LDL increased 2.5-fold at 24 h and 2.3-fold at 48 h. Incubation with 50 μg/ml LDL increased cholesteryl ester concentrations 5.9- and 6.0-fold at 24 and 48 h, respectively. Neither LDL treatment resulted in a significant change in cellular free cholesterol. Incubation with oleate and 11 μg/ml LDL resulted in changes that were additive of the two treatments. Cellular triglyceride content increased linearly to a value 62-fold over control levels. Cholesteryl ester concentrations increased 6.5-fold at 24 h and then 7.5-fold at 48 h. Again, free cholesterol levels were unchanged.

Experiments were conducted to determine the products of oleate esterification in the MA-10 cells. After incubation for 6 h with 1 mM [9,10-³H]oleic acid, the cell lipids were extracted and separated by thin-layer chromatography. It was found that cholesteryl esters, diglycerides, phospholipids, and triglycerides contained 19, 27, 70, and 593 nmol, respectively, of the oleic acid precursor, corresponding to 2.7, 3.8, 9.9, and 83.6% of the total radioactivity in the esterified lipids.

Fatty acids entering liver can partition between two pathways (31). Some fatty acid will be oxidized while other fatty acids become esterified as triglyceride. Generally, fatty acids will not be esterified in abundance until the oxidation pathway is saturated. To determine the concentration of oleate necessary for significant triglyceride accumulation and to examine the relationship between triglyceride and cholesteryl ester accumulation, the experiments of **Fig. 2** were performed. In these experi-
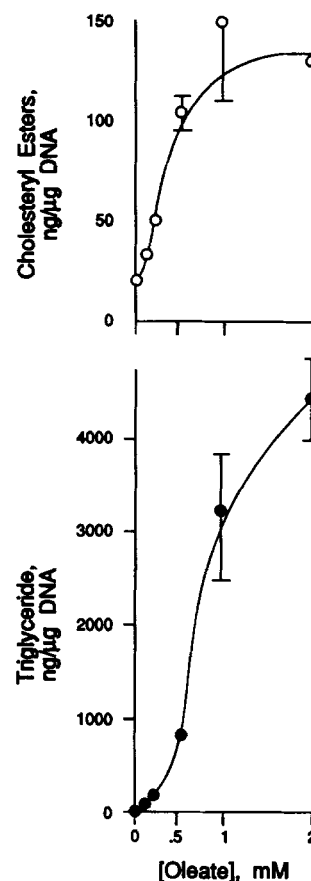

Fig. 2. Dose response of triglycerides and cholesteryl esters to oleate incubation. Dishes of MA-10 cells were placed in culture at T = -24 h. At T = 0, the medium was replaced and oleate was added at the concentrations shown on the figure. At T = 24, the medium and additions were renewed. At T = 48 h, the cell monolayer was harvested and extracted as described in the legend to Fig. 1. Low concentrations shown correspond to 0, 0.1, and 0.2 mM oleate. Data presented are the mean ± range of two determinations.

ments, MA-10 cells were incubated with various concentrations of oleate for 48 h. Illustrated are the concentrations causing significant changes in the triglyceride or cholesteryl ester concentration. Oleate concentrations lower than 0.2 mM increased triglyceride concentrations minimally and did not increase cholesteryl ester concentrations. The data shown in Fig. 2 indicate that significant triglyceride accumulation required oleate concentrations greater than 0.2–0.5 mM. High cell triglyceride concentrations were obtained with oleate concentrations of 1–2 mM. Triglyceride accumulation exhibited a sigmoid response to the oleate concentration. Cholesteryl ester deposition appeared to follow this pattern. The dose-response curve for cholesteryl ester accumulation was shifted from the curve for triglyceride accumulation. Thus maximal cholesteryl ester accumulation occurred in cells that contained about one-sixth of the highest triglyceride content achieved.

The cholesterol that becomes esterified and accumulates in triglyceride-loaded MA-10 cells could have one of two sources. This cholesterol could either be synthesized by the cell or taken up from the growth medium which contained horse serum. The MA-10 cells normally synthesize cholesterol at a high rate (23) but can take up LDL cholesterol as well (19, 23). To determine whether new cholesterol synthesis might supply this cholesterol, the rates of $[^3H]H_2O$ incorporation into cholesterol were measured for control and oleate-treated MA-10 cells.
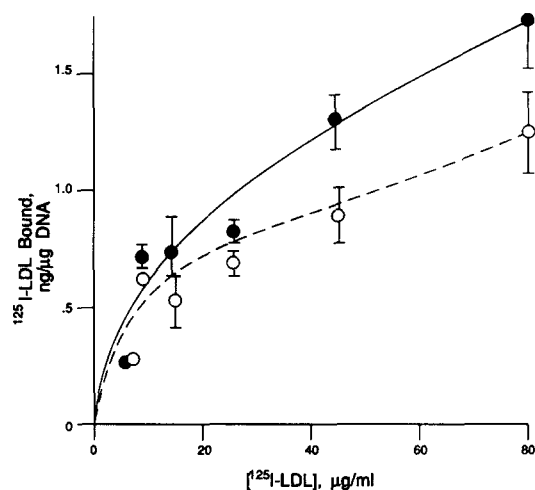
Tritiated water was incorporated into digitonin-precipitable sterols in control cells at 4.8 nmol $[^3H]H_2$/flask per h and into oleate-treated cells at 4.6 nmol $[^3H]H_2O$/flask per h. Since there was no discernible effect of oleate pretreatment and accordingly of triglyceride accumulation, on de novo synthesis of cholesterol the effect of oleate on LDL binding was quantitated. The binding of ^{125}I-labeled LDL by control cells and cells treated with 1 mM oleate for 48 h is shown in **Fig. 3**. Oleate treatment clearly had a profound effect on LDL binding. Control cells maximally bound 2.45 ± 0.15 ng LDL/μg DNA with a K_d of 18.3 ± 1.8 μg/ml. Oleate-treated cells maximally bound only about one-half as much LDL, 1.36 ± 0.19 ng LDL/μg DNA, but with twice the affinity, K_d = 9.9 ± 5.2 μg/ml. The binding data would predict that the changes with oleate treatment would result in no augmentation in LDL uptake at 37°C. Incubation of control and oleate-treated MA-10 cells with 8–10 μg/ml ^{125}I-labeled LDL resulted in equivalent cell-associated and degraded radioactivity (data not shown). These data taken together suggest that the excess cholesterol must have come from the serum-containing growth medium but not from LDL.

Mobilization of cellular triglyceride and cholesteryl ester in unstimulated and dibutryryl-cAMP-stimulated MA-10 cells

In MA-10 cells containing low basal concentrations of cholesteryl ester, or in cells in which the cholesteryl ester has been augmented by incubating the cells with LDL, dibutryryl-cAMP induces rapid mobilization of cholesteryl esters (7, 23). In either case cholesteryl esters are mobilized in cells containing only small amounts of triglyceride. If triglyceride and cholesteryl ester hydrolysis are both catalyzed by the same enzyme (1, 11–15), it might be expected that triglyceride-loaded cells would mobilize cholesteryl esters differently than in control cells with low triglyceride contents. The experiments of **Fig. 4** were designed to allow comparison between cholesteryl ester hydrolysis in cells with high triglyceride concentrations and cells that do not contain this additional lipid. For these experiments cells were incubated for 48 h with either 1 mM oleate or 50 μg/ml LDL. This resulted in two groups of cells with similar cholesteryl ester contents but triglyceride contents that varied by 18-fold. With three ester bonds per triglyceride molecule, the LDL-loaded cells contained twice as many triglyceride-fatty acid esters as cholesterol-fatty acid esters while triglyceride-loaded cells contained 34-fold more triglyceride-fatty acid esters than cholesterol-fatty acid esters. Mobilization was measured after a subsequent incubation in defined medium alone or medium containing dibutryryl-cAMP. Dibutryryl-cAMP caused mobilization of cholesteryl ester in both the oleate- and LDL-treated cells. The amount of cholesteryl ester mobilized as well as
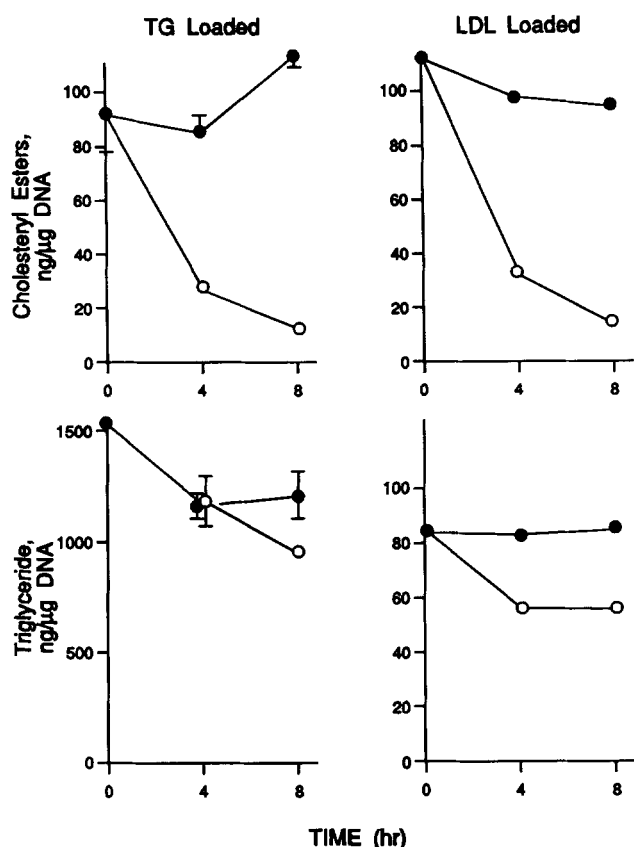

Fig. 3. Effect of oleate treatment on cellular ^{125}I-labeled LDL receptor binding. Dishes of MA-10 cells were left as controls (●) or treated with 1 mM oleate (○) for 48 h as described in the legend to Fig. 1. At 48 h, all cells were washed twice with 2 ml of ice-cold assay medium. The tray of cells was placed on ice in a 4°C room for 30 min at which time ^{125}I-labeled LDL was added at the concentrations indicated. After 2 h at 4°C, the dishes were washed 5 × with 2 ml cold assay medium and the monolayer was dissolved in 0.5 N NaOH. This total cell-associated radioactivity was quantitated by counting on a gamma counter. Cellular content of DNA was also measured. The data depicted are the mean ± SD of data from two independent experiments.

TG Loaded   LDL Loaded

TIME (hr)

Fig. 4. Mobilization of cholesteryl esters and triglycerides in control and dibutyryl-cAMP-stimulated MA-10 cells. Dishes of MA-10 cells were cultured and treated either with 1 mM oleate (left panels) or 50 μg/ml LDL (right panels) as described by the protocol in the legend to Fig. 1. At the beginning of the experiment the medium containing additions was removed, the dishes were washed twice with assay medium, and then placed in 4 ml assay medium either with no additions (●) or containing 1 mM dibutyryl-cAMP (○). After either 4 or 8 h, the cell monolayers were scraped and extracted as described in the legend to Fig. 1. The data depicted are the mean ± range of two experiments.

the rate of mobilization were similar for both groups. Thus, in oleate-treated cells 79 ng/μg DNA of cholesteryl ester was mobilized in 8 h while in LDL-treated cells 97.1 ng/μg DNA of cholesteryl ester was mobilized over the same time. The rate of cholesteryl ester hydrolysis during the first 4 h was 16.2 ng/μg DNA per h for the oleate-treated cells and 19.5 ng/μg DNA per h for the LDL-treated cells. While the effect of cAMP on cholesteryl ester mobilization was striking, its effects on triglyceride mobilization were less prominent. On average in two experiments, the triglyceride contents of control and cAMP-stimulated cells (loaded with triglyceride by incubation with oleate) declined to the same extent at four h and differed only slightly at 8 h (76% of time = 0 in controls and 70% in stimulated cells). In LDL-treated cells, which contained only 6–12% as much triglyceride as the oleate-treated cells, there was on average a moderate effect of dibutryryl-cAMP on triglyceride mobilization. Thus,

stimulated cells contained 76% as much triglyceride at 4 h and 81% as much at 8 h as the zero-time control. Unstimulated cells contained 102% at 4 h and 98% at 8 h, as much triglyceride as the zero-time control. Thus, there appeared to be quantitatively a very small effect of cAMP on triglyceride mobilization.

A cAMP-responsive triglyceride lipase should hydrolyze triglyceride more rapidly in cAMP-treated than in control cells. Greater net mobilization of triglyceride would not occur, however, unless sufficient free fatty acid acceptors were present in the medium. In the experiments of **Fig. 5** the cells were incubated for 48 h with [³H]oleate after which time they were washed and placed in medium
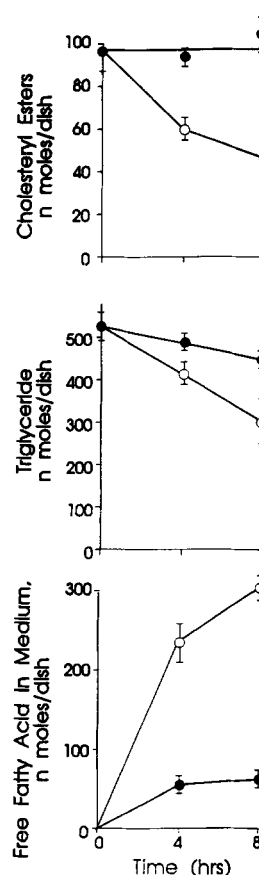

Time (hrs)

Fig. 5. Mobilization of cholesteryl esters and triglycerides under conditions of excess free fatty acid acceptors in control and dibutyryl-cAMP-stimulated MA-10 cells. Dishes of MA-10 cells were cultured and treated with 1 mM [³H]oleate (SR 807 DPM/nmol) as described in the legend of Fig. 1. At T = 0 the dishes were washed twice with 2 ml assay medium and placed in 4 ml of assay medium with BSA (40 mg/ml final concentration) either without (●) or with 1 mM dibutyryl-cAMP (○). At the times indicated, dishes were harvested, the medium was saved for assay of free fatty acids, and the cells were extracted for lipid analysis. After the lipids were separated by thin-layer chromatography, the areas of the plate corresponding to triglyceride and cholesteryl ester were scraped into liquid scintillation counting vials and the radioactivity was quantitated. The data shown are the mean ± SD of triplicate points from one of three similar experiments.

containing 40 mg/ml BSA either with or without dibutyryl-cAMP. The presence of the fatty acid acceptor had little effect on the mobilization of cholesteryl esters. Cholesteryl ester content of control cells declined slightly, 10 nmol over 8 h, while dibutyryl-cAMP-stimulated cells mobilized 50 nmol cholesteryl esters over the same time period. The presence of the acceptors did, however, influence triglyceride mobilization. Although there was significant rate of loss of triglyceride in unstimulated cells, 80 nmol lost over 8 h, there was a much greater loss from stimulated cells, 213 nmol over 8 h. Dibutyryl-cAMP caused increased amounts of free fatty acids to accumulate in the medium as well. Unstimulated cells released 68 nmol free fatty acid into the medium in 8 h while stimulated cells released 302 nmol free fatty acid in the same time period. Most of the released free fatty acid had to be derived from triglyceride since only 10 nmol in control and 50 nmol in stimulated cells could have originated from cholesteryl esters. Dibutyryl-cAMP stimulation had at least one other effect on fatty acids, stimulation increased fatty acid oxidation. Control cells oxidized 37 nmol free fatty acid at 4 h and 49 nmol at 8 h. Stimulated cells oxidized 58 nmol at 4 h and 99 nmol at 8 h. Thus cAMP promotes triglyceride mobilization by two mechanisms: it facilitates release of free fatty acid from the triglyceride (increased hydrolysis) and also inhibits resynthesis of triglyceride by removing fatty acid from the resynthesis pool (oxidation and binding to extracellular albumin).

It seemed possible that free cholesterol might partition into the triglyceride-rich droplets in cells incubated with oleate. If this were true, cholesteryl esters may be hydrolyzed but the resulting cholesterol would not become as available for other functions. It also seemed possible that the triglyceride might compete for transport proteins within the cell. Steroidogenesis is critically dependent on cholesterol mobilization and transport (21, 22). Thus steroid hormone production might be expected to be inhibited in oleate-treated cells compared to LDL-treated cells containing similar concentrations of cholesterol and cholesteryl esters. This hypothesis was tested by measuring the steroid hormone content in the medium of the cells displayed in Fig. 5. The steroid hormone data are shown in **Table 1**. The MA-10 cells produced two major steroid products, progesterone and 20α-dihydroprogesterone. These two products accounted for more than 95% of all steroid hormones synthesized by these cells. As the data of Table 1 show, oleate-treated cells actually synthesized more steroid products than LDL-treated cells. Thus oleate-pretreated cells synthesized 41.9 ng/μg DNA of total steroid in 4 h and 52.7 ng/μg DNA of total steroid in 8 h. MA-10 cells pretreated with LDL synthesized 22.8 ng/μg DNA of total steroid at 4 h and 34.1 ng/μg DNA of total steroid at 8 h.

TABLE 1.   Steroid hormone synthesis by oleate- or LDL-treated MA-10 cells

Treatment	Progesterone	20α-Dihydroprogesterone	Total
	ng/μg DNA		
Oleate			
4 h	12.3 ± 5.3	29.6 ± 7.0	41.9
8 h	12.8 ± 5.7	39.9 ± 3.7	52.7
LDL			
4 h	9.2 ± 3.9	13.6 ± 3.6	22.8
8 h	10.7 ± 2.5	23.4 ± 4.6	34.1

Dishes of cells were incubated in complete growth medium supplemented either with oleic acid (1 mM) or LDL (50 μg/ml) for 48 h. At this time all dishes were washed twice and then placed in 4 ml of assay medium containing 1 mM dibutyryl-cAMP. After either 4 or 8 h at 37°C, the medium was removed and the medium content of progesterone or 20α-dihydroprogesterone was determined by specific radioimmunoassay. The results are the mean ± SD of three determinations. The steroid values correspond to the lipid values depicted in Fig. 4.

DISCUSSION

In the present studies we showed that the steroid hormone-synthesizing MA-10 cells would respond to incubation with oleate by synthesizing triglyceride. The accumulation of triglyceride was associated with changes in the cellular cholesteryl ester content, alterations in LDL receptor binding, and doubtless other changes that were not measured. The coexistence of triglyceride and cholesteryl ester within the cell impaired neither the mobilization of cholesteryl esters nor the utilization of cholesterol for steroid hormone synthesis.

The MA-10 cells seem to take up and esterify fatty acids into triglycerides in much the same way as liver cells do (31). In these studies fatty acid oxidation was not measured but must be active since triglyceride accumulation showed a sharp rise over a very narrow dose range of oleate. Fairly clearly, this competing process became saturated at 0.2-0.5 mM oleate. Just above this range triglyceride accumulation increased rapidly to the 1-mM level and then gradually decelerated between 1 and 2 mM oleate. This esterification process did not become saturated.

It is reasonable to conclude that in the MA-10 cells, as in liver cells, the uptake of long-chain free fatty acids from the albumin-bound complex in the culture medium is accompanied by immediate intracellular utilization, thereby preventing the accumulation of free fatty acids within the cells. Triglyceride synthesis provides a mechanism for the rapid removal of free oleic acid and its storage within the cells for subsequent use. In liver cells triglyceride synthesis was never saturated, even at the highest achievable concentrations of the long chain fatty acid substrate (at a molar ratio of fatty acid to albumin of 8:1) (24). In the

present study the molar ratio of fatty acid to albumin was approximately 6 at the oleate concentration of 2 mM.

The excess cholesterol esterified in the oleate-treated MA-10 cells had to have been derived from the serum in the growth medium. De novo synthesis of cholesterol was not increased. This cholesterol was not derived from LDL cholesterol as no net increase in LDL uptake was documented and because horse serum is relatively deficient in LDL. Cholesterol uptake must have been mediated by some processes not measured in the present experiments.

It is clear that incubating MA-10 cells with oleate caused some complex changes in cellular functioning. One such change uncovered in these studies was in the LDL receptor binding of the cell. Oleate-treated cells had about half the receptor number but twice the receptor affinity of control cells. One possible explanation for such a change is that the oleate replaces other fatty acids in phospholipids in the cell membrane. Alteration in the phospholipid composition or fluidity of membranes can effect the function of membrane bound proteins (30, 32, 33).

An interesting observation made in these studies was that triglyceride did not significantly inhibit mobilization of cholesteryl esters. This finding is surprising in that cholesteryl esters and triglycerides are believed to be broken down by the same hormone-sensitive lipase enzyme (1, 11, 12, 14, 34–36). That an 18-fold molar excess of triglyceride had no effect on cholesteryl ester hydrolysis might be explained in one of four ways. First, it is possible that the MA-10 cells express a separate cholesteryl ester hydrolase that is not identical to the hormone-sensitive lipase. The evidence for a hormone-sensitive lipase in steroidogenic cells is convincing (34–36); however, these studies in no way show that other lipase enzymes are not present. A second possible but unlikely explanation is that the triglycerides and cholesteryl esters are segregated into different lipid droplets. Compartmentalization would explain the data; however, isolated lipid droplets from liver contain both cholesteryl esters and triglyceride (37). A third possibility is that triglycerides preferentially occupy the core of the droplets and cholesteryl esters occupy the periphery. In this way the cholesteryl esters would have preferential access to the cytosolic lipase enzyme. A fourth possibility must be considered. Adding triglyceride to the lipid droplet has been shown to increase the rate of cholesteryl ester hydrolysis (38, 39). The results probably could not explain the present data as triglyceride fully dissolved the cholesteryl esters but increased the hydrolysis rate less than 2-fold, not the 18-fold required to explain the present data.

The results of the present experiments suggest another topic for further study, the interaction of steroidogenic cells with triglyceride-rich lipoproteins. Triglyceride accumulation increases the quantity of cholesteryl ester stored by the cell which, in turn, can be hydrolyzed and used to synthesize steroid hormones. Since triglyceride-rich lipoproteins are a physiological source of cellular triglyceride, it may be of interest to characterize such interaction in some detail. ■

REFERENCES

1. Khoo, J. C., D. Steinberg, J. J. Huang, and P. R. Vagelos. 1976. Triglyceride, diglyceride, monoglyceride, and cholesterol ester hydrolases in chicken adipose tissue activated by adenosine 3', 5' monophosphate-dependent protein kinase. *J. Biol. Chem.* **251:** 2882–2890.
2. Steinberg, D. 1966. Catecholamine stimulation of fat mobilization and its metabolic consequence. *Pharmacol. Rev.* **18:** 217–235.
3. Butcher, R. Y. 1966. Cyclic 3', 5' AMP and the lipolytic effects of hormones on adipose tissue. *Pharmacol. Rev.* **18:** 237–241.
4. Davis, W. W., and L. D. Garren. 1966. Evidence for the stimulation by adrenocorticotropic hormone of the conversion of cholesterol esters to cholesterol in the adrenal. *Biochem. Biophys. Res. Commun.* **24:** 805–810.
5. Beckett, E. J., and G. S. Boyd. 1977. Purification and control of bovine adrenal cortical cholesterol ester hydrolase and evidence for the activation of the enzyme by phosphorylation. *Eur. J. Biochem.* **72:** 223–233.
6. Naghshineh, S., C. R. Treadwell, L. L. Gallo, and G. V. Vahouny. 1978. Protein kinase-mediated phosphorylation of a purified sterol ester hydrolase from bovine adrenal cortex. *J. Lipid Res.* **19:** 561–569.
7. Freeman, D. A. 1987. Regulation of the cholesterol ester cycle of cultured Leydig tumor cells. *Eur. J. Biochem.* **164:** 351–356.
8. Bernard, D. W., A. Rodriquez, G. H. Rothblat, and J. M. Glick. 1991. cAMP stimulates cholesteryl ester clearance to high density lipoproteins in J774 macrophages. *J. Biol. Chem.* **266:** 710–716.
9. Schade, D. F., W. F. Woodside, and R. P. Eaton. 1979. The role of glucagon in the regulation of plasma lipids. *Metabolism.* **18:** 874–886.
10. Woodside, W. F., and J. A. Ontko. 1989. Regulation of triglyceride mobilization in isolated hepatocytes by dibutyryl cyclic AMP and epinephrine. *J. Cardiovasc. Pharmacol.* **13 (Suppl. 2):** 538–544.
11. Pittman, R. C., J. C. Khoo, and D. Steinberg. 1975. Cholesterol esterase in rat adipose tissue and its activation by cyclic adenosine 3',5'-monophosphate-dependent protein kinase. *J. Biol. Chem.* **250:** 4505–4511.
12. Holm, C., P. Belfrage, and G. Fredrikson. 1987. Immunological evidence for the presence of hormone-sensitive lipase in rat tissues other than adipose tissue. *Biochem. Biophys. Res. Commun.* **148:** 99–105.

13. Holm, C., T. G. Kirchgessner, K. L. Svenson, G. Fredrikson, S. Nilsson, C. G. Miller, J. E. Shively, C. Heinzmann, R. S. Sparks, T. Mohandas, A. J. Lusis, P. Belfrage, and M. C. Schotz. 1988. Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-q13.3. *Science.* **241:** 1503–1506.

14. Yeaman, S. J. 1990. Hormone-sensitive lipase—a multipurpose enzyme in lipid metabolism. *Biochim. Biophys. Acta.* **1052:** 128–132.

15. Small, C. A., M. P. Rogers, J. A. Goodacre, and S. J. Yeaman. 1991. Phosphorylation and activation of hormone-sensitive lipase in isolated macrophages. *FEBS Lett.* **279:** 323–326.

16. Ascoli, M. 1981. Characterization of several clonal lines of cultured Leydig tumor cells: gonadotropin receptors and steroidogenic responses. *Endocrinology.* **108:** 88–95.

17. Freeman, D. A., and M. Ascoli. Desensitization in cultured Leydig tumor cells involves loss of gonadotropin receptors and decreased capacity for steroidogenesis. *Proc. Natl. Acad. Sci. USA.* **78:** 6309–6313.

18. Ascoli, M. 1981. Regulation of gonadotropin receptors and gonadotropin responses in a clonal strain of Leydig tumor cells by epidermal growth factor. *J. Biol. Chem.* **256:** 179–183.

19. Freeman, D. A., and M. Ascoli. 1983. The low density lipoprotein pathway of Leydig tumor cells. *Biochim. Biophys. Acta.* **754:** 72–81.

20. Nagy, L., and D. A. Freeman. 1990. Cholesterol movement between the plasma membrane and the cholesteryl ester droplets of cultured Leydig tumor cells. *Biochem. J.* **271:** 809–814.

21. Freeman, D. A. 1987. Cyclic AMP-mediated modification of cholesterol traffic in Leydig tumor cells. *J. Biol. Chem.* **262:** 13061–13068.

22. Nagy, L., and D. A. Freeman. 1990. Effect of cholesterol transport inhibitors on steroidogenesis and plasma membrane cholesterol transport in cultured MA-10 Leydig tumor cells. *Endocrinology.* **126:** 2267–2276.

23. Freeman, D. A., and M. Ascoli. 1982. Studies on the source of cholesterol used for steroid biosynthesis in cultured Leydig tumor cells. *J. Biol. Chem.* **257:** 14231–14238.

24. Ontko, J. A. 1972. Metabolism of free fatty acids in isolated liver cells. *J. Biol. Chem.* **247:** 1788–1800.

25. Ontko, J. A., and C-S. Wang. 1989. Elevation of liver diacylglycerols and molecular species of diacylglycerols in rats fed a lipogenic diet. *J. Lipid Res.* **30:** 691–699.

26. Kuksis, A., J. J. Myher, L. Marai, and K. Geher. 1975. Determination of plasma lipid profiles by automated gas chromatography and computerized data analysis. *J. Chromatogr. Sci.* **13:** 423–430.

27. Wang, C. S., D. Weiser, P. Alaupovic, and W. J. McConathy. 1982. Studies on the degradation of human very low density lipoproteins by human milk lipoprotein lipase. *Arch. Biochem. Biophys.* **214:** 26–34.

28. Dashti, N., J. L. Kelley, R. H. Thayer, and J. A. Ontko. 1983. Concurrent inductions of avian hepatic lipogenesis, plasma lipids, and plasma apolipoprotein B by estrogen. *J. Lipid Res.* **24:** 368–379.

29. Burton, K. 1968. Determination of DNA concentration with diphenylamine. *Methods Enzymol.* **128:** 163–166.

30. Yuli, I., W. Wilbrandt, and M. Shinitzky. 1981. Glucose transport through cell membranes of modified lipid fluidity. *Biochemistry.* **20:** 4250–4256.

31. Zaleski, J., and J. A. Ontko. 1985. Reciprocal effects of energy utilization on palmitate oxidation and esterification in hepatocytes of fed rats. *Biochim. Biophys. Acta.* **836:** 134–142.

32. Brasitus, T. A., and P. K. Dudeja. 1985. Regional differences in the lipid composition and fluidity of rat colonic brush-boarder membranes. *Biochim. Biophys. Acta.* **819:** 10–17.

33. Friedlander, G., M. Shahedi, C. LeGrimellec, and C. Amiel. 1988. Increase in membrane fluidity and opening of tight junctions have similar effects on sodium-coupled uptakes in renal epithelial cells. *J. Biol. Chem.* **263:** 11183–11188.

34. Cook, K. G., S. J. Yeaman, P. Strafors, G. Fredrikson, and P. Belfrage. 1982. Direct evidence that cholesteryl ester hydrolase from adrenal cortex is the same enzyme as hormone-sensitive lipase from adipose tissue. *Eur. J. Biochem.* **125:** 245–249.

35. Cook, K. G., R. J. Colbran, and S. J. Yeaman. 1983. Cytosolic cholesterol ester hydrolase from bovine corpus luteum, its purification, identification, and relationship to hormone sensitive lipase. *Biochim. Biophys. Acta.* **752:** 46–53.

36. Kraemer, F. B., K. Tavanger, and A. R. Hoffman. 1991. Developmental regulation of hormone-sensitive lipase mRNA in the rat: changes in steroidogenic tissues. *J. Lipid Res.* **32:** 1303–1310.

37. Ontko, J. A., L. W. Perrin, and L. S. Horne. 1986. Isolation of hepatocellular lipid droplets: the separation of distinct subpopulations. *J. Lipid Res.* **27:** 1097–1103.

38. Adelman, S. J., J. M. Glick, M. C. Phillips, and G. H. Rothblat. 1984. Lipid composition and physical state effects on cellular cholesteryl ester clearance. *J. Biol. Chem.* **259:** 13844–13850.

39. Minor, L. K., G. H. Rothblat, and J. M. Glick. 1989. Triglyceride and cholesteryl ester hydrolysis in a cell culture model of smooth muscle foam cells. *J. Lipid Res.* **30:** 189–197.