Oleate Acutely Stimulates the Secretion of Triacylglycerol by Cultured Rat Hepatocytes by Accelerating the Emptying of the Secretory Compartment

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ABSTRACT: The acute effects of addition of oleate on the rate of triacylglycerol (TAG) secretion by cultured rat hepatocytes were studied by monitoring the use of endogenous (14C-prelabeled) acyl moieties and exogenous (3H-labeled) oleate for the synthesis of secreted TAG simultaneously. Inclusion of exogenous oleate in the medium stimulated the secretion of the endogenous 14C-labeled acyl moieties by 55–100%. To find out whether the stimulation was due to increased endogenous TAG mobilization or an increased rate of processing of TAG within the endoplasmic reticulum (ER) secretory machinery, use was made of the inhibition of apolipoprotein B (apoB) synthesis (but not degradation) by Ca2+ mobilization from the ER. Inhibition of apoB synthesis stopped entry of acyl moieties (from endogenous and exogenous sources) into the secretory pathway. However, even when entry of acyl moieties into the secretory pathway was totally inhibited, exogenous oleate was still able to stimulate twofold the secretion [14C]TAG, indicating that oleate stimulates the emptying of prelabeled TAG from the secretory compartment at a point distal to apoB synthesis and nascent particle formation. These data indicate that exogenous oleate, besides providing additional acyl moieties for incorporation into secreted TAG, stimulates the secretion of endogenous TAG in a manner (i) that is independent of effects on apoB synthesis and/or degradation and (ii) that involves the enhanced processing of TAG resident within the ER secretory pathway.


Triacylglycerol (TAG) secretion by hepatocytes is affected by several factors, including the availability of fatty acids and of apolipoprotein B (apoB) for assembly into very low density lipoprotein (VLDL) particles. Through the phosphatidate pathway, exogenous fatty acids are incorporated into diacylglycerols (DAG), which are then partitioned between either the synthesis of cytosolic TAG or, after permeation through the endoplasmic reticulum (ER) membrane, into TAG destined for secretion (1). Within the ER, TAG occur within apoB-containing nascent lipoprotein particles in the rough ER (rER), and as apoB-free TAG droplets within the smooth ER (sER) (2). Additionally (fusion) of TAG from this sER pool to the nascent particles (step two of lipidation) at regions of fusion between the rER and sER membrane populations results in the full-sized VLDL particles that are secreted (3,4). Cytosolic TAG undergo a continuous cycle of lipolysis (5) [mostly to DAG (6,7)] and resynthesis. DAG formed through cytosolic TAG lipolysis also contribute to the synthesis of ER luminal (secreted) TAG (6). Therefore, DAG required for TAG synthesis are contributed both directly through the phosphatidate pathway and from the hydrolysis of cytosolic droplet TAG (1,8).

In freshly prepared rat or rabbit hepatocytes, in which the endogenous cytosolic TAG stores are depleted during the cell isolation procedure, the rate of TAG secretion is almost totally dependent on the addition of oleate (9,10). However, in cultured rat hepatocytes, in which the accumulation of cytosolic TAG is maximized by preculture for up to 24 h with fatty acids, the rate of TAG secretion is less dependent on added fatty acids (11), and the DAG and acyl moieties formed by hydrolysis of cytosolic TAG make a major contribution (about 80%) toward the synthesis of secreted TAG, even in the presence of exogenous oleate (7). Therefore, it appears that in addition to the ability of oleate to stimulate TAG secretion directly, by providing additional acyl moieties, it also stimulates secretion of endogenous acyl groups through an unknown mechanism. The secretion of TAG occurs within VLDL particles, the nascent form of which are produced by rapid but partial lipidation of newly synthesized apoB. Therefore, an experimental approach that distinguishes between the stimulation of the supply of acyl moieties and DAG proximal to apoB synthesis, and of the rate of secretory steps distal to apoB synthesis would be useful in identifying the site of oleate action.

One such approach is provided by the inhibition of TAG secretion when intracellular (ER) Ca2+ stores are mobilized (12). The mechanism of this inhibition has not been studied, although it has been suggested that ER luminal Ca2+ is required for the correct folding of apoB (12). However, because ER Ca2+ mobilization is known to inhibit protein synthesis (13,14), we considered it possible that TAG secretion may be inhibited as the result of the inhibition of apoB synthesis. If this could be shown to be the case, it would provide the opportunity experimentally to investigate the effect of the addition of exogenous oleate on the rate of TAG secretion by hepatocytes at a step(s) distal to apoB synthesis (and step 1 lipidation). Therefore, in the present study we combined the use of this approach to the

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Abbreviations: Apo, apolipoprotein; DAG, diacylglycerols; TAG, triacylglycerols; EBSS, Earls balanced salts solution; ER, endoplasmic reticulum; rER, rough endoplasmic reticulum; SDS, sodium dodecyl sulfate; sER, smooth endoplasmic reticulum; VLDL, very low density lipoproteins.
previously described dual-labeling technique for the quantification of the utilization of endogenous and exogenous acyl moieties for the synthesis of secreted TAG (7) to study the mechanism of the effect of exogenously added oleate on TAG secretion by cultured rat hepatocytes. The data show that exogenous oleate stimulates the rate of secretion of a pool(s) of TAG resident within the secretory pathway.

MATERIALS AND METHODS

Preparation and culture of hepatocytes. Hepatocytes were isolated from female Wistar rats (200–220 g) by a two-step collagenase perfusion protocol, as described previously (7). They were plated onto 6-cm diameter plastic dishes and allowed to attach in 3 mL of Earls balanced salts solution (EBSS) containing 10 mM HEPES (pH 7.4) and supplemented with new-born calf serum, amino acids, and antibiotics for 4 h, as described previously (7). The medium was then removed, and fresh serum-free EBSS medium was added, supplemented with 0.75 mM oleate complexed to albumin (final concentration 1%), 1 mM pyruvate, 10 mM lactate, 10 mM dexamethasone, and antibiotics. When [14C]oleate was added the final specific radioactivity of the fatty acid was 0.035 µCi/µmol. After 18 h, the medium was removed and the cells were washed. Fresh medium containing different supplements, as indicated, was added, and the cellular and secreted radiolabeled lipids were quantified for the periods indicated, also as described previously (7).

Measurement of TAG secretion rates from exogenous and endogenous sources. The respective contributions of exogenous and endogenous acyl moieties to the secreted TAG were quantified, after extraction and separation of lipids, as described by Lankester et al. (7). Cellular TAG were quantified at each experimental time-point so as to obtain the specific activity of endogenous acyl moieties. Secretion rates were expressed in terms of nanomoles of fatty acid equivalent, so that utilization of endogenously generated oleoyl moieties would be directly comparable to that calculated for exogenously added oleate. Preliminary experiments established that all the secreted TAG were associated with VLDL; therefore, routinely no separation of the lipoprotein particles was performed. Incorporation of [3H] and [14C] labels into cellular and secreted phospholipid was also measured. Routinely, secretion of labeled phospholipid was very low and was negligible compared to that of TAG. Acid-soluble oxidation products, which under these conditions represented >95% of the total oxidation products, were measured after acid precipitation of the fatty acid substrate from solution (7).

Measurement of apoB synthesis and degradation rates. Hepatocytes cultured for 18 h as described above were washed and incubated in 3 mL of methionine-free EBSS medium. For the measurement of the rate of synthesis of apoB, the medium was then replaced by 1 mL of methionine-free supplemented medium to which was added 0.75 nmol of [35S]methionine (1.14 mCi/µmol); the incorporation of label into cellular protein was allowed to proceed for 20 min, at which time the cells were solubilized by addition of 1.5 mL of a solution containing 100 mM NaCl, 5 mM EDTA, 10 mM K2HPO4, 1% Triton X-100, 0.5% deoxycholate, 0.1% sodium dodecylsulfate (SDS), and protease inhibitors: 1 µg each of antipain, leupeptin, 5 µg N-acetyl-Leu-Leu-Nle-CHO and 1 mM 4-(2-aminoethyl)benzenesulfonfluoride·HCl. The cell extract was sonicated for 30 s and incubated at 40°C for 30 min to complete solubilization. Aliquots (20 µL) of the solubilized cells were acidified with trichloroacetic acid to precipitate total protein. After being washed twice with cold 95% acetone/water, the precipitated protein pellets were used to quantify incorporation of [35S] into total protein.

ApoB was immunoprecipitated from the rest of the solubilized cell material by incubation with sheep anti-rat apoB antibody (4 h at 0°C) followed by incubation (4°C for 18 h) with donkey anti-sheep IgG antiserum. The immune precipitates were dissolved in medium containing 62 mM Tris-HCl (pH 6.8), 8 M urea, 16 mM dithiothreitol, 2% SDS, and 10% mercaptoethanol, aided by sonication. ApoB48 and apoB100 were separated by SDS-polyacrylamide gel electrophoresis as described in Reference 15. Quantification of the radioactivity associated with intact apoB48 and apoB100 was performed by phosphorimage analysis.

Materials. Culture media and amino acid, vitamin, and antibiotic solutions were obtained from Gibco (Irvine, Scotland). TAG assay kits were obtained from Sigma (Dorset, United Kingdom). Radiochemicals were obtained from NEN Life Science Products (Hounslow, United Kingdom).

RESULTS AND DISCUSSION

Effect of exogenous oleate on the use of endogenous acyl moieties for TAG secretion. When cells were preincubated overnight with [14C]oleate, they accumulated [14C]TAG and phospholipid intracellularly (7,16). After washing and re-incubation with fresh medium from which oleate was absent, the cells secreted [14C]-labeled TAG linearly for at least 3 h (basal rate; Fig. 1A). Addition of oleate (0.75 mM) to the fresh incubation medium at the start of this 3 h period increased the rate of secretion of endogenous ([14C]-labeled) moieties within TAG by 55% (Fig. 1A). In addition, when the exogenously added oleate was [3H]-labeled, it too contributed acyl moieties (about 20% of the total, Fig. 1B) toward TAG secretion (Fig. 1B). Consequently, the overall rate of TAG secretion (from endogenous and exogenous acyl moieties) was increased twofold by the addition of oleate (compare filled symbols in Figs. 1A and 1B), with more than half the stimulation being due to the enhanced secretion of endogenous, prelabeled acyl moieties.

Secretion of TAG after depletion of ER Ca2+. Depletion of the thapsigargin-sensitive Ca2+ store of the ER resulted in inhibition of the utilization of both endogenous and exogenous acyl moieties for TAG secretion (Fig. 2A,B, respectively). However, the pattern of inhibition was different for the two sources. Secretion of [14C]oleoyl moieties continued for at least 30 min, after which it was totally inhibited. By contrast,