Fatty Acid Ethyl Ester Synthesis by the Isolated Perfused Rat Heart

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Fatty acid ethyl esters (FAEEs), nonoxidative by-products of ethanol metabolism, are found in various tissues and plasma after ethanol ingestion and may be responsible for some of the pathological changes observed in alcohol-consuming individuals. Previous studies demonstrated that several different enzymes, including lipoprotein lipase (LPL), can catalyze FAEE synthesis in vitro. We report that LPL catalyzes FAEE synthesis in isolated rat hearts perfused with chylomicrons in the presence of ethanol. Most of the FAEEs accumulated in the perfusate, suggesting that in vivo, plasma FAEEs derive from LPL-mediated synthesis. Our results are the first demonstration of the direct involvement of a specific enzyme, LPL, in FAEE synthesis under physiological conditions.

MOST OF THE ETHANOL absorbed from the gastrointestinal tract is cleared from the circulation by the liver, where it is metabolized through well-characterized oxidative pathways.[1] Oxidative metabolites of ethanol, eg, acetaldehyde and acetate, have been implicated in hepatic pathological manifestations associated with ethanol abuse.[1] Pathological changes also occur in organs with a limited capacity to oxidize ethanol (eg, heart, brain, and pancreas), suggesting that in these organs ethanol-induced abnormalities may be caused by the ethanol itself or by nonoxidative products of ethanol metabolism. Fatty acid ethyl esters (FAEEs), nonoxidative by-products of ethanol metabolism, have been proposed to induce pathological changes in various organs.[2] The basis for this proposal rests mainly on observations that FAEEs are found in organs often damaged as a result of excessive alcohol consumption.[2-4] FAEEs are also found in human plasma after ethanol ingestion, in concentrations that appear to correlate with blood ethanol levels.[5] Doyle et al[5] and Gorski et al[6] suggested that FAEEs may be secreted into the plasma by the liver, extrahepatic tissues, and/or leukocytes and could therefore be used to monitor tissue FAEE accumulation. However, the origin of plasma FAEEs has not been determined.

Several enzymes have been reported to catalyze FAEE synthesis in vitro. These include glutathione S-transferase,[7] pancreatic cholesterol esterase,[8] adipose tissue carboxylesterase,[9] pancreatic carboxylester lipase,[10] and pancreatic triglyceride lipase.[8] Whether any of these enzymes are involved in FAEE synthesis in vivo has not yet been established. Tsujita and Okuda[11] reported that purified lipoprotein lipase (LPL) can also catalyze FAEE synthesis in vitro. LPL is a key enzyme in the catabolism of plasma triglyceride-rich lipoproteins.[12] Its physiological site of action is on the lumenal surface of capillary endothelium of cardiac and skeletal muscle, adipose tissue, and several other extrahepatic tissues.[12] LPL catalyzes an essential step in the process of clearance of chylomicrons and very–low-density lipoproteins from the circulation, namely the hydrolysis of their core triglycerides to generate chylomicron remnants.[12] Unesterified fatty acids generated by triglyceride hydrolysis in the presence of ethanol may become esterified to form FAEEs.[11]

In the present investigation, we asked whether LPL can catalyze FAEE synthesis in vivo. For this purpose, we used the isolated perfused rat heart, a preparation that has been successfully used as a physiological model of LPL function in vivo.[13] We report here that in hearts perfused with chylomicrons in the presence of ethanol FAEE is synthesized as a result of LPL activity. Most of the FAEE accumulates in the perfusate, suggesting that in vivo FAEE found in the plasma after ethanol consumption derives from LPL-mediated synthesis.

MATERIALS AND METHODS

Chylomicron and Emulsion Preparation

^{14}C-labeled chylomicrons were obtained by cannulating the thoracic duct of male Sprague-Dawley rats (180 to 200 g), which were then fed by stomach intubation 1 mL corn oil containing 70 μCi [1-^{14}C]-palmitic acid (57 Ci/mol; Amersham, Arlington Heights, IL) as previously described.[14] The lymph was collected in the ensuing 6 hours at room temperature in the presence of penicillin and streptomycin, and chylomicrons were then collected by ultracentrifugation.[14] ^{3}H-labeled triglyceride emulsion was prepared by sonication of 2 mL Lyposin II 10% (Abbott Laboratories, Chicago, IL) with 20 μCi [9, 10-^{3}H] (*N*) triolein (28 Ci/mmol; New England Nuclear, Boston, MA) as previously described.[15] Half of the sonicated emulsion was mixed with 0.75 mL saline, and the other half was mixed with 0.75 mL rat serum. After 20 minutes' incubation at room temperature, the emulsions were layered under 2 mL 0.15-mol/L NaCl and centrifuged at 80,000 × *g* for 60 minutes at 15°C in a Beckman (Fullerton, CA) model L5-75 centrifuge, and the floating lipid layer was collected and redispersed in saline. The serum-incubated ^{3}H-labeled triglyceride emulsion acquired apoproteins, including apoprotein CII, by transfer from the serum lipoproteins.[16]

Heart Perfusion

The perfusion techniques were previously described.[17] Briefly, male Sprague-Dawley rats (250 to 280 g) were fasted overnight and anesthetized, and the hearts were removed, immediately tied to the perfusion cannula, and preperfused for 1 minute in a nonrecirculatory mode with oxygenated Krebs-Henseleit bicarbonate buffer containing 1 mg/mL glucose. The hearts were then switched to a recirculatory perfusion system and perfused for up to 30 minutes with 20 mL oxygenated Krebs-Henseleit bicarbonate buffer containing 1 mg/mL glucose with the following additions as appropriate: ^{14}C-labeled chylomicrons, ^{3}H-labeled triglyceride emulsion, ethanol, and/or fatty acid–depleted bovine serum albumin (A-7030; Sigma, St Louis, MO). At the

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end of the perfusion, the hearts were switched back to the nonrecirculatory perfusion system and perfused for 1 additional minute with the Krebs-Henseleit bicarbonate buffer containing 1 mg/mL glucose.

Lipid Analysis

The perfusates and homogenized hearts were extracted by the procedure of Folch et al.[18] The extracted lipids, as well as FAEE and oleic acid standards, were separated by thin-layer chromatography on glass plates (20 × 20 cm) coated with siliga gel G (Fisher Scientific, Pittsburgh, PA). The solvent system used was petroleum ether: diethyl ether: acetic acid (90:10:1). The lipid spots were visualized by iodine vapor, and FAEEs and unesterified fatty acids were scraped from the plates into vials containing scintillation fluid for radioactivity counting. In preliminary experiments, we determined that the efficiency of ^{14}C-labeled FAEE recovery was 90% to 95%. This was determined by adding ^{3}H-labeled ethyl oleate to the perfusates and heart homogenates before the extraction procedure. ^{3}H-labeled ethyl oleate was synthesized essentially as described by DePergola et al.[19]

Other Procedures

Chylomicron and Liposyn triglyceride levels were measured with the Synchron CX-7 Analyzer (Beckman).

Data Analysis

Curves were determined by analyzing X-Y pairs of variables and then fitting Y by X either by least-squares (straight-line linear regression) or by two-degree polynomial (nonlinear) curve-fitting routines to determine an R^2 using *JMP* Statistical Software (version 3.1.6, SAS Institute, Cary, NC). In all cases, R^2 was greater than .945, except for the curve in Fig 1a, which was .095.

RESULTS

In a series of in vitro experiments, Tsujita and Okuda[11] demonstrated that in addition to its main function in the hydrolysis of chylomicron triglycerides, LPL catalyzes the synthesis of FAEEs when ethanol is present. To determine whether LPL-mediated FAEE synthesis occurs physiologically, isolated rat hearts were perfused with chylomicrons labeled with ^{14}C triglyceride fatty acids in the presence of increasing amounts of alcohol. Figure 1a shows that LPL-mediated hydrolysis of chylomicron triglycerides is unaffected by the presence of ethanol (0.1 to 0.2 mol/L) in the perfusate. The extent of the observed LPL-induced hydrolysis is probably underestimated, since some of the fatty acids generated were presumably taken up by the heart and oxidized or incorporated into tissue lipids.[17,20] Figure 1b shows that FAEE is synthesized when ethanol is present in the perfusate. FAEE was detected in both the perfusate and in the heart, with the majority (>80%) present in the perfusate.

To determine whether LPL-mediated FAEE synthesis occurs in a concentration-dependent manner, isolated rat hearts were perfused with increasing chylomicron concentrations in the presence of 0.15 mol/L ethanol. Figure 2 shows that after a 30-minute perfusion, unesterified fatty acids and FAEEs accumulate in the perfusate as a function of chylomicron concentration. To determine the relationship between LPL-mediated chylomicron triglyceride hydrolysis and FAEE synthesis, hearts were perfused in the presence of 0.15 mol/L ethanol for 30 minutes, and ^{14}C-labeled unesterified fatty acids and FAEEs in the perfusate were determined at 10-minute intervals. Figure 3

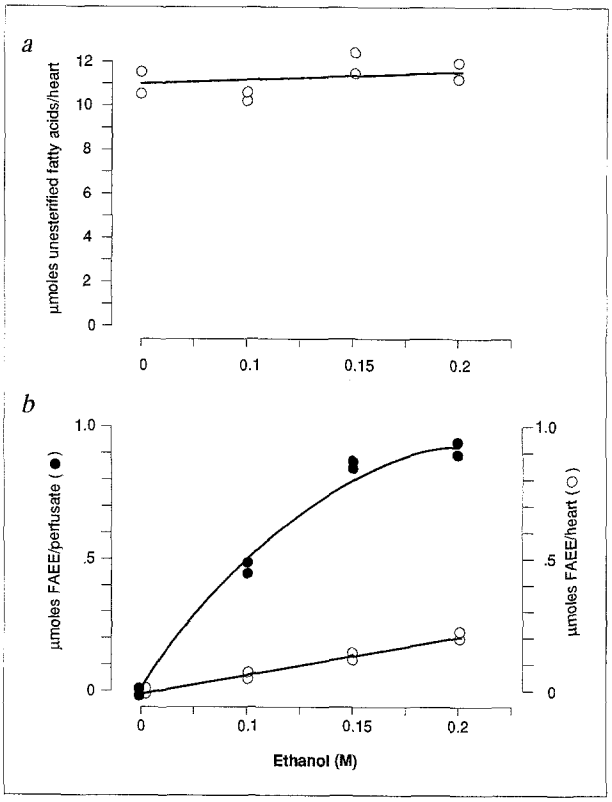

Fig 1. Generation of FAEEs and unesterified fatty acids by isolated heart in the presence of an ethanol-containing perfusate. Hearts were perfused with 20 mL Krebs-Henseleit bicarbonate buffer containing ^{14}C-labeled chylomicrons (1.7 µmol triglyceride fatty acids/mL), glucose (1 mg/mL), albumin (0.6 g/mL), and ethanol as indicated for 30 minutes. Data obtained from individual hearts are plotted.

shows that both products increase as a function of time over the 30-minute period.

To confirm that FAEE synthesis by the isolated perfused rat heart is catalyzed by LPL, we took advantage of the fact that

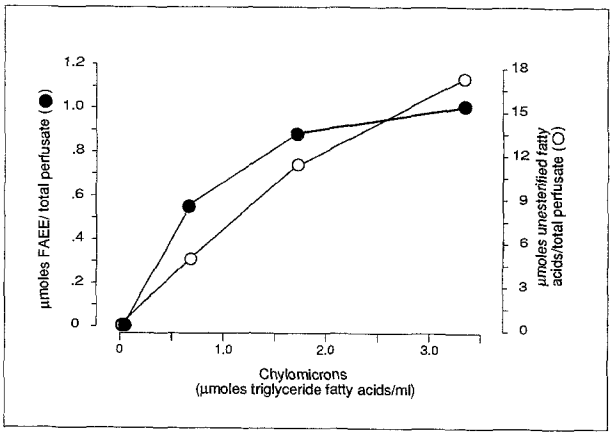

Fig 2. Effect of chylomicron concentration on generation of FAEEs and unesterified fatty acids by the isolated heart in the presence of an ethanol-containing perfusate. Hearts were perfused with 20 mL Krebs-Henseleit bicarbonate buffer containing glucose (1 mg/mL), albumin (0.6 g/mL), ethanol (0.15 mol/L), and ^{14}C-labeled chylomicrons as indicated for 30 minutes. Data obtained from individual hearts are plotted.

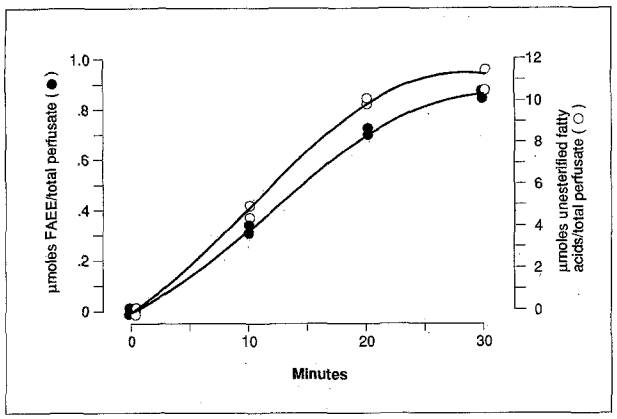

Fig 3. Effect of perfusion time on generation of FAEEs and unesterified fatty acids by the isolated heart in the presence of an ethanol-containing perfusate. Hearts were perfused with 20 mL Krebs-Henseleit bicarbonate buffer containing ^{14}C-labeled chylomicrons (1.7 µmol triglyceride fatty acids/mL), glucose (1 mg/mL), albumin (0.6 g/mL), and ethanol (0.15 mol/L) for the times indicated. Data obtained from individual hearts are plotted.

apoprotein CII is a required cofactor for optimal LPL activity.[12] Rat hearts were perfused either with an apoprotein CII–free, chylomicron-like emulsion labeled with ^{3}H-triolein or with the same emulsion containing apoprotein CII acquired by incubating the emulsion with rat serum, as described earlier. Figure 4 shows that the addition of apoprotein CII increases both the hydrolysis of triglyceride fatty acids and the synthesis of FAEEs, demonstrating the essential role of LPL in these catalytic processes.

Previous study[21] demonstrated that LPL is released into the perfusate when isolated hearts are perfused with a medium containing chylomicrons. When albumin is present in the perfusate, the activity of released LPL is significantly increased.[21] Figure 5 shows that when hearts are perfused with ^{14}C-labeled chylomicrons and ethanol, the accumulation of both unesterified fatty acids and FAEEs in the perfusate is a function of albumin concentration. These results indicate that both triglyceride hydrolysis and FAEE synthesis occur mainly in the perfusate and that the released LPL activity is dependent on the amount of albumin present in the perfusate.

DISCUSSION

Tsujita and Okuda[11] demonstrated that LPL, like several other enzymes,[7-10] can act as an FAEE synthase. The present investigation extends their findings by demonstrating that LPL catalyzes FAEE synthesis in the isolated perfused rat heart preparation. The present results constitute the first demonstration of the direct involvement of a specific enzyme, LPL, in FAEE synthesis under physiological conditions. This was demonstrated by perfusing rat hearts with chylomicrons, a physiological substrate for LPL, in the presence of ethanol. Under these conditions, FAEE was detected both in the perfusate and in the heart. The involvement of LPL as an FAEE synthesis was confirmed by demonstrating that FAEE synthesis was significantly increased upon acquisition from the serum of apoprotein CII, a specific cofactor for LPL, by a chylomicron-like triglyceride emulsion (Fig 5).

Under the conditions used, only a small proportion of the FAEEs synthesized were detected in the perfused heart (Fig 1). The origin of heart FAEEs could not be determined under the experimental conditions used in the present study. Heart FAEEs may have originated (1) from FAEEs synthesized either on the endothelial surface or in the perfusate and subsequently taken up by the heart and/or (2) from the esterification of ethanol to ^{14}C fatty acids within the myocardial cells. FAEEs present in the

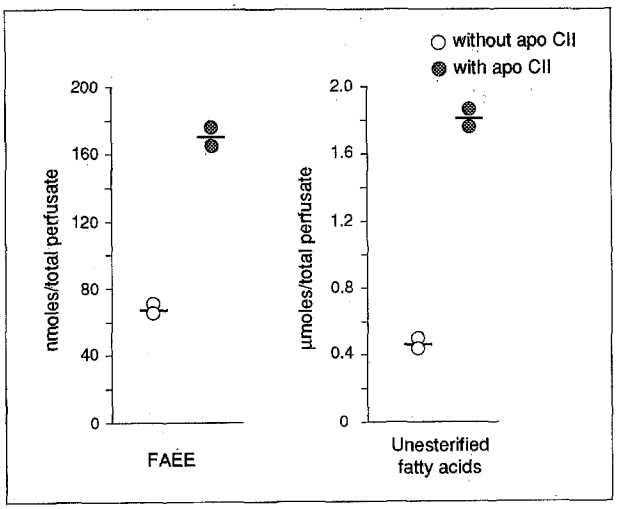

Fig 4. Effect of LPL activation by apoprotein CII on generation of FAEEs and unesterified fatty acids by the isolated heart in the presence of an ethanol-containing perfusate. Hearts were perfused with 20 mL Krebs-Henseleit bicarbonate buffer containing glucose (1 mg/mL), albumin (0.6 g/mL), ethanol (0.15 mol/L), and ^{3}H-labeled triglyceride emulsion (1.7 µmol triglyceride fatty acids/mL) for 30 minutes. Data obtained from individual hearts are plotted.

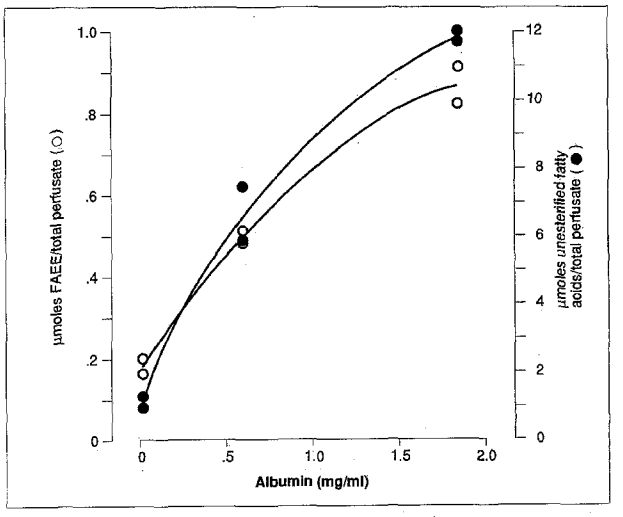

Fig 5. Effect of albumin on generation of FAEEs and unesterified fatty acids by the isolated heart in the presence of an ethanol-containing perfusate. Hearts were perfused with 20 mL Krebs-Henseleit bicarbonate buffer containing ^{14}C-labeled chylomicrons (1.7 µmol triglyceride fatty acids/mL), glucose (1 mg/mL), ethanol (0.15 mol/L, and albumin as indicated for 30 minutes. Data obtained from individual hearts are plotted.

perfusate are presumably derived both from synthesis on the endothelial surface and from synthesis in the perfusate. In the perfused heart, as well as in vivo, LPL-mediated triglyceride hydrolysis initiates when chylomicrons first bind to LPL anchored on the endothelial surface. In the presence of ethanol, FAEEs may be synthesized at this site. However, LPL-chylomicron binding is believed to be transient,[22] some of the endothelium-bound LPL may be transferred to the chylomicron surface, and in the presence of ethanol, FAEE synthesis occurs when the lipoprotein is no longer attached to the endothelial surface. LPL release into the perfusate was confirmed by the demonstration that after 30 minutes of heart perfusion the perfusate continued to accumulate unesterified fatty acids and FAEEs in the absence of the heart (data not shown). Doyle et al[5] and Gorski et al[6] suggested that FAEEs synthesized within various tissues and cells may be the source of plasma FAEEs. Our findings suggest instead that FAEEs present in the plasma of ethanol-consuming individuals are synthesized in the vascular space by LPL bound to the endothelial surface or released into the circulation bound to chylomicrons. Doyle et al[5] reported that plasma FAEEs in ethanol-consuming individuals are bound mainly to albumin. The present investigation did not determine what proportion of FAEEs in the heart perfusate were bound to albumin or to the lipolyzed chylomicrons. However, the observation that the FAEE concentration in the perfusate greatly increased when albumin was present (Fig 5) suggests that only a small proportion of the FAEEs remained bound to the lipolyzed particles.

Our results demonstrate that LPL plays an active role in the nonoxidative metabolism of ethanol resulting in the generation of FAEE. This FAEE may then be incorporated into the tissue, where LPL is located, or transported in the plasma to other tissues to be further metabolized or incorporated into intracellular lipids. The precise role that intracellular FAEE may have in mediating pathological cellular changes remains to be determined.

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