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Trans fatty acids alter the lipid composition and size of apoB-100-containing lipoproteins secreted by HepG2 cells

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Abstract

This study was conducted to determine the secretion rate and composition of lipoproteins secreted by HepG2 cells as influenced by the type of fatty acid present in the incubation medium. Cells were preincubated for 24 h with palmitic, oleic, elaidic, linoleic or conjugated linoleic acid (CLA), and the lipoproteins secreted during a subsequent incubation period of 24 h were collected for analysis. The secretion rate of apolipoprotein B-100 (apoB) was significantly greater in HepG2 cells preincubated with elaidic acid compared with those preincubated with palmitic or oleic acid; apoB secretion was greater in cells preincubated with CLA compared with those preincubated with linoleic acid. The lipid composition of secreted lipoproteins was also influenced by fatty acid treatment, resulting in significantly smaller lipoprotein particles secreted by cells preincubated with elaidic acid and CLA compared with those secreted by cells treated with oleic acid and linoleic acid, respectively. Our results are relevant to human metabolism for the following reasons: (1) the size of plasma low-density lipoproteins (LDLs) is determined, at least in part, by the composition of apoB-containing lipoproteins secreted by the liver; (2) small plasma LDL particles are associated with an increased risk of coronary heart disease; and (3) specific dietary fatty acids can affect the composition and size of plasma LDLs, thereby imparting a relative atherogenicity to plasma LDLs independent of LDL cholesterol concentration. The present study therefore suggests that elaidic acid and CLA promote the hepatic secretion of small apoB-containing lipoproteins, which could lead to an increased production of small plasma LDL particles.

Keywords: HepG2 cells; ApoB secretion; Elaidic acid; CLA; *Trans* fatty acid

1. Introduction

Elevated plasma total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations have long been regarded as primary risk factors for the development of atherosclerosis and coronary heart disease (CHD) [1]. Prevention and treatment strategies for CHD are therefore aimed at lowering LDL cholesterol concentration; the effects of dietary fatty acids on plasma cholesterol levels are well understood in this regard [1–3]. More recently, the size and composition of plasma LDLs have been linked to CHD. Current evidence suggests that individuals with small, dense LDLs have an increased risk of CHD [4,5]. Despite the increasing awareness of LDL size as a risk factor for CHD, little is known about the influence of dietary fatty acids on LDL size and composition.

Plasma LDLs are largely derived from the intravascular degradation and modification of very low-density lipoproteins (VLDLs) secreted by the liver [6]. Apolipoprotein B-100 (apoB) is synthesized and secreted by the human liver and is a major protein constituent of plasma VLDLs and LDLs. ApoB is the sole apolipoprotein associated with plasma LDLs and, as expected, there is a direct correlation between plasma apoB and LDL cholesterol concentration [7]. Consequently, the dietary factors that control the rate of secretion of apoB-containing lipoproteins by the liver may also control, at least in part, plasma LDL composition and particle size.

HepG2 cells in culture have been used extensively as a model for studying the secretion of apoB-containing lipoproteins from the human liver [8,9]. In addition to apoB, HepG2 cells synthesize and secrete all the major apolipoproteins, enzymes, receptors and transport proteins that are essential to normal lipoprotein metabolism [8,9]. While the apoB-containing lipoproteins secreted by HepG2 cells tend

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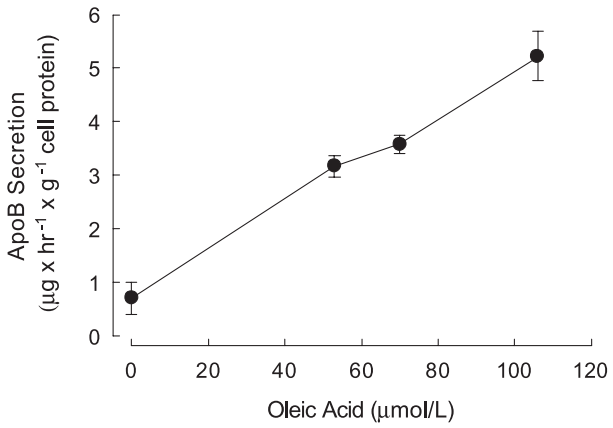

Fig. 1. ApoB secretion by HepG2 cells preincubated with increasing concentrations of oleic acid. Cells were preincubated for 24 h with DMEM-HAM's F-10 medium containing 0, 53, 70 or 106 µmol/L of oleic acid complexed with 180 µmol/L of BSA. After the preincubation, fresh medium (without fetal bovine serum) was added to all flasks and incubated for 24 h. Each point represents the mean±S.E.M. ($n=10$).

to be smaller and more dense than circulating plasma VLDLs, inclusion of fatty acids in the culture medium causes the secreted VLDLs to be larger and less dense, so that the secreted particles resemble plasma VLDLs [10]. In this way, HepG2 cells have proven to be an excellent model of VLDL assembly and secretion because they retain many of the normal biochemical functions of human liver cells and because they are responsive to fatty acids in a manner consistent with normal liver function.

Therefore, we have utilized HepG2 cells to examine the size and lipid composition of secreted lipoproteins as influenced by fatty acids added to the culture medium. We have focused our attention on *trans* fatty acids, namely, elaidic acid and conjugated linoleic acid (CLA). Elaidic acid is a major *trans* fatty acid found in hydrogenated vegetable oils and is thus a significant component of the American food supply. Elaidic acid is thought to promote hypercholesterolemia and increase the risk of CHD [11,12]. CLAs are also present in the food supply but are less abundant; their effect on lipoprotein and cholesterol metabolism is not well defined [13,14].

2. Methods and materials

2.1. HepG2 cell growth and maintenance

Stock cultures of HepG2 cells were grown in 75-cm^2 flasks containing 10 ml of DMEM-HAM's F-10 phenol red-free medium containing 2.5% fetal bovine serum and 1% antibiotic. The stock cultures were incubated in a humidified chamber and maintained at 37°C in a 95% air/5% CO_2 atmosphere. Cells were used for experiments when confluent monolayers were observed by light microscopy.

2.2. Preparation of FFA/BSA complex

Free form of long chain fatty acids (FFAs) was added to the culture medium as a complex with bovine serum albumin (BSA) using the procedure described by Watanabe et al. [15]. Specifically, free fatty acids (Nu-Chek Prep, Inc., Elysian, MN, USA) were dissolved in 10 ml of acetone. Exactly 33 µL of 5 N NaOH was added to each fatty acid and dried under nitrogen. When the sample was dry, 3.33 ml of 150 mmol/L NaCl was added and heated for 3–5 min at 60°C. Samples were then cooled and placed on a stir plate without heat and 4.2 ml of 24% BSA (ice cold) was rapidly added and stirred for 10 min. The final volume was adjusted to 8.33 ml with 150 mmol/L NaCl. The final concentration for each fatty acid was 106 mmol/L. Each FFA/BSA complex was applied to a PD-10 column (Amersham Biosciences, Piscataway, NJ, USA) to eliminate unbound fatty acids. To calculate the FFA/BSA molar ratio of each complex, a small amount of the complex was extracted then the fatty acids were analyzed by gas chromatography and the albumin was quantified by the Lowry et al. procedure [16]. If necessary, free albumin was added to the FFA/BSA solutions so that the FFA/BSA molar ratio was equivalent for all treatments in order to maintain a comparable concentration of both fatty acid and albumin in the medium. The FFA/BSA complex was sterilized by passing through a 0.45-µm filter.

2.3. Experimental design

When cells reached confluence, they were preincubated for 24 h with 10 ml of medium containing 106 µmol/L of fatty acid complexed with 180 µmol/L of BSA. (Preliminary experiments were conducted using oleic acid to attain optimal incubation conditions, which are presented in the Results section.) After the preincubation, fresh medium (without fetal bovine serum) was added to all flasks and incubated for 24 h. The 10 ml of medium was concentrated to 2 ml by ultrafiltration (Millipore Corp., Bedford, MA, USA) and analyzed for apoB and lipids as described below. Lipoprotein particle size was calculated according to Van Heek and Zilversmit [17].

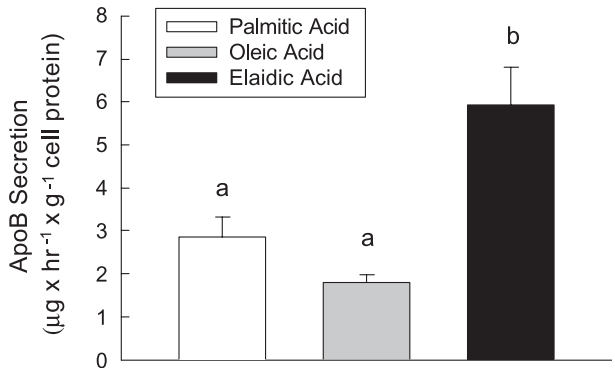

Fig. 2. ApoB secretion by HepG2 cells incubated with palmitic, oleic or elaidic acid. Cells were preincubated for 24 h with DMEM-HAM's F-10 medium containing 106 µmol/L of fatty acid complexed with 180 µmol/L of BSA. After the preincubation, fresh medium (without fetal bovine serum) was added to all flasks and incubated for 24 h. Values represent the mean±S.E.M. ($n=10$). Means having different superscripts are significantly different ($P<.05$). Data were log transformed prior to ANOVA.

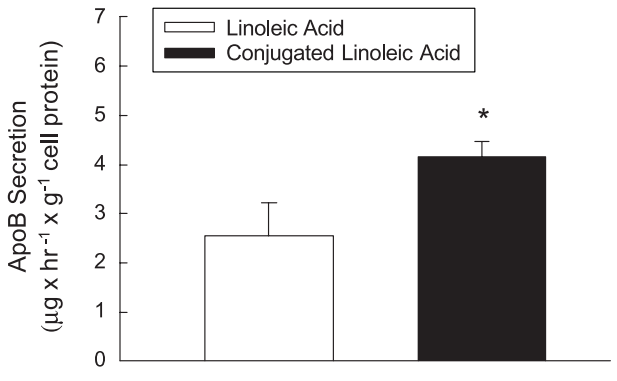

Fig. 3. ApoB secretion by HepG2 cells incubated with linoleic or CLA. Cells were preincubated for 24 h with DMEM-HAM's F-10 medium containing 106 µmol/L of fatty acid complexed with 180 µmol/L of BSA. After the preincubation, fresh medium (without fetal bovine serum) was added to all flasks and incubated for 24 h. Values represent the mean±S.E.M. ($n=10$). *Means are significantly different ($P<.05$). Data were log transformed prior to ANOVA.

2.4. ApoB determination

ApoB was determined by immunoturbidimetry (Sigma Chemical Co., St. Louis, MO, USA) and modified for use with 96-well microplates.

2.5. Lipid determination

Triglyceride, phospholipid, total cholesterol (Roche Diagnostics, Indianapolis, IN, USA) and free cholesterol (Wako, Richmond, VA, USA) were determined by an enzymatic method and adapted to microplates [18]. Esterified cholesterol was calculated by subtracting the amount of free cholesterol from the amount of total cholesterol in each sample.

2.6. Statistical analyses

Data were expressed as the mean±S.E.M. Effect of treatment was determined by one-way ANOVA and Fisher's post hoc test or Student's *t* test when only two treatment groups were compared (SigmaStat, Chicago, IL, USA). Differences were considered significant at $P<.05$.

3. Results

Preliminary experiments were performed to determine the optimal incubation conditions with regard to incubation time, BSA concentration and oleic acid concentration. ApoB accumulation in the medium was used as the primary indicator of lipoprotein secretion, assuming that each lipoprotein particle contains only one apoB molecule. Fig. 1 shows apoB accumulation to be linear when cells were preincubated with oleic acid up to 106 µmol/L of medium. In similar experiments, the optimal concentration of the BSA carrier was determined to be 180 µmol/L; therefore, all subsequent experiments were conducted with FFA/BSA complexes that delivered 106 µmol/L of FFA and 180 µmol/L of BSA during preincubation. After switching to fresh medium devoid of fatty acid, BSA and fetal bovine serum, we found that apoB accumulation was linear up to 24 h (data not shown); therefore, 24-h incubation times were used in all subsequent experiments for the analysis of lipoprotein secretion and composition.

To test the differential effects of fatty acids on lipoprotein (apoB) secretion, HepG2 cells were preincubated with palmitic, oleic or *trans* elaidic acid. Elaidic acid caused a significantly greater apoB secretion than palmitic acid and oleic acid (Fig. 2). Although elaidic acid and oleic acid differ only as geometric isomers, the *trans* double bond in elaidic acid caused a threefold increase in apoB secretion. A similar comparison was made between linoleic acid and CLA, in which CLA caused significantly greater apoB secretion (Fig. 3). These data indicated that fatty acids containing *trans* double bonds (i.e., elaidic and CLA) increase the secretion of apoB-containing lipoproteins by HepG2 cells when compared with their counterpart fatty acids with *cis* double bonds.

Lipoprotein particle composition was also measured and expressed as the number of lipid molecules per apoB molecule, assuming one apoB molecule per particle (Table 1). Cells preincubated with oleic acid exhibited greater amounts of lipids relative to apoB, indicating larger lipoprotein particles. Using the Van Heek and Zilversmit method for calculating particle size [17], oleic acid treatment resulted in the secretion of lipoproteins with significantly larger diameters compared with palmitic and elaidic acid treatments.

In a similar manner, we compared the composition of lipoproteins secreted by cells preincubated with linoleic acid or CLA (Table 2). Linoleic acid treatment resulted in

Table 1
Composition and size of lipoproteins secreted by HepG2 cells preincubated with palmitic, stearic, oleic or elaidic acid

	Surface lipid		Core lipid		
	Phospholipid	Free cholesterol	Triglyceride	Cholesteryl ester	Lipoprotein diameter
	Lipid/apoB molar ratio				Angstroms
Palmitic	744±110[a]	123±37	255±66[a]	1399±230[b]	210±38[a]
Oleic	1483±172[b]	338±96	2166±517[c]	2148±255[c]	331±37[b]
Elaidic	536±109[a]	73±44	506±56[b]	586±82[a]	208±22[a]

Values represent means±S.E.M. ($n=10$). Means within a column having different superscripts are significantly different ($P<.05$). Data were log transformed prior to ANOVA.
HepG2 cells were preincubated for 24 h with DMEM-HAM's F-10 medium containing 106 µmol/L of fatty acid and 180 µmol/L of BSA added as a complex.

Table 2
Composition and size of lipoproteins secreted by HepG2 cells preincubated with linoleic or CLA

	Surface lipid		Core lipid		
	Phospholipid	Free cholesterol	Triglyceride	Cholesteryl ester	Lipoprotein diameter
	Lipid/apoB molar ratio				Angstroms
Linoleic acid	2979±571	483±122	2127±399	1806±566	268±37
CLA[a]	590±90[b]	117±20[b]	285±102[b]	915±96	179±19[b]

Values represent means±S.E.M. ($n=10$). Data were log transformed prior to analysis by Student's t test.
HepG2 cells were preincubated for 24 h with DMEM-HAM's F-10 medium containing 106 μmol/L of fatty acid and 180 μmol/L of BSA added as a complex.
[a] CLA was an equimolar mixture of 18:1Δ9*c*,11*t* and 18:1Δ10*t*,12*c*.
[b] Mean of the CLA treatment is significantly lower compared with that of the linoleic acid treatment.

significantly more lipids relative to apoB and, consequently, larger particle diameters compared with CLA treatment.

4. Discussion

The primary objective of this study was to determine the secretion rate and composition of lipoproteins secreted by HepG2 cells as influenced by the type of fatty acid present in the incubation medium. Previous studies have shown that the addition of oleic acid to the culture medium stimulates apoB (i.e., lipoprotein particle) secretion by HepG2 cells [19–22]. Our results are consistent with other reports [23,24] indicating that cells incubated with oleic acid increase apoB secretion in a dose-dependent manner (Fig. 1). The rate of cellular triglyceride synthesis is also an important regulatory factor that governs lipoprotein secretion [25–27] and is correlated with oleic acid concentration in the medium [23]. These data confirm that the delivery of exogenous fatty acid increases the availability of cellular triglyceride for lipoprotein assembly, thus increasing lipoprotein secretion.

The secretion of apoB by cells appears to be regulated by the type of fatty acid present in the incubation medium. HepG2 cells incubated with elaidic acid secreted 2–3 times more apoB than cells incubated with palmitic or oleic acid (Fig. 2). Dashti et al. [28,29] also reported that elaidic acid caused greater apoB secretion compared with palmitic acid but that elaidic acid and oleic acid were equivalent in their effect on apoB secretion [28]. In agreement with our results, studies using rat hepatoma cells, McA-RH7777, indicated greater apoB secretion by cells incubated with elaidic acid compared with those incubated with oleic acid [30]. Among these common dietary fatty acids, it appears that elaidic acid promotes apoB secretion to the greatest extent. In other experiments, we found that CLA also increased apoB secretion compared with linoleic acid (Fig. 3). Similar results were reported by McLeod et al. [31] using McA-RH7777 cells. In contrast, Yotsumoto et al. [32] reported that the CLA isomer (18:2Δ10*t*,12*c*) decreased apoB secretion compared with linoleic acid. Despite these inconsistent results, the data demonstrate that both the position and geometric configuration (*cis* vs. *trans*) of double bonds affect the extent to which fatty acids regulate apoB secretion by hepatic cells.

Fatty acids appear to exert their influence on apoB secretion through posttranslational mechanisms including intracellular degradation of apoB and, possibly, recruitment of newly synthesized triglycerides for lipoprotein assembly [20,30,33–36]. Degradation of apoB is increased (and apoB secretion is thus reduced) by eicosapentaenoic acid relative to oleic acid [36], whereas myristic acid inhibits apoB degradation compared with oleic acid [34]. Although not measured directly in the present study, our data imply that elaidic acid (and perhaps CLA) promotes apoB secretion by inhibiting apoB degradation relative to the other fatty acids tested. Another possibility, given that triglyceride availability is an important factor in lipoprotein assembly and secretion [33,37], is that elaidic acid and CLA stimulate triglyceride synthesis and thus promote apoB secretion. Dashti et al. [29] have reported in HepG2 cells that elaidic acid promotes triglyceride synthesis and secretion compared with palmitic acid. However, regulation of triglyceride synthesis by fatty acid type is probably a minor factor compared with the regulation of apoB degradation [38]. Also, the secretion of triglyceride mass was not increased by elaidic acid or CLA in the present study (data not shown), further suggesting that regulation of apoB degradation is the most likely mechanism by which fatty acids mediate apoB secretion.

The composition and size of plasma LDLs are determined in large part by the composition of precursor lipoproteins secreted by the liver (reviewed in Ref. [39]). We therefore measured the composition and size of apoB-containing lipoproteins secreted by HepG2 cells as affected by the type of fatty acid in the incubation medium (Tables 1 and 2). Whereas cells incubated with oleic acid secreted relatively low amounts of apoB, lipid secretion was relatively high, resulting in lipoproteins with the largest particle diameter due to their lipid enrichment. The same observation was true for cells incubated with CLA, in which apoB secretion was significantly higher compared with that in linoleic acid treatment, but the lipid/apoB ratios and particle diameters were reduced. Dashti et al. [28,29] have also reported a significant shift in the lipid composition of apoB-containing lipoproteins secreted by HepG2 cells incubated with elaidic, oleic, palmitic and linoleic acids. Similarly, cells incubated with CLA alter the secretion of lipid relative to apoB [31,32,40]. Thus, our findings further demonstrate that the extent to which lipid associates with apoB during lipoprotein assembly is regulated by the type of fatty acid present in hepatic cells.

In humans, the size of plasma LDL particles has been investigated for its role in CHD. Several epidemiological and clinical studies have indicated an association between small LDLs and CHD [4,5,41–44], although this has not been shown to be fully independent of other metabolic factors such as elevated triglyceride levels [45]. Disagreement also exists regarding the effect of dietary fatty acids on LDL size. For example, Kim and Campos [46] reported an increase in LDL size with increasing *trans* fatty acid intake, whereas Mauger et al. [47] showed an association between small LDLs and consumption of *trans* fatty acids. These disparities notwithstanding, the present study suggests that individual dietary fatty acids may elicit their differential responses on plasma lipoproteins by mediating, at least in part, the composition and size of lipoproteins secreted by the liver.

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