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

Susan Hazels Mitmesser, Timothy P. Carr*

Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE 68583, USA

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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

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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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* Corresponding author. Tel.: +1 402 472 7940; fax: +1 402 472 1587.
E-mail address: tcarr2@unl.edu (T.P. Carr).

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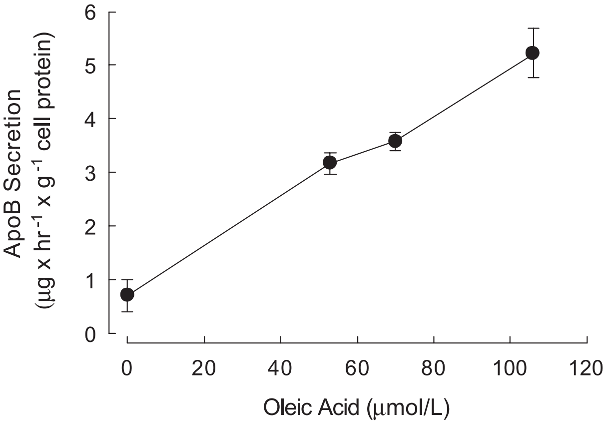

Fig. 1. ApoB secretion by HepG2 cells preincubated with increasing concentrations of oleic acid. Cells were preincubated for 24 h with DMEM-HAM's F-10 medium containing 0, 53, 70 or 106 µmol/L of oleic acid complexed with 180 µmol/L of BSA. After the preincubation, fresh medium (without fetal bovine 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].

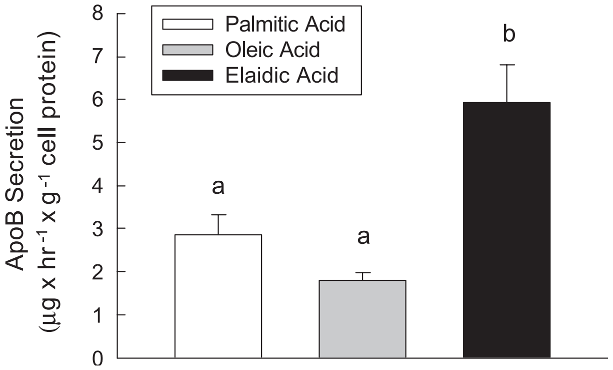

Fig. 2. ApoB secretion by HepG2 cells incubated with palmitic, oleic or elaidic acid. Cells were preincubated for 24 h with DMEM-HAM's F-10 medium containing 106 µmol/L of fatty acid complexed with 180 µmol/L of BSA. After the preincubation, fresh medium (without fetal bovine serum) was added to all 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.