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Nutrition Research 25 (2005) 387–399

NUTRITION RESEARCH

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Effects of *trans*-10,*cis*-12 conjugated linoleic acid and cognates on apolipoprotein B secretion in HepG2 cells

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Received 15 October 2003; revised 21 July 2004; accepted 7 December 2004

Abstract

Conjugated linoleic acid (CLA) has been shown to decrease apolipoprotein B (apoB) secretion in HepG2 cells. The purpose of this study was to determine the activity of individual CLA isomers—and to test cognates that are structurally related to CLA—with regard to apoB secretion. *Trans*-10,*cis*-12 CLA decreased apoB secretion whereas *cis*-9,*trans*-11, *cis*-9,*cis*-11, *trans*-9,*trans*-11, and the chloride, alcohol, or amide forms of CLA had no effect on apoB secretion. *Trans*-9,*cis*-12 octadecadienoic acid had no effect whereas *cis*-9,*cis*-12 octadecadienoic acid (linoleic acid) enhanced apoB secretion. Among 18-carbon monounsaturated fatty acids tested, only *trans*-10 octadecenoic acid decreased apoB secretion. *Trans*-11, *trans*-12, *trans*-13, *cis*-9, *cis*-11, and *cis*-13 octadecenoic acids increased apoB secretion whereas *trans*-9 and *cis*-12 octadecenoic acids were without effect. None of the 20-carbon compounds tested or *cis*-12 octadecen-10-ynoic acid had an effect on apoB secretion. Conjugated nonadecadienoic acid decreased apoB secretion whereas *cis*-10,*cis*-13 nonadecadienoic acid did not. The reduction of apoB secretion by CLA mixture is caused by the unique structural features of *trans*-10,*cis*-12 CLA. A *trans* double bond at the 10th position appears to be a key structure involved in the inhibition of apoB secretion.

Keywords: Conjugated linoleic acid; Apolipoprotein B; HepG2 cells; CLA; CNA; *Trans* fatty acids

Abbreviations: apoB; apolipoprotein B; CEA; *cis*-11,*trans*-13/*trans*-12,*cis*-14 eicosadienoic acid; CLA; conjugated linoleic acid; CNA; conjugated nonadecadienoic acid; LA; linoleic acid; LDL; low-density lipoprotein; LPL; lipoprotein lipase; NA; *cis*-10,*cis*-13 nonadecadienoic acid.

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doi:10.1016/j.nutres.2004.12.008

1. Introduction

Conjugated linoleic acid (CLA) is the term used for a group of positional and geometric conjugated dienoic isomers of linoleic acid (LA). Originally isolated from grilled ground beef, CLA occurs naturally in food, most notably dairy products and meat from ruminant animals [1,2]. Alkali isomerization of pure LA is used to produce the CLA typically used in experiments. This CLA consists mainly of the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers in approximately equal amounts (ranging from 40.8% to 47.6% each, depending on preparation), along with minor amounts of *trans*,*trans* and *cis*,*cis* isomers. Conjugated linoleic acid exhibits several biologically beneficial activities including decreased carcinogenesis, decreased adverse effects of immune stimulation, reduction of body fat with concurrent increase in lean body mass, and reduced development of atherosclerosis in various animal models [3]. It has been suggested that the *trans*-10,*cis*-12 CLA isomer is responsible for body composition changes in mice and decreased lipoprotein lipase (LPL) activity in 3T3-L1 adipocytes [4] as well as decreased hepatic stearoyl-CoA desaturase activity [5] and messenger RNA expression [6]. The *cis*-9,*trans*-11 and *trans*-9,*trans*-11 CLA isomers had no effect on these parameters [5,6].

The development of atherosclerosis is associated with elevated levels of low-density lipoproteins (LDL) and/or very low-density lipoproteins. HepG2 cells, a human hepatoma cell line, are often used to study effects on apolipoprotein B (apoB) because they synthesize and secrete lipoproteins in a manner that is similar to normal liver cells [7,8]. These cells secrete lipoprotein that has the density of LDL, with apoB being the principle apolipoprotein [7,9].

Previous reports by Lee et al [10] (in rabbits) and Nicolosi et al [11] (in hamsters) showed that CLA, fed as a mixture of isomers, reduced the development of atherosclerosis in animals fed with atherogenic diets. The mechanism by which CLA reduces atherosclerosis involves, at least in part, effects on lipoprotein metabolism. It has been reported that CLA [12] and, in particular, the *trans*-10,*cis*-12 isomer of CLA [13] decrease apoB secretion in HepG2 cells. Treatment with CLA did not affect apolipoprotein A1 secretion (indicative of HDL cholesterol) in HepG2 cells [12]. The purpose of this study was to test individual CLA isomers and other structurally related compounds to determine the key structure responsible for the effects of CLA on apoB secretion.

2. Methods and materials

2.1. Materials

HepG2 cells were purchased from the American Type Culture Collection (Rockville, Md). Bovine serum albumin (BSA; fatty acid free), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, Sigma Diagnostics Apo B kit, and human LDL were purchased from Sigma Chemical Co (St Louis, Mo). Unconjugated and peroxidase-conjugated sheep antihuman apoB antibodies for enzyme-linked immunosorbent assay (ELISA) were purchased from The Binding Site (Birmingham, UK).

Conjugated linoleic acid and *cis*-11,*trans*-13/*trans*-12,*cis*-14 eicosadienoic acid (CEA) were prepared by chemical synthesis using the procedure previously described [2], but with different starting materials (*cis*-9,*cis*-12 octadecadienoic acid and *cis*-11,*cis*-14 eicosadienoic