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Conjugated docosahexaenoic acid inhibits lipid accumulation in rats

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Abstract

Conjugated linoleic acid (CLA), which contains a conjugated double-bond system, and n-3 highly unsaturated fatty acids such as docosahexaenoic acid (DHA) are widely known to improve lipid metabolism. To examine the possibility that a fatty acid with a combination of these structural features might have stronger physiological effects, we prepared conjugated DHA (CDHA) by alkaline isomerization of DHA and examined its effects on lipid and sugar metabolism in rats. Rats were force fed with 200 mg of test oils [linoleic acid (LA), DHA, CLA or CDHA] everyday for 4 weeks. Compared with the animals from the other groups, those in the CDHA group showed a significant weight loss in white adipose tissue (57% of adipose tissue weight in the LA group) and significant decreases in the levels of liver triacylglycerol (TG; 65% of TG level in the LA group) as well as total cholesterol (TC; 88% of TC level in the LA group), indicating suppression of lipid accumulation in the liver and adipose tissue. In addition, plasma TG and TC levels significantly decreased (69% of TG level and 82% of TC level in the LA group), indicating improved lipid metabolism. In the liver, the fatty acid synthesis system was inhibited and the fatty acid β-oxidation system was activated, whereas the free fatty acid, glucose and tumor necrosis factor α levels in the plasma were lowered following CDHA administration. Hence, intake of CDHA appears to suppress the accumulation of fat in the liver and epididymal adipose tissue and improves lipid and sugar metabolism in rats.

Keywords: Conjugated docosahexaenoic acid; Conjugated linoleic acid; Conjugated fatty acid; Antiobesity

1. Introduction

Increased intake of fat is a factor in the recent growth of obesity in advanced countries. Obesity is caused by an excessive increase in white adipose tissue and has an important role as the basis for so-called lifestyle-related diseases such as diabetes mellitus, hyperlipidemia and arteriosclerosis. Accumulation of triacylglycerol (TG), which accounts for most dietary lipids, causes changes in liver function and is strongly associated with the development of pathological conditions such as fatty liver, hyperlipidemia and obesity [1]. Therefore, preventing fat accumulation in white adipose tissue and the liver is an approach to preventing lifestyle-related diseases.

Recent reports have shown that conjugated linoleic acid (CLA; 18:2), a fatty acid containing conjugated double bonds, has an antiobesity effect as a result of body fat accumulation suppression [2–5]. CLA is a geometric and optical isomer of linoleic acid (LA; 9*Z*12*Z*-18:2), is found widely in natural products and is particularly abundant in ruminant-derived oils and fats such as beef tallow and milk fat [6]. However, because the content of CLA in these sources is only approximately 1%, such natural oils and fats have not found use as CLA-containing lipids. Thus, CLA-containing oils and fats resulting from alkaline isomerization of plant oils such as safflower oil are currently available as commercial products and CLA has been reported to have various physiological effects, such as antiobesity, anticancer and antiarteriosclerotic properties [6–9].

Conjugated fatty acids other than CLA are known to occur naturally; however, only a few studies on their physiological functions have been performed [10–12]. We have studied the physiological function, metabolism,

Abbreviations: ACO, acyl–CoA oxidase; CLA, conjugated linoleic acid; CDHA, conjugated docosahexaenoic acid; DHA, docosahexaenoic acid; FAS, fatty acid synthase; FFA, free fatty acid; LA, linoleic acid; ME, malic enzyme; PL, phospholipid; TC, total cholesterol; TG, triacylglycerol; TNF-α, tumor necrosis factor α.

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analysis and oxidative stability of these molecules [13–19] and found that their tumoricidal effect is stronger than that of CLA in vivo and in vitro [15,16,19]. Furthermore, we have shown that rats fed with a diet containing a fatty acid with a conjugated triene system metabolize this fatty acid to one with a conjugated diene system [13,18]. We have also developed analytical methods in which isomerization of conjugated fatty acids is avoided and in which the stability of fatty acids against oxidation is maintained [14,17].

It has been reported that n-3 highly unsaturated fatty acids such as docosahexaenoic acid (DHA; 4*Z*7*Z*10*Z*13*Z*16*Z*19*Z*-22:6) may improve lipid metabolism [20]. This observation, and the relative lack of information regarding the physiological functions of conjugated fatty acids other than CLA, led us to speculate that a fatty acid with a combination of conjugated double bonds and an n-3 highly unsaturated structure might have stronger physiological effects. A fatty acid with such a structural character does not exist in nature. Therefore, to examine this possibility, we prepared conjugated DHA (CDHA) from DHA by alkaline isomerization and examined its effects on lipid and sugar metabolism in rats.

2. Materials and methods

2.1. Materials

DHA (89% purity) was donated by Bizen Chemical (Okayama, Japan). Safflower oil and CLA (80% purity) were obtained from Rinoru Oil Mills (Nagoya, Japan).

2.2. Preparation of safflower oil fatty acid

Safflower oil fatty acid was prepared from safflower oil by saponification [14,15]. After bubbling with nitrogen gas for 15 s, 90 mg of oil was saponified with 15 ml of 0.3N KOH in 90% methanol at 37°C for 2 h. After cooling to room temperature, the reaction mixture was added to 5 ml of 90% methanol and 15 ml of hexane, and the mixture was vigorously shaken. The methanolic aqueous layer was further washed twice with 15 ml of hexane to exclude nonsaponaceous material. The recovered washed methanolic aqueous layer was added to 9 ml of 6N HCl, and the fatty acids were then extracted twice with 15 ml of hexane. The combined hexane extracts were evaporated under a nitrogen stream, and the concentrate was passed through Sep-Pak Silica (Waters, MA, USA), with 10 ml of hexane–diethyl ether (95:5, vol/vol) as the eluant to collect the safflower oil fatty acid.

2.3. Preparation of CDHA

CDHA was prepared from DHA by alkaline isomerization using the AOAC method with slight modifications [16,19,21]. DHA (100 mg) in a test tube (100-ml volume) was mixed with 10 ml of potassium hydroxide at a concentration of 21% (wt/wt) in ethylene glycol. Nitrogen gas was bubbled through the mixture, and the tube was then screw capped and allowed to stand for 5 min at 180°C. The reaction mixture was cooled, then 10 ml of methanol was added. The mixture was acidified to below pH 2 with 20 ml of 6N HCl, and the conjugated fatty acid was extracted with 5 ml of hexane after dilution with 2 ml of distilled water. The hexane extract was washed with 3 ml of 30% methanol and with 3 ml of distilled water before being evaporated under a nitrogen gas stream. The concentration of the conjugated fatty acid was determined by UV/VIS spectrophotometric analysis, which was performed using a Shimadzu UV-2400PC (Shimadzu, Kyoto, Japan). The spectrophotometric readings confirmed the occurrence of conjugated fatty acids [22] and showed that approximately 90% of the DHA had been isomerized to CDHA. CDHA was stored at −20°C after being purged with nitrogen gas.

2.4. Animals and treatments

Male Sprague–Dawley rats (4 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The commercial diet (MF) used for the animal trial was purchased from Oriental Yeast (Chiba, Japan). The nature of the conjugated double bonds in the CDHA samples prepared as described above was determined by UV/VIS spectrophotometric analysis; these data are summarized in Table 1. The prepared test oils contained the main fatty acid as 60% of the total fatty acid content, based on gas chromatography and UV/VIS spectrophotometry (Table 1). The gas chromatography conditions have been described in our previous reports [13,17,18]. The test oils were prepared as follows: LA oil was prepared with only safflower oil fatty acids; CLA oil, with safflower oil fatty acid and CLA (safflower oil fatty acid/CLA=13:87, vol/vol); DHA oil, with safflower oil fatty acid and DHA (safflower oil fatty acid/DHA=27:73,

Table 1
Fatty acid composition of the dietary oil mixtures

	LA[a] (%)	CLA[b] (%)	DHA[c] (%)	CDHA[d] (%)
16.0	8.0	8.8	2.1	2.0
18.0	3.0	5.2	1.0	0.7
18:1 (n-9)	17.3	14.6	14.2	11.2
18:2 (n-6)	70.8	10.3	22.3	20.7
CLA				
9*Z*11*E*	–	24.0	–	–
10*E*12*Z*	–	25.8	–	–
Others	–	10.2	–	
22:6 (n-3)	–	–	60.0	4.3
CDHA				
Diene	–	–	–	34.9
Triene	–	–	–	12.9
Tetraene	–	–	–	9.9
Pentaene	–	–	–	1.9
Hexaene	–	–	–	0.4
Others	0.9	1.1	0.4	1.1

CLA was donated by Rinoru Oil Mills. CDHA was prepared from DHA by the AOCS method. 9*Z*11*E* indicates 9*Z*11*E*-18:2; 10*E*12*Z*, 10*E*12*Z*-18:2; diene, conjugated diene; triene, conjugated triene; tetraene, conjugated tetraene; pentaene, conjugated pentaene; hexaene, conjugated hexaene.

[a] Safflower oil fatty acid.
[b] Safflower oil fatty acid/CLA=13:87 (vol/vol).
[c] Safflower oil fatty acid/DHA=27:73 (vol/vol).
[d] Safflower oil fatty acid/CDHA=21:79 (vol/vol).