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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).

Table 2
Effects of conjugated fatty acid on body weight, liver weight and epididymal adipose tissue weight of rats

	Groups			
	LA	CLA	DHA	CDHA
Body weight (g)	349±26.0	345±20.4	349±7.9	335±16.1
Liver weight (g/100 g body weight)	4.99±0.32	5.00±0.32	5.07±0.15	5.20±0.20
Epididymal adipose tissue (g/100 g body weight)	2.81±0.51[a]	2.36±0.41[b]	2.06±0.31[b]	1.61±0.25[c]

Values are expressed as mean±S.D. (n=8). Mean values in a row with different superscript letters are significantly different at P<.05.

vol/vol); and CDHA oil, with safflower oil fatty acid and CDHA (safflower oil/CDHA=21:79, vol/vol). The test oils were stored at −20°C until they were fed to the animals. After acclimatization to a commercial diet for 1 week, 32 rats were randomly divided into four groups according to the administered test oils: safflower oil fatty acid supplement (LA group, n=8), 60% CLA supplement (CLA group, n=8), 60% DHA supplement (DHA group, n=8) and 60% CDHA supplement (CDHA group, n=8). Eight rats were assigned to each group in this examination in reference to the subject allocation in the nutritional evaluation examination of conjugated fatty acids that we conducted before [13]. The initial body weight of the rats was 140±4 g in all groups. Two hundred milligrams of test oil per rat was administered daily by oral intubation. Two rats were housed in each cage. They were given free access to commercial diets and distilled water and were housed in a temperature- and humidity-controlled room with light cycles of 12 h on and 12 h off. All procedures were performed in accordance with the animal experiment guidelines of the Tohoku University.

2.5. Sample preparation and body weight measurement

During the 4-week test period, we measured diet intake in each group. The diet was given in a feeding box for powdered diets excluding an inside lid. The feeding box was fixed so that it would not be dropped by the rats. The diet intake was obtained by measuring the feeding box everyday and calculating the decrement of the diet. Feces during the 4 days preceding the end of the test period were collected as samples for determining the percentages of TG and free fatty acids (FFAs) that were absorbed. At the end of the test period, the rats were weighed and their blood samples were collected following decapitation. The liver and epididymal adipose tissue were removed and weighed. The blood was treated with EDTA, and plasma was isolated by cold centrifugation at 1000×g for 15 min at 4°C [13] and kept at −30°C until use.

2.6. Measurement of the absorption ratio of TG and FFAs

The TG and FFA absorption ratios were obtained from the amount of diet lipid and test oil consumed as well as from the total fatty acid content. Total lipids were extracted from the feces and diet during the final 4 days of the test period using the method of Folch et al. [23], and FFAs were prepared by saponification, as described above. The total fatty acid content was measured by the ACS/ACOD method, using an NEFA C-test Wako kit (Wako Pure Chemicals Industries, Tokyo, Japan). The TG and FFA absorption ratios were calculated using the following formula:

Absorption ratio (%) = (amount of fatty acids in diet + amount of fatty acids in test oil − amount of fatty acid in feces)/(amount of fatty acids in diet + amount of fatty acids in test oil) × 100.

2.7. Lipid determination

The liver was homogenized with four volumes of ice-cold saline. Total lipids from the liver homogenate and plasma were extracted using the procedure of Folch et al. [23]. TG and total cholesterol (TC) in total lipids of the plasma and liver were measured using commercially available enzyme kits [Triacylglycerol Test Wako and Total Cholesterol Test Wako (Wako Pure Chemicals Industries), respectively] according to the manufacturer's protocol. Phospholipid (PL) content in the total lipids of the plasma and liver was determined using the method described by Bartlett [24].

2.8. Assays of enzyme activity in the liver

Fatty acid synthase (FAS) activity in the liver was determined spectrophotometrically from the rate of malonyl–CoA-dependent NADPH oxidation [25]. Malic enzyme (ME) activity in the liver was determined as previously described [26]. FAS and ME activities were expressed as nanomoles of NADPH/min per mg protein. Acyl–CoA oxidase (ACO) activity in the liver was measured from the rate of palmitoyl–CoA-dependent H_2O_2 production coupled with dichlorofluorescein oxidation, as described by Small et al. [27]. The liver was homogenized to prepare a peroxisome-enriched fraction. The homogenate was centrifuged at 6000×g for 10 min at 6°C; the pellet was resuspended and recentrifuged as above. The supernatants were combined and centrifuged for 30 min

Table 3
Effects of conjugated fatty acid intake on the plasma and liver lipids of rats

	Groups			
	LA	CLA	DHA	CDHA
Plasma (μmol/ml)				
TG	2.48±0.45[a]	2.02±0.12[b,c]	2.08±0.23[b]	1.71±0.23[c]
TC	2.97±0.33[a]	2.59±0.33[b]	2.66±0.24[a,b]	2.45±0.14[b]
PL	3.16±0.65	2.77±0.40	3.02±0.46	2.72±0.23
Liver (μmol/g)				
TG	45.3±2.68[a]	39.1±3.76[b]	41.1±3.40[a,b]	29.5±4.54[c]
TC	8.95±0.50[a]	8.61±0.44[a,b]	8.32±0.54[a,b]	7.90±0.59[b]
PL	14.1±2.41	14.1±3.43	12.9±2.31	14.2±1.00

Values are expressed as mean±S.D. (n=8). Mean values in a row with different superscript letters are significantly different at P<.05.

Table 4
Activity of enzymes in fatty acid metabolism in the liver of rats fed with conjugated fatty acids for 4 weeks

	Groups			
	LA	CLA	DHA	CDHA
FAS (nmol/min per mg protein)	84.2±9.8[a]	88.7±8.1[a]	73.8±11.3[a]	54.8±5.2[b]
ME (nmol/min per mg protein)	85.0±7.6[a]	86.1±6.4[a]	80.8±8.3[a,b]	61.0±11.8[b]
ACO (nmol/min per mg protein)	7.7±0.9[a]	9.7±1.6[a,b]	9.1±0.8[a,b]	12.0±1.3[b]

Values are expressed as mean±S.D. ($n=8$). Mean values in a row with different superscript letters are significantly different at $P<.05$.

at 30,000×g; the pellet was resuspended. The supernatants were used as a reaction liquid. ACO activity was expressed as nanomoles of dichlorofluorescein/min per mg protein. The protein concentration in the liver was determined using a DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA, USA), with bovine serum albumin used as the standard.

2.9. Assays of leptin, tumor necrosis factor α, insulin, glucose and FFA concentration in the plasma

In accordance to the manufacturer's protocol in each case, the leptin concentration in the plasma was determined with a rat leptin enzyme immunoassay kit (Immuno-Biological Lab, Gunma, Japan); tumor necrosis factor α (TNF-α), with a rat TNF-α ultrasensitive ELISA kit (BioSource International, CA, USA); and insulin, with a rat insulin ELISA kit (Shibayagi, Gunma, Japan). The glucose and FFA contents were measured enzymatically with commercial kits supplied by Wako Pure Chemicals Industries, again according to the manufacturer's protocol.

2.10. Statistical analysis

Statistical analysis was performed using one-way ANOVA, followed by a Newman–Keules test for multiple comparisons among several groups. A difference was considered to be significant at $P<.05$.

3. Results

Rats were divided into four groups of eight animals each, and each group received a different test oil. The LA group was fed with only safflower oil; the remaining three groups were fed with a diet containing fatty acids, 60% of which was CLA, DHA or CDHA (Table 1). CLA contains only conjugated diene structures, whereas CDHA has a mixture of conjugated diene, triene, tetraene, pentaene and hexaene structures (Table 1). Each rat was force fed with 200 mg of the test oil everyday for 4 weeks. The diet plus 200 mg of the test oil for a rat is equivalent to a 7% fat diet, which is not a high-fat diet and is within the normal limits for lipid content. The conjugated fatty acids accounted for an average of 0.6% of the daily diet.

The amount of diet intake was 23.1±2.0 g/day in the LA group, 22.1±2.5 g/day in the CLA group, 21.5±1.5 g/day in the DHA group and 20.0±1.6 g/day in the CDHA group (values are expressed as mean±SD; $n=8$). There was no significant difference in the average diet intake during the test period. In addition, the amount of diet intake showed a tendency to decrease in the CDHA group. The digestibility of TG and FFA was 96.1±0.6% in the LA group, 95.1±0.8% in the CLA group, 96.2±0.6% in the DHA group and 95.0±0.8% in the CDHA group ($n=8$). There was no significant difference in the TG and FFA absorption ratios among the groups, with absorption of more than 95% of the fatty acids in the diet. The body weight at the end of the test period did not differ significantly among the four groups but showed a tendency to decrease in the CDHA group, as compared with the remaining groups (Table 2). The liver weight showed a tendency to increase in the CDHA group, as compared with the remaining groups, but the change was not significant (Table 2). The weight of epididymal adipose tissue, a visceral white adipose tissue, significantly decreased in the CDHA group, as compared with the remaining groups (Table 2); the adipose tissue weight in the CDHA group was 57% of that in the LA group by the end of the test period. These results suggest that CDHA intake prevented the growth of white adipose tissue and influenced lipid metabolism in rats.

The levels of TG, TC and PLs were determined in the plasma and liver to further explore the effects of CDHA on lipid metabolism. The plasma TG level in the CDHA group

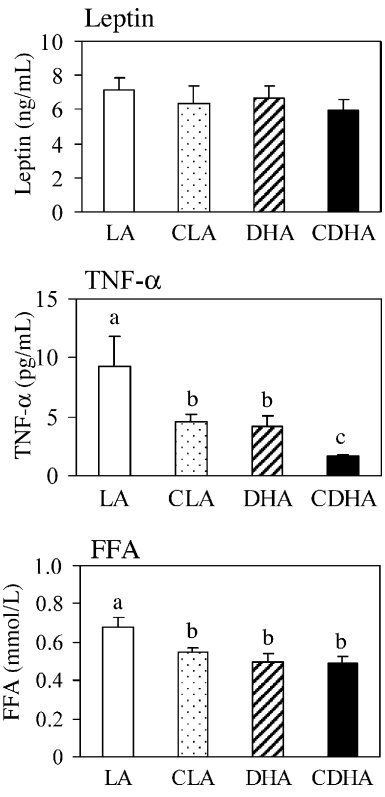

Fig. 1. Leptin, TNF-α and FFA concentrations in the plasma of rats fed with CDHA. Values are expressed as mean±S.D. ($n=8$). Mean values in a row with different superscript letters are significantly different at $P<.05$.

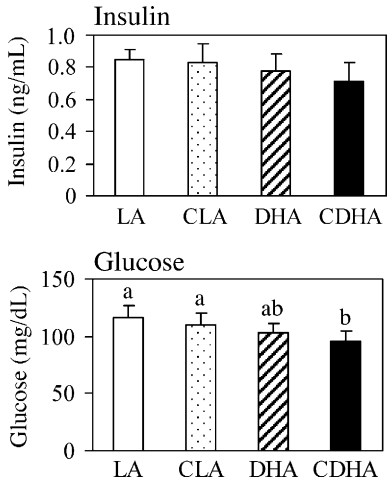

Fig. 2. Insulin and glucose concentrations in the plasma of rats fed with CDHA. Values are expressed as mean±S.D. ($n=8$). Mean values in a row with different superscript letters are significantly different at $P<.05$.

significantly decreased, as compared with the remaining groups, and reached a level of 69% of that in the LA group (Table 3). Similarly to the TG level, the plasma TC and PL levels showed a tendency to decrease in the CDHA group, as compared with the remaining groups (Table 3), and the TC level in the CDHA group significantly decreased to 82% of that in the LA group. There was no significant difference in the PL levels among the four groups. The lipid composition in the liver showed a behavior similar to that in the plasma, showing a tendency to decrease in the CDHA group (Table 3). The liver TG level in the CDHA group significantly decreased to 65% of that in the LA group, and the liver TC level in the CDHA group significantly decreased to 88% of that in the LA group. There was no significant difference in the PL levels among the four groups. These results suggest that CDHA reduces plasma and liver lipid levels and prevents accumulation of fat in the liver and white adipose tissue. This may be caused by an effect of CDHA on the fatty acid-metabolizing system in the liver, which could lead to changes in the levels of various hormones secreted from white adipose tissue.

To examine possible CDHA-induced changes in fatty acid metabolism in the liver, we measured the activities of fatty acid-synthesizing enzymes such as FAS and ME as well as the activity of the fatty acid β-oxidation enzyme ACO (Table 4). FAS activity in the CDHA group decreased to 65% of that in the LA group, and the CDHA group showed a significant decrease in FAS activity, as compared with the remaining groups. ME activity in the CDHA group was also significantly lower, at 72% of that in the LA group. In contrast to the activities of these enzymes, ACO activity showed the opposite tendency. Compared with the LA group, the CDHA group showed a significant increase in ACO activity to 157%. These results indicate that CDHA inhibits fatty acid synthesis and promotes fatty acid β-oxidation in the liver.

Next, we measured changes in the levels of leptin (an antiobesity hormone), TNF-α (known to induce insulin resistance) and FFAs in the blood (Fig. 1). There was no significant difference in the leptin levels among the four groups. However, the TNF-α level in the CDHA group was significantly decreased to 17% of that in the LA group, and the CDHA group also showed a reduced level of FFA to 72% of that in the LA group. The CLA and DHA groups also showed a significant decrease in the FFA level, as compared with the LA group. These results suggest that CDHA promotes energy consumption and sugar catabolism in the liver and muscles by reducing FFA levels; the decrease in the TNF-α level also suggests an effect of CDHA on sugar metabolism.

The blood insulin and glucose levels were measured to examine the effect of CDHA on sugar metabolism (Fig. 2). The plasma insulin level showed a tendency to decrease in the CDHA group, but the change was not significant. The glucose level also tended to be lower in the CDHA group and reached 83% of that in the LA group, which was a significant change. Hence, these results indicate that CDHA not only increases lipid metabolism but also affects sugar metabolism.

4. Discussion

In this study, we examined the effects of CDHA, a fatty acid containing conjugated double bonds and an n-3 highly unsaturated structure, on lipid and sugar metabolism in rats and compared them with those induced by LA, DHA and CLA. The results show that CDHA suppresses fat accumulation in the liver and adipose tissue and increases fat and sugar metabolism.

Although the mechanism of suppression of fat accumulation may involve inhibition of TG absorption, the TG and FFA absorption ratios did not differ among the groups receiving LA, DHA, CLA and CDHA. More than 95% of fat was absorbed in each group, indicating that orally consumed conjugated fatty acids do not influence the digestion and absorption of fat. Because suppression of fat absorption is involved in the development of colorectal cancer, this particular property of conjugated fatty acids is desirable.

The plasma TG level tended to decrease in the CDHA group, as compared with the other groups (Table 3). The rats were not fasted before autopsy, and, in general, blood TG in unfasted mammals is known to be present mainly in chylomicrons and VLDL. Chylomicron-TG, which is absorbed from dietary Fat and reconstituted in the small intestinal epithelium, is secreted into the blood through the lymphatic system. The finding that fat digestion and absorption did not differ among the four groups suggests that the level of chylomicron-TG does not vary with the diet. On the other hand, VLDL is produced in liver parenchymal cells and then secreted into the blood.

Therefore, the decrease in blood TG after intake of CDHA presumably resulted from a decrease in VLDL secretion and the promotion of hydrolysis and uptake of VLDL-TG in peripheral tissue. The plasma TC decrease was probably coupled with the decrease in TG (Table 3).

The activities of the liver fatty acid-synthesizing enzymes FAS and ME were significantly reduced in the CDHA group (Table 4), whereas the activity of ACO, the rate-limiting enzyme in fatty acid β-oxidation in liver peroxisomes, was significantly enhanced by CDHA intake (Table 4). These results indicate that CDHA suppresses fat accumulation in the liver and adipose tissue through inhibition of fatty acid synthesis and promotion of fatty acid catabolism in the liver peroxisomal β-oxidation system. The fatty acid β-oxidation system is present in mitochondria and peroxisomes, and fatty acids are usually thought to undergo β-oxidation in mitochondria. However, the peroxisomal β-oxidation system is also known to be sensitive to drugs, including antihyperlipidemic drugs such as fibrates, and is more easily activated compared with the mitochondrial oxidation system [28]. Therefore, enhancement of peroxisomal β-oxidation may be associated with CDHA-induced activation of fatty acid β-oxidation; the tendency of the liver to increase in weight also supports the activation of peroxisomes by CDHA (Table 2).

Transcription of the ACO gene is regulated by ligand-inducing transcription factors called transcription factor peroxisome proliferator-activated receptors (PPARs) [29]. Polyunsaturated fatty acids and their metabolites, eicosanoids, are endogenous ligands for PPARs, and fibrates and thiazolidinedione derivatives (antidiabetic drugs) are also known to be PPAR ligands [30–33]. In addition to ACO, PPAR-regulated transcription of various enzymes related to catabolism and utilization of fatty acids, such as mitochondrial β-oxidation system enzymes, acyl–CoA-synthesizing enzymes and lipoprotein lipases, is associated with suppression of fat accumulation [31]. CLA has been reported to act as a high-affinity ligand for PPAR and to activate PPARα in the regulation of lipid metabolism [34–36]. Hence, our results suggest that CDHA may also regulate lipid metabolism through the PPAR pathway and may have a higher affinity for PPAR compared with CLA. However, a recent study using PPARα knockout mice has shown that when PPARα knockout mice and wild-type mice are fed with a CLA-containing diet, genes related to hepatic lipid metabolism respond similarly, suggesting that CLA regulation of lipid metabolism may proceed via a pathway other than PPARα-mediated transcription [37]. Therefore, conjugated fatty acids may regulate lipid metabolism by a PPARα-independent mechanism and their PPAR-mediated effects on lipid metabolism remain unclear and require further study.

Recent studies have reported increased glucose metabolism in muscle tissue with a delay in the development of diabetes mellitus following CLA intake in Zucker diabetic fatty rats [38,39]. Our data suggest that CDHA lowers blood glucose levels more potently than CLA (Fig. 2), suggesting that CDHA may increase sugar metabolism and exert a more potent antidiabetic effect than CLA. This effect is presumably secondary to the decrease in blood TG levels with suppression of FFA release from adipose tissue and promotion of sugar consumption in muscle tissue (Table 3; Figs. 1 and 2). It has also been reported that CLA enhances the effect of insulin in mice, swine and humans [40,41] and that the levels of FFAs and TNF-α, both of which cause insulin resistance, are elevated in the blood of obese patients [42]. In the CDHA group, the blood insulin level did not change but the FFA and TNF-α levels significantly decreased (Figs. 1 and 2), presumably leading to enhancement of the effect of insulin and contributing to the CDHA-induced increase in sugar metabolism.

Our data show that CDHA exerted stronger effects than CLA, suggesting that the structure of conjugated double bonds in fatty acids can influence their effects. We have previously reported that fatty acids with a conjugated triene structure have a stronger tumoricidal effect than those with a conjugated diene structure and that the effect varies with the structure of the conjugated double bonds [15,16,19]. Although we are unable to conclude which type of conjugated double bond system leads to the strongest effects, it seems likely that the configuration of the conjugated double bonds, the number of carbon atoms and the number of double bonds all influence the effects of fatty acids (Table 1).

In conclusion, CDHA, a fatty acid containing conjugated double bonds and an n-3 fatty acid structure, showed a stronger effect on fat accumulation compared with CLA and DHA. CDHA was found to inhibit the hepatic fatty acid-synthesizing system, activate the hepatic fatty acid β-oxidation system, lower blood TG and TC levels, reduce secretion of FFAs from white adipose tissue, suppress the accumulation of fat in the liver and adipose tissue, enhance the action of insulin and lower blood glucose levels. These properties suggest that CDHA may be effectively used as a medicine, although further studies will be needed to ascertain the effects and safety of its long-term administration. In addition, an advanced study on the relationship between chemical structures and lipid accumulation inhibitory action is now underway in our laboratory.

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