Combined Effects of Dietary Conjugated Linoleic Acid and Sesamin on Triacylglycerol and Ketone Body Production in Rat Liver

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Summary The effects of a combination of dietary conjugated linoleic acid (CLA) supplemented with sesamin on hepatic ketogenesis and triacylglycerol secretion were compared using the livers of rats fed diets containing 1% CLA or linoleic acid (LA) in combination with 0.2% sesamin for 14 d, respectively. The feeding of CLA, as compared to LA, caused a significant reduction in the weight of perirenal adipose tissue but not that of epididymal adipose tissue, and affected neither growth parameters nor hepatic lipid concentration. Hepatic production of ketone bodies was consistently higher in rats fed CLA than in those fed LA, while triacylglycerol secretion was reversed. No significant difference was noted in the hepatic secretion of cholesterol among the groups. Although there was no effect of the dietary combination of CLA with sesamin on adipose tissue weight, hepatic lipid parameters and ketone body production were observed: i.e., triacylglycerol secretion tended to be reduced. These results suggest that the dietary combination of CLA with sesamin may be an effective approach for lowering serum triacylglycerol levels. The decreased hepatic secretion of triacylglycerol is, in part, due to enhanced fatty acid oxidation in the liver.

Key Words conjugated linoleic acid, sesamin, triacylglycerol, ketone body

CLA, a mixture of the positional and geometrical isomers of linoleic acid that occurs naturally in small quantities in meat and dairy products, has recently been shown to have a variety of beneficial effects on health, including anticarcinogenic, antiatherogenic, antidiabetic, and antiobesity activities (1). In addition, we have recently reported that CLA is a potent stimulator for fatty acid oxidation rate in rat liver, as characterized by an elevation of ketone body production possibly by an increase in the activity of liver mitochondrial carnitine palmitoyltransferase (2).

On the other hand, sesamin is one of the lignans occurring in sesame seed and oils, and it exhibits a number of beneficial effects including protection against tumor development (3), liver damage caused by carbon tetrachloride or alcohol (4), and lipid and lipoprotein disorders caused by dietary manipulations (5–11). We have previously reported that sesamin is probably a potent peroxisome proliferator, and thereby exhibits a specific action toward fatty acid metabolism as characterized by enhanced ketone body production in rat liver (12, 13). Thus, the mechanism underlying the elevated fatty acid oxidation rate as reflected by the enhanced ketone body production in the livers of rats fed diets containing CLA or sesamin appears to be different in some manners. The same may be the case for the reciprocal decrease in triacylglycerol secretion by the livers of rats fed diets containing either of these dietary factors (2, 12, 13).

These observations led us to examine whether or not a dietary combination of CLA and sesamin exerts a combined effect on lipid and lipoprotein metabolisms. In this context, Sugano et al. recently reported that the body fat-reducing activity of dietary CLA was increased by the simultaneous ingestion of sesamin (14). The present study examined the possibility that the combination of CLA and sesamin exerts a beneficial effect on liver lipid metabolism using a rat liver perfusion system.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Kyudo Co., Kumamoto, Japan) weighing 140–150 g (5-wk-old) were individually housed in hanging wire mesh cages and maintained at a constant temperature (22–24°C) with lights on 7:00 to 19:00. Rats were acclimatized for 6 d and then divided to 4 groups with equal body weights: the first and second groups were fed powdered commercial chow (Type CE-2, Nihon Clea, Tokyo, Japan) supplemented with 1.0% linoleic acid (LA) or 1.0% conjugated linoleic acid (CLA), and the third and fourth groups were fed LA and CLA diets supplemented with 0.2% sesamin (sesamin: episesamin, 1:1, w/w, 99.5% purity as total lignans; kindly supplied by Suntory Co.,
Tokyo, Japan), respectively. The CLA and LA used were the same preparations as reported previously (2). The LA and CLA preparations included 73.3% linoleic acid and 73.8% CLA (consisting mainly of 34.6% 9cis, 11trans and 35.9% 10trans, 12cis isomers), respectively. These diets were given ad libitum for 14 d. Food intake and body weight of each animal were recorded every other day.

On the day of the perfusion experiments, rats were given an intraperitoneal injection of sodium pentobarbital (5 mg/100 g body weight) around 8:30. The livers were then isolated and perfused with 120 mL of recirculating Krebs-Henseleit perfusion medium (pH 7.4) containing 25 mM glucose and 1.5% (w/v) bovine serum albumin (Fraction V, Boehringer Mannheim), and 25% (v/v) washed bovine erythrocytes at the rate of 20 mL/min at 37°C. After establishing recirculation, 5 mL of 20 mM potassium oleate (100μmol) as the exogenous fatty acid substrate was added, and the same solution was continuously infused at the rate of 4.5 mL/h (90μmol/h). Every 1 h, 20 mL of perfusate was removed for analysis of ketone bodies and lipids, and the same amount of fresh perfusion medium was added to maintain a recirculating volume of 120 mL. Two livers were selected at random from either of the four groups of rats, and perfused at the same time with different liver perfusion apparatus for a total of 4 h, as described in detail previously (15–18). After removal of the livers for perfusion experiments, perirenal and epididymal adipose tissues were excised into ice-cold saline, dissected free of connective tissues and weighed. We followed the guidelines of Miyazaki University in the care and use of laboratory animals.

The methods employed for the lipid and ketone body analyses in perfusates and post-perfused livers were described previously (12, 13, 15–18). Data was analyzed using ANOVA (19), and the statistical significance of the differences in the means among each group was evaluated by Tukey’s multiple comparison test at the level of p<0.05.

RESULTS AND DISCUSSION

There were no significant differences in food intake, body weight gain and relative weight of the post-perfused livers among the groups. On the other hand, the weight of perirenal adipose tissue was significantly lower in the rats fed CLA as compared to those fed LA, while the weight of the epididymal adipose tissue was comparable between the groups (Table 1). Supplementation of sesamin to diets containing LA, but not CLA, also caused a reduction in perirenal adipose tissue weight but it did not influence epididymal adipose tissue weight (Table 1). No dietary combination effect of CLA and sesamin was observed. The body fat-reducing potential of CLA appears to be greater than that of sesamin. Thus, the effects of dietary CLA or sesamin on the reduction of adipose tissue weight are more marked in the perirenal adipose tissue than in the epididymal tissue. The reasons for the different effects on these two adipose tissues are not known at present. However, the weight reduction in perirenal adipose tissue induced by dietary CLA was consistent with previous observations (20, 21). Rahman et al. (21) measured the activities of both rate-limiting enzyme for fatty acid oxidation (carnitine palmitoyltransferase, CPT) and triacylglycerol synthesis (Mg²⁺-dependent phosphatidate phosphohydrolase) in the perirenal adipose tissue of OLETF rats fed diets containing CLA as compared to LA, and found that CPT activity was stimulated and triacylglycerol synthetic enzyme activity was conversely reduced. On the other hand, Park et al. (20) reported a reciprocal response of dietary CLA on these two parameters in mice. In addition, other studies have demonstrated an increase in adipose tissue hormone-sensitive lipase activity and a reduction in lipoprotein lipase activity, which causes a net reduction in fatty acids to store the triacylglycerol in this tissue (20, 22, 23). These observations therefore suggest that the reduced weight of the perirenal adipose tissue induced by CLA feeding is, in part, due to reciprocal response in fatty acid oxidation and triacylglycerol synthesis. On the other hand, sesamin-dependent reduction in perirenal adipose tissue weight in this experiment was consistent with the results reported by Sugano et al. (14), although the mechanism underlying this effect remains unclear.

No significant difference was noted in the concentration of hepatic triacylglycerol and cholesterol among the 4 groups (Table 2).

Ketone bodies and triacylglycerol accumulated for 4 h in the perfusate of various livers from the 4 groups

| Table 1. | Food intake, body weight gain, adipose tissue weight and liver weight of rats. |
| --- | --- | --- | --- | --- |
| | LA | LA + sesamin | CLA | CLA + sesamin |
| Food intake (g/d) | 23.9±0.5 | 25.3±0.6 | 25.0±0.7 | 25.0±0.2 |
| Body weight gain (g/d) | 8.64±0.56 | 8.22±0.60 | 8.12±0.44 | 8.26±0.23 |
| Adipose tissue weight (g) | | | | |
| Epididymal | 3.22±0.36 | 2.68±0.34 | 2.88±0.22 | 2.82±0.28 |
| Perirenal | 2.79±0.27a | 2.00±0.23b | 1.49±0.10b | 1.85±0.24b |
| Liver weight (g) | 17.7±0.3 | 17.1±0.9 | 17.0±0.9 | 17.6±0.6 |

Mean±SE of 5 rats. Liver weight was measured at the end of perfusion.

a Significantly different between values with different superscript letter at p<0.05. Rats weighing about 165 g were fed diets supplemented with either 1% LA or CLA with or without 0.2% sesamin for 14 d. The livers were then isolated and perfused in the presence of an oleic acid substrate.
Table 2. Lipid concentrations of post-perfused liver.

|                      | LA          | LA + sesamin | CLA      | CLA + sesamin |
|----------------------|-------------|--------------|----------|---------------|
| Total cholesterol    | 6.39±0.33   | 6.84±0.28    | 6.45±0.15| 6.49±0.14     |
| Free cholesterol     | 4.50±0.15   | 4.79±0.13    | 4.44±0.09| 4.67±0.27     |
| Triacylglycerol      | 12.3±1.9    | 13.4±2.3     | 10.8±1.4 | 13.0±1.1      |

Mean ± SE of 5 rats. Liver weight was measured at the end of perfusion. Rats weighing about 165 g were fed diets supplemented with either 1% LA or CLA with or without 0.2% sesamin for 14 d.

Fig. 1. Ketone body production (A) and triacylglycerol secretion (B) by perfused rat liver. Mean ± SE of 5 rats. At each point, means with different letters are significantly different at p<0.05. Rats weighing about 165 g were fed diets supplemented with either LA or CLA with or without sesamin for 14 d: open circle, LA; closed circle, LA + sesamin; open square, CLA; closed square, CLA + sesamin. The livers were then isolated and perfused in the presence of an oleic acid substrate.

are shown in Fig. 1. These components accumulated in the perfusate linearly during 4-h perfusion periods, as has been reported previously (12, 13, 15-17), suggesting that perfused liver functions normally during the entire period of perfusion. Under this situation, we could therefore compare the efficacy of dietary CLA, sesamin and combination thereof on ketogenesis and secretion rate of triacylglycerol and cholesterol by the perfused liver.

We have previously reported that CLA causes elevated ketogenesis and conversely reduces the secretion rate of triacylglycerol by perfused livers of rats fed diets containing 1% CLA as compared to 1% LA. Consistent with these observations, we again observed a significant 1.4-fold elevation in ketone body production by the livers following the feeding of CLA as compared to LA (Fig. 1), indicating a stimulatory effect of dietary CLA on ketone body production in the liver, probably due to increased liver mitochondrial carnitine palmitoyltransferase activity. No significant difference was observed in the secretion of cholesterol among the four groups (data not shown).

CLA is a mixture of positional and geometrical isomers of linoleic acid (LA), and 9cis,11trans- and 10trans,12cis-octadecadienoic acid are major constituents of commercially available CLA preparations, as described previously (2). The 10trans,12cis isomer appears to be responsible for the altered hepatic ketogenesis and triacylglycerol secretion observed in this experiment, since this isomer is capable of reducing the adipose tissue weight (22), suppressing the secretion of apoB-containing lipoproteins (24) and lowering the serum lipid concentration (1).

On the other hand, sesamin is known to be a potent proliferator of peroxisomes, another site for fatty acid oxidation in the liver (25). Ide et al. (26) observed a marked increase in hepatic mitochondrial and peroxisomal fatty acid oxidation enzyme activities such as mitochondrial carnitine acyltransferase I, acyl-CoA dehydrogenase and peroxisomal acyl-CoA oxidase in the livers of rats fed sesamin as compared to those without the lignan. Umeda-Sawada et al. (27) also observed similar results in sesamin-fed rat liver. These observations therefore suggest that sesamin feeding causes enhanced fatty acid oxidation as reflected by the increase in ketone body production, which is probably due to peroxisomal and mitochondrial fatty acid oxidation in the rat liver. We previously reported that 0.2% sesamin feeding results in a 1.2-fold increase in hepatic production of ketone bodies, and this is due to the enhanced metabolism of exogenous fatty acids added during the perfusion process. In the present experiment, we again con-
firmed a 1.25-fold increase in ketone body production in the livers of rats fed 0.2% sesamin as compared to those fed LA. Although the elevation was not statistically significant, the extent of elevation was similar to that previously observed (12, 13). On the other hand, we were unable to find a combined effect of dietary CLA and sesamin on hepatic ketogenesis; only a marginal increase was noted as compared to CLA alone. The reasons for the lack of combined effects are not known, however, it is likely that the ketone body production observed in the livers of rats fed CLA may already attain the maximum rate of fatty acid oxidation in the liver.

A reciprocal response in hepatic ketogenesis and triacylglycerol secretion under various nutritional and physiological conditions has long been recognized (17, 18). Fatty acid oxidation rate is a critical determinant in regulating hepatic triacylglycerol synthesis and secretion in the rat (12, 13). Consistent with these observations, the present experiment clearly showed that the hepatic secretion rate of triacylglycerol was inversely related to the production of ketone bodies (Fig. 1): triacylglycerol secretion by the livers of rats fed CLA was 24% less than that in those fed LA, which is consistent with previous observations (2). On the other hand, a similar result was obtained using HepG2 cells: the addition of CLA to the medium causes a marked reduction in the secretion rates of triacylglycerol and apoB 100-containing lipoproteins (24). In the present study, dietary sesamin, as compared to LA, resulted in a 30% reduction in the secretion rate of triacylglycerol (Fig. 1). Furthermore, the dietary combination of CLA and sesamin, as compared to LA, caused a more marked reduction of hepatic triacylglycerol secretion (43%), suggesting that the triacylglycerol secretion-reducing potential of CLA may be increased by a combination with dietary components that reduce the secretion of this lipid molecule. The observed reduction in triacylglycerol secretion caused by feeding CLA, sesamin, or a combination of CLA and sesamin, may, in part, be due to enhanced fatty acid oxidation, since it has been known that increased fatty acid oxidation, as reflected by elevated ketone body production, implies a diversion of endogenous and exogenous free fatty acids from esterification to the oxidation pathways, and subsequently decreases synthesis and secretion of this lipid-bearing lipoprotein (12, 13, 15–17).

In summary, the effects of a dietary combination of CLA and sesamin on ketone body production and triacylglycerol secretion may be beneficial for alleviating hypertriglyceridemia.

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