Dietary Manipulations of Body Fat-reducing Potential of Conjugated Linoleic Acid in Rats

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Received June 28, 2001; Accepted July 31, 2001

To study whether the body fat-reducing potential of conjugated linoleic acid (CLA) could be increased through dietary manipulations, the effects of the combination of CLA with different proteins, fats, and sesamin were examined in rats. Male rats were fed diets containing 1% CLA or linoleic acid (LA) in combination with different proteins (20% of casein or soybean protein), fats (7% perilla oil or soybean oil) and 0.2% sesamin (SES) for 3 or 4 weeks. When the dietary fat source was soybean oil, CLA, as compared with LA, significantly reduced weights of epididymal and perirenal adipose tissues, irrespective of the dietary protein sources. However, the highest reducing effect was shown when soybean protein was given as a protein source. SES stimulated the reduction of epididymal and perirenal adipose tissue weights in both protein diets. In contrast, CLA increased the weight of brown adipose tissue, and SES further increased it in combination with soybean oil but not with perilla oil. No effect of dietary manipulation was observed on serum leptin and TNF-α levels. Thus, the body fat-reducing potential of CLA can be increased by an appropriate combination with food factors that may stimulate fatty acid β-oxidation.

Key words: conjugated linoleic acid; adipose tissue; dietary protein and fat; sesamin; leptin

Conjugated linoleic acid (CLA) has diverse physiological functions and its effect on adiposity is currently attracting attention. CLA appears to reduce body fat in various experimental animals through a series of metabolic consequences including the reduction of lipoprotein lipase activity, the increase in hormone-sensitive lipase activity and presumably the increase in hepatic and extrahepatic β-oxidation of fatty acid. The 10t, 12c-isomer is possibly the active component primary involved in the induction of body composition changes. Although no mechanistic differences among animal species are evident, the body fat-reducing potential is not necessarily confirmative in humans. Hence, an appropriate approach to increase the potential of CLA seems significant. A dietary factor that can stimulate fatty acid β-oxidation particularly in the muscle and liver is one such candidate, since CLA marginally effects on this metabolic event. In this context, soybean protein compared with casein moderately stimulates fatty acid oxidation in the liver of rats. Dietary fat also is a modulator of fatty acid metabolism and it has been shown that n-3 polyunsaturated fatty acids (PUFA) reduce body fat. Sesamin (SES), a lignan abundantly occurring in sesame seed and sesame oil, has been shown to markedly stimulate hepatic fatty acid β-oxidation in mitochondria and in particular in peroxisomes in rats. It is therefore reasonable to examine the combined effect of these food factors with CLA.

The present study deals with this issue using rats. In one experiment in which the effects of dietary protein were examined, perilla oil was used as a source of n-3 PUFA, α-linolenic acid. In other experiments, soybean oil, the oil recommended in the AIN-93G formula was used to compare the effect of SES and the interaction of dietary protein with SES. We measured serum leptin and TNF-α levels in one experiment. These cytokines are produced by the adipocytes and may be useful indicators of the change in body fat content and metabolism.

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Abbreviations: CLA, conjugated linoleic acid; SES, sesamin; CAS, casein; SOY, soybean protein; PUFA, polyunsaturated fatty acids
Materials and Methods

Animals and diets. Male Sprague-Dawley rats, 4 weeks old (Seac Co., Yositomi, Fukuoka, Japan) were individually housed in an air-conditioned room (21-23°C, lights on 0800 to 2000) and acclimatized for 5 days with a commercial non-purified diet (Type NMF, Oriental Yeast Co., Tokyo, Japan). The number of rats in the following experiments was 8 per group. In experiment 1, diets made according to the AIN-93G formula17 containing 7% soybean oil in combination with 1% of either linoleic acid (LA, as safflower oil) or CLA (both donated by Rinnoru Oil Mill Co., Nagoya, Japan) were freely given to rats. The fatty composition of CLA was in weight%, 16:0, 6.9; 18:0, 2.4; 18:1n-9, 15.3; 18:2n-6, 0.7; and CLA, 74.1 (9c, 11t, 34.1; 10t, 12c, 35.9; 9c, 11c/10c; 12c, 2.5; 9t, 11t/10t, 12, 1.6). The protein source was casein (CAS, Wako Pure Chemicals, Co., Osaka, Japan) at the 20% level. Sesamin (SES) was added at the 0.2% level at the expense of cornstarch. Sesamin was a gift from Takekoto Oil Mill Co., Gamagori, Japan and composed of a 53:47 mixture (w/w) of SES and epi-SES. The dietary groups in this experiment were LA, LA+SES, CLA, and CLA+SES. In experiment 2, to AIN-93G formula diets containing soybean oil (7%) and 1% CLA were added sesamin at the 0.2% level at the expense of cornstarch. Dietary protein sources were CAS and soybean protein (SOY, Fujiopro R, Fuji Oil Co., Osaka, Japan) (both at the 20% level). The dietary groups in this experiment were CAS, CAS+SES, SOY and SOY+SES. In experiment 3, rats were fed diets containing 7% perilla oil as a source of α-linolenic acid and either 1% LA or CLA. The dietary protein sources were either CAS or SOY. The dietary groups in this experiment were CAS+LA, CAS+CLA, SOY+LA, and SOY+CLA. After feeding diets for 4 weeks in experiments 1 and 2, and 3 weeks in experiment 3, rats were deprived of diets overnight, and under diethyl ether anesthesia, blood was withdrawn from the abdominal aorta and serum was harvested. Visceral tissues were excised immediately, blotted, and weighed. Brown adipose tissue was excised from underneath the dorsal scapula. These studies were done in accordance with the Guidelines of Animal Experiments approved by the Prefectural University of Kumamoto, Siebold University of Nagasaki, and Kyushu University School of Agriculture.

Analyses. Tissue lipids were extracted by the method of Folch et al.21 and their fatty acid compositions were measured by gas-liquid chromatography as fatty acid methyl esters.22 The activity of carnitine palmitoyltransferase in brown adipose tissue and liver mitochondria was measured by the method of Bieber et al.23 The rate of fatty acid β-oxidation in liver peroxisomes was measured using palmitoyl-CoA (Sigma Chemical Co., St. Louis, MO) as a substrate and 13,000 × g sediment as an enzyme source.24 Serum lipids and thiobarbituric acid reactive substances (TBARS) were analyzed by enzymatic kits (Wako Pure Chemicals, Co., Osaka, Japan) as reported elsewhere.25 The concentration of serum TNF-α and leptin was measured using commercial rat ELISA kits (Yanaihara Institute Inc., Shizuoka, Japan).

Statistics. Results were given as means ± SE. Statistical difference of means among groups was analyzed by one-way or two-way ANOVA followed by Duncan’s new multiple test to identify significant difference among the groups. Differences were considered significant at p < 0.05.24

Results

Interaction of dietary sesamin with CLA (Expt. 1) In this experiment, rats were fed approximately 21 g/day of diets and gained body weight of approximately 220 g for 4 weeks. No diet-dependent difference was observed in these parameters. The weight of tissues was comparable among the groups, except for a slight enlargement of liver in rats fed sesamin (SES) irrespective of LA or CLA (4.36 ± 0.40, 4.88 ± 0.41, 4.40 ± 0.26 and 5.15 ± 0.28 g/100 g body weight for LA, LA+SES, CLA and CLA+SES groups, respectively, p < 0.05).

SES significantly reduced serum cholesterol in both LA and CLA groups and the value was lowest in the CLA-SES combination (Table 1). A similar response pattern was observed on serum phospholipid. There was no clear difference in the concentration of serum triglyceride among the groups, although it tended to be lower in two groups of rats fed SES.

As shown in Fig. 1, dietary CLA compared with LA significantly reduced the weight of perirenal adi-

| Table 1. Effects of Dietary CLA and Sesamin on Serum Lipid Levels (Expt. 1) |
|----------------|--------------|--------------|--------------|--------------|
|                | LA           | LA + SES     | CLA          | CLA + SES    |
| Serum lipid    | mmol/L       | mmol/L       | mmol/L       | mmol/L       |
| Total cholesterol | 2.58 ± 0.23⁹ | 1.87 ± 0.14²⁹ | 2.21 ± 0.08²⁹ | 1.63 ± 0.14²⁹ |
| Triglyceride   | 1.40 ± 0.18  | 1.02 ± 0.14  | 1.34 ± 0.14  | 1.09 ± 0.20  |
| Phospholipid   | 2.88 ± 0.19⁹ | 2.24 ± 0.13²⁹ | 2.66 ± 0.10²⁹ | 2.13 ± 0.18²⁹ |

Means ± SE of 8 rats in each group. Values without a common superscript letter are significantly different at p < 0.05.
Conjugated Linoleic Acid and Body Fat

Fig. 1. Effects of Dietary CLA and SES on Relative Weight of Adipose Tissues of Rats (Expt. 1).
Values are means ± SE of 8 rats in each group. In each tissue, values without a common letter are significantly different at p<0.05.

pose tissue. SES itself also tended to reduce the weight, and the combination of CLA with SES gave the lowest figure among four dietary groups. No difference in the weight of epididymal adipose tissue was observed between rats fed LA and CLA, although SES tended to reduce the weight in both dietary fat groups. In contrast, CLA in relation to LA significantly increased weight of brown adipose tissue, but no significant additional effect of SES was observed.

The activity of carnitine palmitoyltransferase of brown adipose tissue expressed as total activity tended to increase by dietary CLA (Fig. 2). SES showed no effect on the activity.

Interaction of dietary protein and sesamin with CLA (Expt. 2)

During the 4 weeks of the feeding period, rats consumed 21 g of diets per day and gained approximately 220 g of body weight. There were no differences in these parameters among the groups. Dietary SES significantly increased liver weight (p<0.05) irrespective of the source of dietary protein (4.36 ± 0.4, 4.88 ± 0.41, 4.40 ± 0.26 and 5.15 ± 0.28 g/100 g body weight for CAS, CAS + SES, SOY and SOY + SES groups, respectively). Relative weights (g/100 g body weight) of other tissues examined (kidney, heart, lung, spleen and brain) were comparable among the groups.

As shown in Fig. 3, there was a trend of lowering weights of perirenal and epididymal adipose tissues in rats fed SOY than in those fed CAS, and the protein-dependent difference was significant in the latter tissue. Dietary SES did not influence the weights of these adipose tissues. CLA did not affect the weight of brown adipose tissue when the protein source was CAS, while in rats fed SOY the weight tended to increase as compared with those fed CAS, and it was significantly higher in the SOY + SES group than in the CAS + SES and SOY groups.

Dietary SES increased the activity of carnitine palmitoyltransferase of liver mitochondria (Fig. 4). The β-oxidation activity of liver peroxisomes also was increased by dietary SES when the protein source was CAS. The peroxisomal activity was significantly higher in rats fed SOY than in those fed CAS, but there was no further increase by the dietary combination of SOY with SES.

Figure 5 shows the concentration of serum TNF-α and leptin. The treatments had no effect on TNF-α and leptin levels, although they tended to be elevated in rats fed a CLA + SOY diet free of SES. There was little relationship between these cytokines and individual adipose tissue weights (both r=0.1, data not shown).

Interaction of dietary protein and fat with CLA (Expt. 3)

Although all groups of rats consumed a compara-
ble amount of diet (17.0 to 19.0 g/day on average), body weight gain was significantly higher ($p<0.05$) in the CAS groups than in the SOY groups (149 ± 5, 143 ± 3, 122 ± 4 and 122 ± 5 g for CAS + LA, CAS + CLA, SOY + LA and SOY + CLA groups, respectively). However, CLA did not influence weight gain. There were no differences in relative weight of tissues examined (liver, kidney, heart, lung, spleen, testis and brain) among the groups.

As shown in Fig. 6, feeding CLA resulted in a significant reduction of the weight of perirenal and epididymal adipose tissues irrespective of the source of dietary protein. The magnitude of reduction was more marked in perirenal than in epididymal adipose tissues, and the weight of the former was lowest in rats fed diets containing CLA in combination with SOY.

CLA was incorporated in various tissues to different extents (Fig. 7), and adipose tissues contained most. The composition of CLA in these tissues reflected that ingested and 9c, 11t- and 10t, 12c-isomers were major constituents. No tissue-dependent difference in the proportion of individual CLA was observed (data not shown).

The concentration of serum TBARS was significantly lower in two groups of rats fed SOY than in those fed CAS (16.8 ± 1.3, 17.2 ± 1.3, 12.9 ± 1.2 and 12.9 ± 0.8 ng/ml for CAS + LA, CAS + CLA, SOY + LA and SOY + CLA groups, respectively, $p<0.05$).

**Discussion**

There is data from tissue culture studies that adipocyte $\beta$-oxidation is increased and whole animal data suggesting increased fatty acid oxidation by CLA. The possibility exists that CLA may stimulate hepatic $\beta$-oxidation. The ketone body production in isolated perfused liver was only slightly higher in rats fed a 0.2% CLA diet than in those fed LA for
14 day. However, there is a species to species difference in the body fat-reducing effect of CLA. In this context, the results of a series of studies suggested that the adiposity reducing activity of CLA is rather weak or little effective in humans. Thus, explanation of the efficacy that dietary manipulation could exert on body fat-reducing effect of CLA seems reasonable for its application to human use.

This study in which the effects of dietary protein were examined using α-linolenic acid-rich perilla oil as a fat source, no statistically significant stimulating effect of SOY on adipose tissue weight in relation to CAS was confirmed, although the extent of weight reduction of perirenal adipose tissue tended to be greater with SOY than with CAS both in LA and CLA diets. There is a possibility that dietary n-3 PUFA erased the protein effect. However, the extent of weight reduction by CLA and its incorporation into adipose tissues currently observed were comparable with those obtained in a similar type of study using soybean oil as a dietary fat source. These observations therefore suggest that the effect of dietary SOY may in a sense practically be moderate to augment the preferable action of CLA, but an appropriate combination with n-3 PUFA or SES may be of use.

SES is a potent stimulator of fatty acid β-oxidation in the liver, and the effect was much more marked on peroxisomes than on mitochondria. This effect was dose-dependent and could be demonstrated at the dietary level as low as 0.1%. As expected, SES at the dietary level of 0.2% cumulatively reduced weight of perirenal adipose tissue. The increase in liver weight by dietary SES is temporary and specific to animal species, and it disappears after feeding SES for more longer periods. SES exerted serum cholesterol-lowering activity even in rats fed CLA (Table 1). Although the effect on the distribution of cholesterol in serum lipoproteins was not measured currently, we previously reported that SES lowers an atherogenic index in rats.

In all experiments, the extent of the weight reduction was more marked in perirenal than in epididymal adipose tissues, although in one experiment (Fig. 3) the weight reduction was statistically significant only in epididymal adipose tissue. The reason for this discrepancy is not clear, but this observation at least suggests a metabolic heterogeneity of different adipose tissues. Anyhow, dietary CLA could be an anti-obese food component. The observation that CLA was incorporated most in white adipose tissues (Fig. 7) may have some relation with its anti-obese activity. The statistically significant difference in the CLA level was observed in brain and lung between CAS and SOY groups. The physiological significance of this observation remains unclear at present, but it is at least unlikely that the small difference observed may influence the functions of these tissues.

CLA increased brown adipose tissue weight and the activity of liver carnitine palmitoyltransferase, a key enzyme in β-oxidation of fatty acids, irrespective of the protein source. The responses of these two parameters were apparently paralleled and were not essentially modified by the type of dietary protein. However, these CLA effects appear to be dependent on the type of dietary fats, either n-6 or n-3 PUFA. In case of perilla oil, the effect of CLA was not influenced by the type of dietary protein. In contrast, when the dietary fat source was soybean oil rich in n-6 linolenic acid, SOY in combination with SES significantly increased the weight of brown adipose tissue. Thus, an appropriate selection of dietary fat seems important to expect an augmentation of the effect that CLA exerts on the weight of brown adipose tissue and hence, futile fatty acid oxidation. In addition, an appropriate selection of dietary protein may also a useful approach to increase the effect of CLA. In one experiment in which soybean oil was the fat source (Fig. 4), SES increased liver peroxisomal β-oxidation of fatty acid when the protein source was CAS, and SOY itself also was a stimulant of this reaction compared to CAS. In all experiments, the color of brown adipose tissue was apparently the same among groups.

Several cytokines are considered to be important regulatory factors for lipid metabolism in adipocytes. TNF-α and leptin exert diverse actions on the fatty acid metabolism of adipocytes and are likely to behave as an adipostat. Turk et al. have reported that CLA reduces basal levels of TNF-α production by resident peritoneal macrophages in rats given soybean oil for 6 weeks. Leptin, a protein hormone produced almost exclusively by adipocytes, reflects in most cases the body fat content, i.e., its serum concentrations are elevated in obese people in comparison with lean people, and there is a strong positive correlation between leptin and body fat content. In this study, however, no clear effect on serum levels of TNF-α and leptin of dietary manipulations including CLA, SES, and the type of dietary protein was observed. Medina et al. reported that 64 days of CLA supplementation in women produced a transient decrease in leptin levels, and CLA did not affect this parameter in a manner that promoted decreases of adiposity. It appears that a higher level of dietary CLA, 2% for example, may be needed to elicit the reduction of serum leptin level in rats. In contrast, we have observed in mice a marked reduction of the serum concentration of both TNF-α and leptin by dietary CLA (unpublished observation).

Basu et al. reported that CLA induced both non-enzymatic and enzymatic lipid peroxidation in humans, although other studies in vitro and in vivo did not necessarily support these observations. This study also showed no sign of lipid peroxidation.
as judged from the serum level of TBARS. However, insofar as the possibility of lipid peroxidation is anticipated, a lower amount of dietary CLA is more appropriate to avoid health hazards if any. In this context, the augmentation of the body fat-reducing potential of CLA by an appropriate combination with food factors having a mechanism different from that of CLA could be highly evaluated. SES is one such candidate.

In conclusion, the body fat-reducing effect of CLA was increased by simultaneous ingestion of SES. The combination of SES with SOY instead of CAS may be one approach to improve the effect of CLA when the dietary fat source was soybean oil but not perilla oil. Insight into the dose-dependency of the combined effect of CLA and SES deserves further study.

Acknowledgment

This work is supported in part by a grant-in-aid (No. 11660130) for Scientific Research (C) from the Ministry of Education, Science, Sports, and Culture of Japan and a Research Grant from the Supporters' Association of the Prefectural University of Kumamoto.

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