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The 10*trans*,12*cis* isomer of conjugated linoleic acid suppresses the development of hypertension in Otsuka Long–Evans Tokushima fatty rats

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Abstract

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid found in beef, lamb, and dairy products. CLA has attracted considerable attention over the past several decades because of its potentially beneficial biological effects, including protective effects against several cancers, atherosclerosis, and obesity. Here we provide the first evidence that the 10*trans*,12*cis*-CLA isomer is able to suppress increases in blood pressure during the onset of obesity in OLETF rats. After 3 weeks of feeding with 10*t*,12*c*-CLA, systolic blood pressure was significantly lowered compared with rats fed linoleic acid or 9*c*,11*t*-CLA. Abdominal adipose tissue weight was also significantly lowered in rats fed 10*t*,12*c*-CLA, but not in those which were fed 9*c*,11*t*-CLA. In addition, we found that the relative mRNA expressions of angiotensinogen and leptin were suppressed by 10*t*,12*c*-CLA in adipose tissue. We speculate that the antihypertensive effect of 10*t*,12*c*-CLA can be attributed to the lowered secretion of hypertensive adipocytokines from abdominal adipose tissues.

Keywords: Conjugated linoleic acid isomers; OLETF rat; Obesity; Blood pressure

Conjugated linoleic acid (CLA) refers to a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. It is found in meat and dairy products, such as beef, milk, and processed cheese [1,2]. CLA has attracted considerable attention because of its potentially beneficial biological effects in inhibiting carcinogenesis, attenuating atherosclerosis, and reducing body fat in animal models [3–7]. Recent studies have also reported that dietary CLA supplementation reduced the percentage of body fat compared to control groups in humans [8–10]. Though there is a substantial amount of research evaluating the effect of CLA on lifestyle-related diseases, as described above, none of the studies have evaluated the effect of CLA on hypertension.

Hypertension and obesity are common pathological states independently associated with an increased risk of cardiovascular diseases. Many epidemiological studies have demonstrated an association between body mass index and blood pressure, and there is evidence to suggest that obesity is a risk factor for the development of hypertension in humans [11,12]. Otsuka Long–Evans Tokushima fatty (OLETF) rats develop a syndrome with multiple metabolic and hormonal disorders that shares many features with human obesity. OLETF rats have hyperphagia, since they lack receptors for cholecystokinin, and become obese, developing hyperlipidemia, diabetes, and hypertension [13–16]. We previously reported that CLA, especially 10*t*,12*c*-CLA, has antiobese and hypolipidemic effects in OLETF rats. These effects were attributed to the enhancement of fatty acid β-oxidation and the suppression of fatty acid synthesis [17–21]. In the present study we evaluate the effect of

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CLA isomers on the development of hypertension in OLETF rats.

Materials and methods

Animals and diets. Male OLETF rats aged 6 weeks were provided by the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). The rats were housed individually in metal cages in a temperature-controlled room (24 °C) under a 12-h light/dark cycle. After a 1-week adaptation period, the rats were assigned to three groups (six rats each) that were fed with one of the three diets: a semi-synthetic diet supplemented with 5% corn oil plus 0.5% high-linoleic safflower oil (control group); a semi-synthetic diet supplemented with 5% corn oil and 0.5% 9*cis*,11*trans*-CLA rich oil (9*c*,11*t*-CLA group); or a semi-synthetic diet supplemented with 5% corn oil and 0.5% 10*trans*,12*cis*-CLA rich oil (10*t*,12*c*-CLA group). Both CLA-isomer rich oils were prepared by lipase-catalyzed selective esterification [22] and other oils were provided by Rinoru Oil Mills (Nagoya, Japan). The composition of the semi-synthetic diets and their fatty acid contents are given in Table 1. The animals received the diets for 3 weeks. At the end of the feeding period, the rats were anesthetized with diethyl ether. Blood from the abdominal aorta was withdrawn with heparinized syringes and centrifuged to separate plasma. Abdominal white adipose tissue was also excised and weighed immediately. All aspects of the experiment were conducted according to the guidelines provided by the ethical committee of experimental animal care at Saga University.

Measurement of systolic blood pressure. Systolic blood pressure in the conscious state was measured by the indirect tail cuff method using a Model MK-2000 BP monitor for rats and mice (Muromachi Kikai, Tokyo, Japan) according to the manufacturer's instructions. The measurement was carried out under room temperature condition (24 °C), because the instrument does not require pre-warming of the animals. Five readings were obtained from each rat and averaged, after the highest and the lowest values were excluded.

Assay of angiotensin converting enzyme activity. The method of Meng et al. was used with modification [23,24]. Fifty microlitres of plasma (containing 20 μM aprotinin and 10 μM chymostatin) was incubated with 200 μl of a reaction mixture containing 5 mM Hip–His–Leu (HHL) as substrate and 0.3 M NaCl in 0.1 M phosphate buffer, pH 8.3, at 37 °C for 30 min. The enzymatic reactions were terminated by the addition of 750 μl of 3% metaphosphoric acid. After centrifugation at 4 °C, 10,000*g* for 5 min, 20 μl of aliquot was applied directly to a reversed-phase column (Cosmosil $5C_{18}$-AR, $\varnothing 4.6 \times 250$ mm; Nacalai Tesque, Kyoto, Japan) for HPLC (Shimadzu LC-9A instrument, Kyoto, Japan), eluted with 35% CH_3CN in 0.1% trifluoroacetic acid at a flow rate of 0.4 ml/min, and the hippuric acid (HA) formed by the action of angiotensin converting enzyme (ACE) on HHL at 228 nm was detected. ACE activity was expressed as units of HA formed per ml plasma (1 U = 1 μmol HA formed per min at 37 °C).

Semi-quantitative analysis of mRNA expression. Total RNA was extracted from 300 mg of the perirenal white adipose tissue, using a TRIZOL reagent (Invitrogen, Tokyo, Japan). Extracts of RNA were kept at −80 °C until reverse transcription polymerase chain reaction (RT-PCR) analyses were performed. A one-step RT-PCR kit (Qiagen, Tokyo, Japan) was used for semi-quantitative analysis of angiotensinogen (AGT), leptin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression [25]. The sense and antisense primers used were 5′-AGGGTGGAAGATGAACTTGC-3′ and 5′-ATGCTG TTGAGAACCTCTCC-3′ for AGT, 5′-GTGGCTTTGGTCCTATCT GTCCTATG-3′ and 5′-TCAGGGCTAAGGTCCAACTGTTGAAG-3′ for leptin, and 5′-ATTCTACCCACGGCAAGTTCAATGG-3′ and 5′-AGGGGCGGAGATGATGACCC-3′ for GAPDH, respectively [26]. They would amplify fragments of different sizes: 507-, 458-, and 224-bp for AGT, leptin, and GAPDH cDNA, respectively. The relative RT-PCR was performed according to the manufacturer's recommendation, using 50 ng of total RNA as a template, 0.6 μM of primers for the target transcript (AGT or leptin), and 0.3 μM of primers for the internal control transcript (GAPDH). Following the reverse-transcription reaction at 50 °C for 30 min, an initial denaturation at 95 °C for 15 min, 25 cycles of denaturation at 94 °C for 0.5 min, annealing at 57 °C for 0.5 min, elongation at 72 °C for 1 min, and a final extension reaction at 72 °C for 10 min proceeded. PCR products were electrophoresed on a 1.5% agarose gel and stained with ethidium bromide. Densitometry was performed using a GENE GENIUS image analyzer (Synoptics, Cambridge, UK). The reliability of the semi-quantitative RT-PCR technique for measuring relative gene expression was verified using the amounts of template RNA (from 25 to 150 ng) and number of amplification cycles (from 15 to 35 cycles). Results were expressed as a relative value after normalization to the GAPDH expression.

Statistical analysis. All values are expressed as means ± SE. Data were analyzed by one-way ANOVA, all differences being inspected by Duncan's new multiple-range test [27]. Differences were considered significant at $p < 0.05$.

Results and discussion

We provide the first evidence that dietary 10*t*,12*c*-CLA isomer can suppress the development of hypertension in OLETF rats.

Table 1
Composition of experimental diet

Ingredients	Control (%)	9*c*,11*t*-CLA (%)	10*t*,12*c*-CLA (%)
Casein	20.0	20.0	20.0
Corn starch	15.0	15.0	15.0
Cellulose	5.0	5.0	5.0
Mineral mixture (AIN 76)	3.5	3.5	3.5
Vitamin mixture (AIN 76)	1.0	1.0	1.0
DL-Methionine	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2
Corn oil	5.0	5.0	5.0
Safflower oil (high linoleic)	0.5	0.0	0.0
9*c*,11*t*-CLA rich oil*	0.0	0.5	0.0
10*t*,12*c*-CLA rich oil**	0.0	0.0	0.5
Sucrose		To make 100	

* Contained different isomers: 96.0% of 9*c*,11*t*; 3.6% of 10*t*,12*c*; and 0.4% of 9*t*,11*t*/10*t*,12*t*.
** Contained different isomers: 3.1% of 9*c*,11*t*; 95.7% of 10*t*,12*c*; 0.6% of 10*c*,12*c*; and 0.7% of 9*t*,11*t*/10*t*,12*t*.

As shown in Figs. 1 and 2, a remarkable increase in the systolic blood pressure was observed during the onset of obesity in OLETF rats. Many epidemiological studies have also shown that there is a highly positive correlation between obesity and hypertension in humans [28,29]. In particular, abdominal obesity was found to be associated with a two- to threefold increase in the risk of hypertension in humans [30]. The OLETF rat is an animal model of lifestyle-related diseases, characterized by abdominal obesity, hypertension, and type-2 diabetes [13–16]. In this study, there was no significant difference in final body weights (control, 315 ± 6; 9*c*,11*t*-CLA, 312 ± 5; 10*t*,12*c*-CLA, 315 ± 4) and amount of food intakes (control, 441 ± 3; 9*c*,11*t*-CLA, 435 ± 7; 10*t*,12*c*-CLA, 435 ± 4) among the three groups. Systolic blood pressure as measured by the indirect tail cuff method increased during the onset of obesity in OLETF rats. The increase, however, was significantly lower in the 10*t*,12*c*-CLA group as compared with the other two groups after 3 weeks feeding.

Because inhibitory effects on ACE activity [31,32], a key enzyme of the renin–angiotensin system, are reported for many hypotensive drugs and functional foods, the effects of CLA isomers on ACE activity were measured (Fig. 3). However, 10*t*,12*c*-CLA does not have an inhibitory effect on ACE, because there was no significant difference among the three groups in ACE activity in plasma.

After 3 weeks feeding with 10*t*,12*c*-CLA, abdominal white adipose tissue weights were significantly decreased as compared with the other two groups (Fig. 4). Recent advances in molecular and cell biology have shown that abdominal adipose tissue not only stores excess energy in the form of fat but also secretes physiologically active substances called adipocytokines [33]. For instance, the obesity gene product leptin, secreted in excess from the enlarged adipose tissues in obesity, activates sympathetic nerve activities, including those regulating blood pressure [34]. AGT, also secreted from adipose tissues, is cleaved by renin to form angiotensin I, the precursor to angiotensin II [35]. Because it has been reported that an

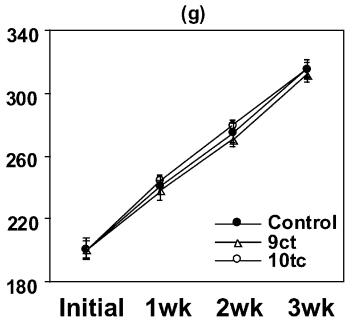

Fig. 1. Effect of dietary CLA isomers on body weight. Rats were fed control diet; 9*c*,11*t*-CLA diet; or 10*t*,12*c*-CLA diet for 3 weeks. Values are expressed as means ± SE of six rats. See Table 1 for composition of diets.

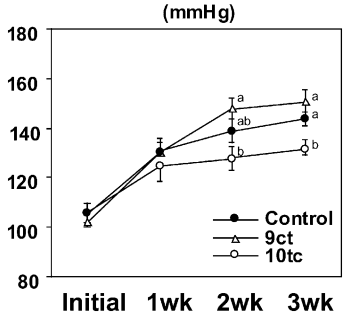

Fig. 2. Effect of dietary CLA isomers on systolic blood pressure. Rats were fed control diet; 9*c*,11*t*-CLA diet; or 10*t*,12*c*-CLA diet for 3 weeks. During the feeding period of the semi-synthetic diets, the systolic blood pressure of each animal was measured by the indirect tail cuff method every week. Values are expressed as means ± SE of six rats. See Table 1 for composition of diets. [ab]Different letters show significant difference at $p < 0.05$.

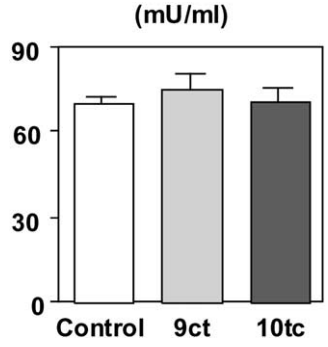

Fig. 3. Effect of dietary CLA isomers on ACE activity in plasma. Rats were fed control diet; 9*c*,11*t*-CLA diet; or 10*t*,12*c*-CLA diet for 3 weeks. ACE activity in plasma was measured by HPLC, as described in Materials and methods. Values are expressed as means ± SE of six rats. See Table 1 for composition of diets.

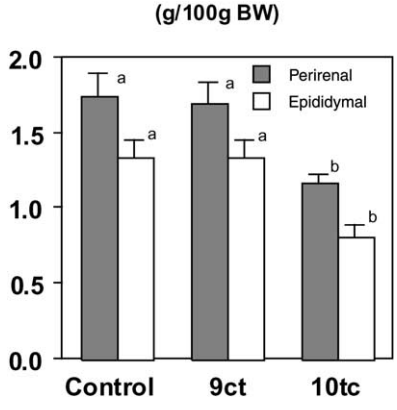

Fig. 4. Effect of dietary CLA isomers on abdominal white adipose tissues. Rats were fed control diet; 9*c*,11*t*-CLA diet; or 10*t*,12*c*-CLA diet for 3 weeks. Values are expressed as means ± SE of six rats. See Table 1 for composition of diets. [ab]Different letters show significant differences at $p < 0.05$.

increase in AGT levels leads to a parallel change in the formation of angiotensin II, increased synthesis due to abundant adipose tissue is suspected in an involvement in the increased blood pressure of obese subjects [11]. The effect of CLA isomers on the gene expressions of hypertensive adipocytokines, AGT, and leptin, was evaluated by semi-quantitative RT-PCR (Fig. 5). The relative expression levels of adipocytokines, especially leptin, were specifically suppressed by 10*t*,12*c*-CLA in abdominal white adipose tissue. Kang et al. [36] previously reported supportable results showing that 10*t*,12*c*-CLA reduced leptin secretion and mRNA levels in 3T3-L1 adipocytes,

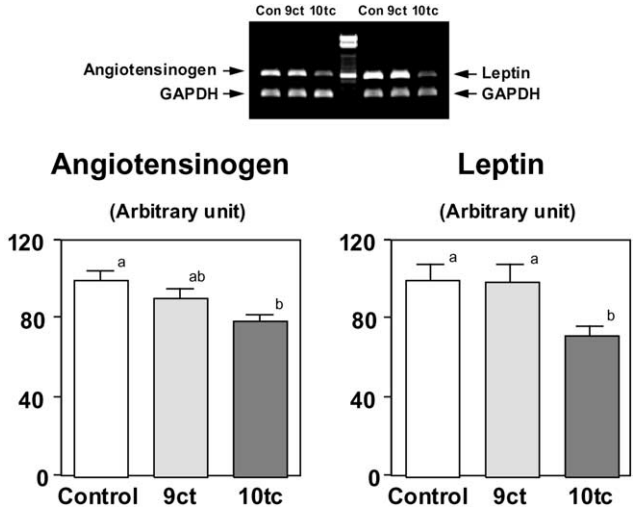

Fig. 5. Effect of dietary CLA isomers on mRNA expressions of adipocytokines in perirenal white adipose tissue. Rats were fed control diet; 9*c*,11*t*-CLA diet; or 10*t*,12*c*-CLA diet for 3 weeks. mRNA expressions of AGT, leptin, and GAPDH were measured by semi-quantitative RT-PCR, as described in Materials and methods. Relative values to GAPDH expression are expressed as means ± SE of six rats. See Table 1 for composition of diets.

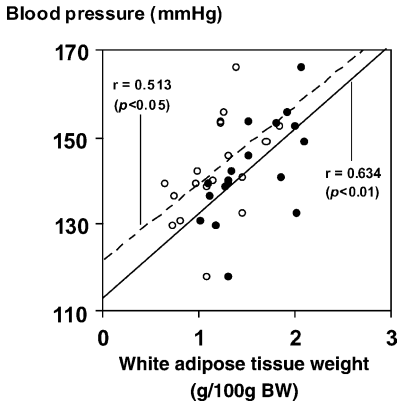

Fig. 6. Correlation between blood pressure and abdominal adipose tissue weight. Rats were fed control diet; 9*c*,11*t*-CLA diet; or 10*t*,12*c*-CLA diet for 3 weeks. •, Perirenal adipose tissue; ○, epididymal adipose tissue; —, vs. perirenal adipose tissue; - - -, vs. epididymal adipose tissue. See Table 1 for composition of diets.

and serum leptin levels and mRNA expression in abdominal adipose tissue in ICR mice. These results suggest that downregulated gene expression of hypertensive adipocytokines implies an overall decrease in those secretions from abdominal adipose tissues, as fat mass is also decreased by 10*t*,12*c*-CLA feeding. The highly positive correlations between perirenal or epididymal white adipose tissue weights and systolic blood pressure may also support that speculation (Fig. 6).

In conclusion, we report that 10*t*,12*c*-CLA suppresses the development of hypertension during the onset of obesity in OLETF rats. Although the detailed mechanism of downregulated gene expression has not been clarified, the antihypertensive effect of 10*t*,12*c*-CLA is due, at least in part, to the lowered secretion of hypertensive adipocytokines from abdominal adipose tissues.

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