Effects of Conjugated Linoleic Acid on Serum Leptin Concentration, Body-Fat Accumulation, and \(\beta\)-Oxidation of Fatty Acid in OLETF Rats

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We investigated the efficacy of a 4-wk supplementation of conjugated linoleic acid (CLA) as free fatty acid (FFA) or triacylglycerol (TG) on serum leptin concentration, body-fat accumulation, and mitochondrial \(\beta\)-oxidation in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. A significant reduction of serum leptin concentration (42%) and a decrease in the wet weights of perirenal, epididymal, and omental/visceral-adipose tissue in TG-CLA and FFA-CLA groups were found in comparison with the OLETF control group. Both forms of CLA supplementation produced a 5.2% decrease in body weight compared with the control even though food intake was similar in the OLETF groups. Moreover, both forms of CLA enhanced carnitine-palmitoyltransferase activity in brown adipose tissue, perirenal adipose tissue, red gastrocnemius muscle, and liver in comparison with the OLETF control group. Serum concentrations of non-esterified fatty acid and TG also were reduced in rats fed diets supplemented with TG-CLA and FFA-CLA. Nutrition 2001;17:385–390. ©Elsevier Science Inc. 2001

KEYWORDS: conjugated linoleic acid, leptin, perirenal adipose tissue, brown adipose tissue, gastrocnemius muscle, carnitine-palmitoyltransferase activity, serum lipids

INTRODUCTION

Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers that have conjugated double bonds and is found in foods such as ruminant meats, pasteurized dairy products, and processed cheeses.\(^1\)\(^2\) CLA is believed to have protective action against cancer and atherosclerosis.\(^3\)\(^-\)\(^5\) We demonstrated that CLA influences apolipoprotein-B secretion and lipid metabolism in HepG2 cells, a human liver-derived cell line.\(^6\) Houseknecht et al. found that CLA improved glucose tolerance in Zucker diabetic fa/fa rats.\(^7\) CLA also was reported to reduce body-fat content in mice.\(^8\)\(^-\)\(^10\) However, the exact mechanism of CLA-induced reduction of fat mass is not clear.

Leptin is an obese gene product and is produced mainly in adipose tissue.\(^11\) Leptin was reported to control food intake and body-energy expenditure through hypothalamic receptors.\(^12\)\(^,\)\(^13\) However, studies about the relation between dietary CLA and its effect on circulating leptin concentration in animals is scarce.

Most of the published animal experiments were conducted with CLA in the form of free fatty acid (FFA).\(^7\)\(^-\)\(^10\) CLA is a component of triacylglycerol (TG) in food,\(^14\) so we compared the physiologic effects of TG-CLA and FFA-CLA. Until now there was no information about the comparative physiologic functions of TG-CLA and FFA-CLA except a study by Ip et al. who reported that the anticancer effects of both forms of CLA are identical. Fatty acid in the forms of TG and FFA were reported to differ from each other in term of digestion, absorption, and transport.\(^15\) Ikeda et al.\(^16\) reported that \(\omega\)-3 polyunsaturated fatty acids in TG form are absorbed more rapidly than those in FFA form. We investigated the efficacy of both forms of CLA on serum leptin concentration, fat mass, and mitochondrial \(\beta\)-oxidation of fatty acids in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. The OLETF rat is an animal model of non-insulin–dependent diabetes mellitus (NIDDM) that is characterized by mild obesity with visceral-fat accumulation and late-onset insulin resistance. This animal model develops obesity and hypertriacylglycerolemia at about 6 wk of age, insulin resistance at around 12 wk, and NIDDM at around 30 wk.\(^17\)\(^-\)\(^19\)

MATERIALS AND METHODS

Animals and Diets

Male OLETF rats and their corresponding controls, 4-wk-old Long-Evans Tokushima Otsuka (LETO) rats were obtained from Tokushima Research Institute (Otsuka Pharmaceutical Company Ltd., Tokushima, Japan). Rats were housed individually in metal cages in a temperature-controlled (24°C) room with a 12-h light/dark cycle. After a 1-wk adaptation period, the rats were assigned to three groups that were fed ad libitum with a semisynthetic diet supplemented with 6.5% safflower oil (control group), a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% TG-CLA (TG-CLA group), or a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% FFA-CLA (FFA-CLA group). Each group consisted of five to six rats. The composition of the semisynthetic diets and fatty acids are presented in Table I. The CLAs and safflower oil were provided by Rinoru Oil Mills (Nagoya, Japan). The fatty-acid profile of the diets was measured with gas liquid chromatography. The CLA contained 33.2% of isomers 9c, 11t/9t, and 11c; 34.2% of isomers 10t and 12c; 2.4% of isomers 9c,
TABLE I.

COMPOSITION OF THE SEMISYNTHETIC DIETS AND FATTY-ACID COMPOSITION OF DIETARY FAT

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>TG-CLA</th>
<th>FFA-CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semisynthetic diet (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>α-Corn starch</td>
<td>15.0</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>6.5</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>CLA</td>
<td>—</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Sucrose added to make 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>6.7</td>
<td>7.3</td>
<td>6.6</td>
</tr>
<tr>
<td>18:0</td>
<td>2.5</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>18:1</td>
<td>15.9</td>
<td>15.2</td>
<td>18.1</td>
</tr>
<tr>
<td>18:2 (LA)</td>
<td>73.0</td>
<td>1.4</td>
<td>0.72</td>
</tr>
<tr>
<td>18:2 (CLA)†</td>
<td>—</td>
<td>73.2</td>
<td>71.6</td>
</tr>
<tr>
<td>Others</td>
<td>1.9</td>
<td>0.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*AIN 93.
† Contained different isomers: 33.2% of 9c, 11b/9b; 11c; 34.2% of 10c, 12c; 2.4% of 9c, 11c/10c; 12c; and 1.8% of 9b, 11b/10b, 12b; the rest were other fatty acids.
CLA, conjugated linoleic acid; FFA, free fatty acid; LA, linoleic acid; TG, triacylglycerol.

Measurement of Serum and Liver Metabolites

Serum insulin concentration was measured with a radioimmunoassay kit (catalog number RF-13K; Linco, St. Charles, MO, USA) standardized against rat insulin. Serum leptin concentration was measured with a radioimmunoassay kit (catalog number RL-83K; Linco) to find rat leptin. TG, total cholesterol, non-esterified fatty acid (NEFA), glucose, and phospholipid concentrations in serum were measured with commercial kits obtained from Wako Pure Chemical Industries (Osaka, Japan). Hepatic lipids were extracted and purified, and lipid concentrations were measured as described previously.20–22

Preparation of Tissue Homogenates

Liver homogenate was prepared as described previously.20,21 Briefly, a piece of liver was homogenized in 6 vol of 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose solution and 1 mM ethylene-diaminetetraacetic acid. The same procedure was followed to homogenate perirenal adipose tissue, BAT, and red gastrocnemius muscle. Protein concentration was measured with the method of Lowry et al.,23 with bovine serum albumin as the standard.

Assay of Carnitine-Palmitoyltransferase (CPT) Activity

CPT (EC 2.3.1.23) activity was assayed by measuring the CoASH formation, as described previously.21,24,25 The reaction mixture contained 116 mM Tris HCl (pH 8.0), 2.50 mM ethylene-diaminetetraacetic acid neutralized to pH 8.0 with Tris, 2.50 mM l-carnitine, 0.5 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), 75 mM palmitoyl-CoA, and 0.2% Triton X-100. The whole solution was equilibrated at 25°C. The reaction was initiated by adding the enzyme source and absorbance was monitored for 2 to 4 min. The l-carnitine-independent rate was measured in a second cuvette by omitting only l-carnitine. The difference between the rates with and without carnitine provided the carnitine-dependent rate for the formation of CoA and was equated to carnitine palmitoyltransferase. The assay was conducted in freeze-thawed homogenate.

Statistical Analyses

All values are expressed as means ± standard error. Data were analyzed by one-way analysis of variance, followed by inspection of all differences by Duncan’s new multiple-range test.26 Differences were considered significant at P < 0.05.

RESULTS

Growth Performance

Body weights and food intakes of all groups are shown in Table II. Body weights and food intakes were higher in OLETF than in LETO rats. In the OLETF groups, supplementation with TG-CLA and FFA-CLA significantly reduced body-weight gain (ca. 18 g, 5.2%) compared with the control group. Daily food intakes were similar in the OLETF groups.

Wet Weight of Visceral-Adipose Tissue

Wet weights of perirenal, epididymal, and omental-adipose tissues were higher in OLETF rats than in LETO rats (Table III). Supplementation with TG-CLA and FFA-CLA significantly reduced wet weights of perirenal, epididymal, and omental-adipose tissue in comparison with OLETF control rats. Supplementation with TG-CLA reduced the wet weights of perirenal and epididymal adipose tissues by 36% and 44%, respectively, compared with the OLETF control group. Similar reductions in the wet weights of perirenal and epididymal adipose tissues were found in rats fed diets supplemented with FFA-CLA. The degree of reduction of the wet weight of omental-adipose tissue was lower (from 14% to 22%) than that of perirenal and epididymal adipose tissue in the CLA groups.

Serum and Liver Metabolites

All biochemical parameters studied in the serum were markedly higher in OLETF rats than in LETO rats (Table IV). OLETF rats fed CLA-supplemented diets showed significant reduction of serum leptin concentration compared with those fed the control diet. Serum TG and NEFA concentrations were lower (20% to 31%) in the CLA groups. Similarly, liver TG concentration was 20% lower in the CLA groups (control: 29.5 ± 2.0 μmol/g of liver; CLA groups: 23.7 ± 1.1 and 23.5 ± 0.8 μmol/g liver). Serum glucose concentration was elevated in the FFA-CLA group, but serum insulin levels did not change across OLETF groups. Phospholipid and cholesterol concentrations in the serum did not differ across OLETF groups.
### TABLE II.

EFFECTS OF TG-CLA AND FFA-CLA ON BODY WEIGHT AND FOOD INTAKE IN OLETF RATS*

<table>
<thead>
<tr>
<th>Group</th>
<th>OLETF</th>
<th>Control</th>
<th>TG-CLA</th>
<th>FFA-CLA</th>
<th>LETO, control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>137 ± 2*a</td>
<td>137 ± 2*a</td>
<td>137 ± 3*a</td>
<td>115 ± 3*b</td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td>345 ± 3*a</td>
<td>327 ± 2*b</td>
<td>326 ± 4*b</td>
<td>271 ± 5*c</td>
<td></td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>25.5 ± 0.1*a</td>
<td>24.5 ± 0.4*a</td>
<td>24.6 ± 0.1*b</td>
<td>17.9 ± 0.3*b</td>
<td></td>
</tr>
</tbody>
</table>

* Rats were fed a semisynthetic diet supplemented with 6.5% safflower oil (control group), a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% TG-CLA (TG-CLA group), or a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% FFA-CLA (FFA-CLA group). Feeding stopped 10 h before the rats were killed. Values are expressed as means ± standard error of five to six rats. Values with different letters are significantly different at P < 0.05.

CLA, conjugated linoleic acid; FFA, free fatty acid; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; TG, triacylglycerol.

### TABLE III.

EFFECTS OF TG-CLA AND FFA-CLA ON WET WEIGHT OF VISCERAL-ADIPOSE TISSUE IN OLETF RATS*

<table>
<thead>
<tr>
<th>Group</th>
<th>OLETF</th>
<th>Control</th>
<th>TG-CLA</th>
<th>FFA-CLA</th>
<th>LETO, control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral-adipose tissue, wet weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perirenal</td>
<td>6.58 ± 0.5*a</td>
<td>4.22 ± 0.1*b</td>
<td>4.31 ± 0.1*b</td>
<td>2.49 ± 0.1*c</td>
<td></td>
</tr>
<tr>
<td>Epididymal</td>
<td>5.63 ± 0.6*a</td>
<td>3.13 ± 0.1*b</td>
<td>3.36 ± 0.1*b</td>
<td>3.10 ± 0.1*b</td>
<td></td>
</tr>
<tr>
<td>Omental</td>
<td>2.78 ± 0.2*a</td>
<td>2.06 ± 0.2*b</td>
<td>2.39 ± 0.1*ab</td>
<td>1.35 ± 0.1*c</td>
<td></td>
</tr>
</tbody>
</table>

* Rats were fed a semisynthetic diet supplemented with 6.5% safflower oil (control group), a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% TG-CLA (TG-CLA group), or a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% FFA-CLA (FFA-CLA group). Feeding stopped 10 h before rats were killed. Values are expressed as means ± standard error of five to six rats. Values with different letters are significantly different at P < 0.05.

CLA, conjugated linoleic acid; FFA, free fatty acid; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; TG, triacylglycerol.

### TABLE IV.

EFFECTS OF TG-CLA AND FFA-CLA ON SERUM METABOLITES IN OLETF RATS*

<table>
<thead>
<tr>
<th>Group</th>
<th>OLETF</th>
<th>Control</th>
<th>TG-CLA</th>
<th>FFA-CLA</th>
<th>LETO, control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/mL)</td>
<td>9.04 ± 1.2*a</td>
<td>5.19 ± 0.5*b</td>
<td>5.03 ± 0.2*b</td>
<td>1.75 ± 0.3*c</td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>2.68 ± 0.4*a</td>
<td>2.06 ± 0.2*a</td>
<td>2.91 ± 0.5*a</td>
<td>0.38 ± 0.12*b</td>
<td></td>
</tr>
<tr>
<td>NEFA (μEq/L)</td>
<td>904 ± 43*a</td>
<td>718 ± 129ab</td>
<td>613 ± 39*b</td>
<td>610 ± 30*b</td>
<td></td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.15 ± 0.03*a</td>
<td>1.72 ± 0.21*ab</td>
<td>1.48 ± 0.10*b</td>
<td>0.61 ± 0.02*c</td>
<td></td>
</tr>
<tr>
<td>T-chol (mmol/L)</td>
<td>4.20 ± 0.15*a</td>
<td>4.32 ± 0.23*a</td>
<td>4.37 ± 0.09*a</td>
<td>3.51 ± 0.06*b</td>
<td></td>
</tr>
<tr>
<td>Phospholipid (mmol/L)</td>
<td>2.67 ± 0.14*a</td>
<td>2.58 ± 0.15*a</td>
<td>2.57 ± 0.08*a</td>
<td>1.91 ± 0.07*b</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>8.48 ± 0.89*a</td>
<td>8.23 ± 1.05*a</td>
<td>12.07 ± 1.19*b</td>
<td>3.55 ± 0.37*c</td>
<td></td>
</tr>
</tbody>
</table>

* Rats were fed a semisynthetic diet supplemented with 6.5% safflower oil (control group), a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% TG-CLA (TG-CLA group), or a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% FFA-CLA (FFA-CLA group). Feeding stopped 10 h before rats were killed. Values are expressed as means ± standard error of five to six rats. Values with different letters are significantly different at P < 0.05.

CLA, conjugated linoleic acid; FFA, free fatty acid; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; NEFA, non-esterified fatty acid; T-chol, total cholesterol; TG, triacylglycerol.
FIG. 1. Effects of TG-CLA and FFA-CLA on CPT activity in different tissues of OLETF rats. (A) Specific activity of CPT (nmol/min/mg protein).
(B) Tissue capacity of CPT (mmol/min/g of wet tissue). Rats were fed a semisynthetic diet supplemented with 6.5% safflower oil (control group), a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% TG-CLA (TG-CLA group), or a semisynthetic diet supplemented with 5.5% safflower oil and 1.0% FFA-CLA (FFA-CLA group). Feeding was stopped 10 h before the rats were killed. Values are expressed as mean ± standard error of five to six rats. Values with different letters were significantly different at P < 0.05. Enzyme activity was measured in freeze-thawed homogenates. BAT, brown adipose tissue; CLA, conjugated linoleic acid; CPT, carnitine palmitoyltransferase; FFA, free fatty acid; LETO, Long-Evans Tokushima Otsuka; OLETF, Otsuka Long-Evans Tokushima Fatty; perirenal, perirenal adipose tissue; muscle, red gastrocnemius muscle; TG, triacylglycerol.
CPT Activity

In the present study, CPT activity was measured in BAT, perirenal adipose tissue, liver, and red gastrocnemius muscle to investigate mitochondrial β-oxidation. Figure 1A shows that both forms of CLA can enhance CPT activity in BAT about 1.6-fold. CPT activity also was elevated by 1.4- to 2.5-fold in perirenal tissue, liver, and red gastrocnemius muscle of CLA-treated OLETF rats versus OLETF controls. The degree of enhancement was higher in the TG-CLA group than in the FFA-CLA group, especially in liver and red gastrocnemius muscle. In addition, CPT activity was higher in BAT and perirenal adipose tissue of LETO rats than of OLETF rats. However, CPT activities in the liver and red gastrocnemius muscle were similar in LETO and OLETF rats. A similar trend was found when tissue capacity of the enzyme was expressed as millimoles per minutes per gram of tissue wet weight (Fig. 1B).

DISCUSSION

This is the first study to examine the effects of both forms of CLA on serum leptin concentration, body-fat mass and β-oxidation of fatty acids in OLETF rats.

Leptin is believed to regulate body-weight homeostasis and energy balance.11,27 and circulating leptin level was found to be proportional to adipose tissue mass.28,29 In the present study, a reduction in the body-fat mass and body weight in CLA groups was found. The present study emphasized visceral-adipose tissue and measured the wet weights of perirenal, epididymal, and omental-adipose tissue because visceral-fat obesity is frequently accompanied by hyperlipidemia, hypertension, and glucose intolerance, which are causes of cardiovascular diseases.30–32 The present study also found significant reductions of serum leptin concentration in rats fed diets supplemented with TG-CLA and FFA-CLA. Circulating leptin concentrations decreased and body-fat mass was significantly reduced in CLA-treated mice.10 Medina et al.33 showed that CLA supplementation (3 g/d) significantly decreases circulating leptin concentrations without changing fat mass in humans.33 Circulating leptin levels in OLETF rats increase during feeding and the obesity in this animal model is supposed to arise from leptin insensitivity.34 Therefore, our results that both forms of CLA reduced body-fat mass and serum leptin concentrations in OLETF rats are significant. The decreased levels of serum leptin and body-fat mass might be coordinated responses. However, there might be reasons for the effects of CLA on serum leptin concentration. First, CLA by activating peroxisome proliferator-activated receptor-γ (PPAR-γ) might decrease leptin gene expression.35 Second, CLA incorporation into membrane phospholipid fractions might have effects on signal-transducing pathways and alter leptin production.4

The effects of CLA on β-oxidation of fatty acids were measured with CPT activity, a rate-limiting enzyme of the mitochondrial β-oxidation system.36,37 We measured CPT activity in major tissues such as the liver, perirenal fraction of visceral-adipose tissue, BAT, and red gastrocnemius muscle.

BAT is believed to produce heat by oxidizing fatty acids and might contribute to maintaining whole-body energy balance.38 The higher CPT activity in the present study in terms of specific (nmol · min⁻¹ · mg⁻¹ of protein) and tissue (nmol · min⁻¹ · g⁻¹ of wet weight) capacity implicate this tissue in the β-oxidation of fatty acids by CLA.

We also measured CPT activity in red gastrocnemius muscle. The red type of muscle fiber was reported to have high mitochondrial content, possess high aerobic capacity to meet energy demands, and have relatively high intracellular TG content and high rate of FA oxidation.39,40 We investigated whether CLA feeding enhanced β-oxidation of fatty acids in this muscle tissue compared with the control. CPT activity was higher in red gastrocnemius muscle with CLA feeding, indicating that dietary CLA might make this tissue more efficient in oxidizing fatty acids. The higher CPT activity also was found in perirenal fraction of visceral-adipose tissue and liver in TG-CLA and FFA-CLA groups. The increase in β-oxidation ultimately might prevent storage of TG in adipose tissues and thereby reduce fat mass. Park et al.4,9 and Martin et al.41 showed that CLA enhances CPT activity in mice and rats. In the present study, we showed that both forms of CLA enhance CPT activity in OLETF rats, an obese NIDDM animal model. In addition, TG-CLA was more effective than FFA-CLA in increasing β-oxidation of fatty acids in muscle and liver of OLETF rats.

With regard to CLA-induced enhancement of CPT activity, CLA might enhance fatty-acid oxidation by affecting PPARs (α and γ).42–44 More studies should explore this possibility.

We measured CPT activity in the homogenate, which might represent overall fatty-acid oxidation.45,46 Two forms of CPT, CPT I and CPT II, have been reported, and CPT I is believed to be the key enzyme for fatty-acid oxidation.36,37 More studies are needed to understand the effect of CLA on CPT I and CPT II activity.

In the present study, both forms of CLA prevented hypertriglyceridemia in OLETF rats. The increase in β-oxidation in the liver might be associated in part with TG decreasing action of CLA in these rats. In this context, we found that 10t,12c-CLA reduces secretion of apolipoprotein B-100, a main apoprotein component of very-low-density lipoprotein in HepG2 cells.4 In addition, serum NEFAs concentration was lower in CLA-treated groups. The decrease in adipose tissue mass in CLA-treated groups might be responsible for the CLA-mediated reduction of serum NEFA because NEFAs is released mainly from adipose tissue.

We did not find any changes in serum insulin concentration among the OLETF groups. Serum glucose concentration was not changed by dietary CLA supplementation, but serum glucose concentration was higher in the FFA-CLA group. This result indicates that, under the present experimental conditions, CLA did not reduce serum glucose and insulin concentrations. Another study suggested that CLA reduces circulating insulin and glucose concentrations in Zucker fatty rats.7 However, Medina et al.33 showed that CLA increases circulating insulin concentrations in humans. Some studies suggested that fatty acids can alter glucose utilization and insulin secretion in animal models.45,46 This apparent discrepancy might be due to the differences in animal models and the effects of CLAs on peripheral glucose utilization versus hepatic production and on insulin release from pancreatic β-cells.

In summary, both forms of CLA can reduce serum leptin concentrations, body-fat mass, and body weight. CPT activity was enhanced in BAT, liver, perirenal adipose tissue, and red gastrocnemius muscle because of supplementation with CLAs.

ACKNOWLEDGMENT

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