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# Prolonged feeding of mice with conjugated linoleic acid increases hepatic fatty acid synthesis relative to oxidation

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**Abstract**

Feeding mice conjugated linoleic acid (9 *cis,*11 *trans*/9 *trans,*11 *cis*-and 10 *trans,*12 *cis*-CLA in equal amounts) resulted in triacylglycerol accumulation in the liver. The objective of this study was to examine whether this steatosis is associated with changes in hepatic fatty acid synthesis and oxidation. Therefore, we measured the activities of key enzymes of fatty acid synthesis, i.e., acetyl-CoA carboxylase and fatty acid synthase and of fatty acid oxidation, i.e., 3-hydroxy-acyl-CoA dehydrogenase and citrate synthase in livers of mice fed a diet with 0.5% (w/w) CLA. CLA (a 1:1 mixture of the 10 *trans*, 12 *cis* and 9 *cis*, 11 *trans* isomers of octadecadenoic acid) was administered for 3 and 12 weeks with high-oleic sunflower oil fed as control. The proportion of body fat was significantly lower on the CLA than on the control diet and this effect was already significant after 3 weeks. The specific activites of 3-hydroxy-acyl-CoA dehydrogenase and citrate synthase were unaffected by CLA both after 3 and 12 weeks. The specific activity of fatty acid synthase was nonsignificantly raised (by 12%) after 3 weeks on the CLA diet but had increased significantly (by 34%) after 12 weeks of feeding. The specific activity of acetyl-CoA carboxylase had also increased both after 3 weeks (by 53%) and 12 weeks (by 23%) on the CLA diet, but this effect did not reach statistical significance. Due to CLA-induced hepatomegaly, the overall capacity for both fatty acid oxidation and synthesis—as evidenced by the total hepatic activities of 3-hydroxy-acyl-CoA dehydrogenase, citrate synthase, acetyl-CoA carboxylase, and fatty acid synthase—was significantly greater in the CLA-fed group after 12 weeks, although the overall capacity for fatty acid synthesis had increased more than that for fatty acid oxidation. Thus, this study indicates that prolonged, but not short-term, feeding mice with CLA increased hepatic fatty acid synthesis relative to oxidation, despite the decrease in body fat and the increase in liver weight seen earlier. It is concluded that the observed CLA-induced changes in hepatic fatty acid synthesis and oxidation are the result, rather than the cause, of the lowering of body fat. 

*Keywords:* Dietary conjugated linoleic acid; Fatty acid synthesis; Fatty acid oxidation; Liver; Mice

## 1. Introduction

It has been well documented that dietary conjugated linoleic acid (CLA) and in particular the *trans*-10, *cis*-12 isomer results in a considerable reduction of the proportion of body fat in mice [1–3]. Food intake is usually not affected by the incorporation of CLA in the diets and therefore the body fat-–lowering effect of CLA is most likely mediated by an enhanced energy expenditure. Energy balance studies in mice have indeed shown that feeding CLA (29.6% 9 *cis,*11 *trans* and 30.1% 10 *trans,*12 *cis* and 2.4% 9 *trans,*11 *trans* and 10 *trans,*12 *trans*) to mice increased energy expenditure [2], and similar results were found when measuring the energy expenditure in metabolic chambers for mice fed CLA (39.1% 9 *cis,*11 *trans* and 9 *trans,*11 *cis* 40.7%10 *trans,* 12*cis,* 1.8% 9 *cis,* 11*cis*, 1.3% 10 *cis,*12 *cis*,1.9% 9 *trans*,11 *trans* and 10 *trans*,12 *trans*) [1]. Furthermore, CLA (9 *cis*,11 *trans*/9 *trans*,11 *cis* and 10 *trans*,12 *cis*) has been found to increase the activity of carnitine palmitoyltransferase-I (CPT-I), an enzyme involved in fatty acid oxidation, in muscles and fat pads of mice [4] and rats [5–7], which suggests an enhanced mitochondrial oxidation of fatty acids. Moreover, several studies have also pointed to an increased activity of uncoupling proteins in CLA (9 *cis*,11 *trans* and 10 *trans*,12 *cis*) fed mice [3,8], which

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enables the dissipation of the energy due to an enhanced oxidation.

The body fat–lowering effect of CLA (9 *cis*,11 *trans* and 10 *trans*,12 *cis*), however, is associated with a considerable increase in absolute and relative liver weight [1,2] and the concentration of liver triacylglycerols (TAG) [3,9–11]. It has been shown that feeding CLA (9 *cis*,11 *trans*/9 *trans*,11 *cis* and 10 *trans*,12 *cis*) to mice resulted in lipodystrophy [3], which, in mice, is associated with an enlarged and fatty liver, as is obesity. In both lipodystrophy and obesity, there is no functional adipose tissue, and functions such as fatty acid synthesis will then be taken over by the liver [12].

Measurement of enzyme activities showed that feeding mice 3 weeks CLA concomitantly up-regulates the pathways of hepatic fatty acid synthesis and oxidation, synthesis more so than oxidation [13]. The question arises whether 3 weeks dietary CLA treatment is long enough to establish a new steady state or whether long-term compensatory mechanisms nullify the short-term effects.

The objective of our study was to test whether prolonged CLA-treatment of mice results in sustained up-regulation of hepatic fatty acid synthesis and fatty acid oxidation. Therefore, we measured the activity of two hepatic key enzymes of fatty acid synthesis, i.e., fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). In addition, we measured the activities of two hepatic key enzymes involved in fatty acid oxidation, i.e., 3-hydroxy-acyl-CoA dehydrogenase (3-HAD), and citrate synthase (CS). In addition, we determined body composition as an index of sustained changes.

## 2. Methods and materials

The experimental protocol was approved by the Animal Experiments Committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

### 2.1. Animals and Diets

A total of 24 mice (Balb-C/OlaHsd), 5 weeks old, were purchased from Harlan (Horst, The Netherlands) and housed in a temperature controlled (21°C) animal room with a 12-hour light–dark cycle (lights on 06:00 to 18:00). On arrival, the mice were placed in polycarbonate cages with wood chips and were fed a pelleted commercial rodent diet (Hope Farms, Woerden, The Netherlands) for 2 days. Subsequently, all mice were transferred to individual polycarbonate cages with wood chips. A polyethylene pipe with an inner diameter of 4.2 cm, outer diameter of 5 cm, and a length of 13.8 cm was added to the cages for environmental enrichment. First, all of the mice were fed the semipurified control diet for 7 days. The mice were then divided into four groups of six animals, each balanced for body weights.

The test diet was a high-fat diet with 0.66% (w/w) TAG with CLA (a 1:1 mixture of the 10 *trans*, 12 *cis* and 9 *cis*, 11 *trans* isomers of octadecadenoic acid) added at the expense of part of the HOSF oil (Table 1). The control diet contained 2% HOSF oil. The test diet without HOSF was prepared by Research Diets Services (Wijk bij Duurstede, The Netherlands). The CLA preparation (Clarinol G-80) and the HOSF oil (Table 2) were donated by Loders Croklaan B.V. (Wormerveer, The Netherlands). Two groups of six mice were fed the control HOSF diet and the CLA diet for 3 weeks. Another two groups were fed the same diets for 12 weeks.

Table 1
Composition of semi-purified diets*

| Ingredient | g/kg | Metabolizable energy % |
|---|---|---|
| Casein | 200.0 | 16.97 |
| Total fat | 200.1 | 38.19 |
| Oil mixture | 180.1 | 34.37 |
| Corn oil | 31.5 | 6.01 |
| Coconut oil | 38.0 | 7.25 |
| Olive oil | 55.3 | 10.55 |
| Palm oil | 55.3 | 10.55 |
| High oleic sunflower oil† | 20.0 | 3.82 |
| Total carbohydrates | 507.2 | 43.03 |
| Corn starch | 253.6 | 21.51 |
| Dextrose | 253.6 | 21.51 |
| Cellulose | 33.6 | |
| $CaCO_3$ | 12.4 | |
| $NaH_2PO_4.2H_2O$ | 15.1 | |
| $MgCO_3$ | 1.4 | |
| KCl | 1.1 | |
| $KHCO_3$ | 7.2 | |
| Mineral premix‡ | 10.0 | 0.82 |
| Vitamin premix‡ | 12.0 | 1.00 |
| Total | 1000.1 | 100 |

* The diet contained a calculated amount of 19.73 kJ metabolizable energy per gram. The calculated polyunsaturated / monounsaturated / saturated (P/M/S) fatty acid ratio of the oil mixture was 16.8 / 41.3 / 41.9 and the calculated polyunsaturated / saturated (P/S) ratio was 0.40 (data obtained from USDA Food Tables on the Internet).

† The conjugated linoleic acid (CLA) diet contained 0.5% CLA (w/w) or 1.78 mmol of total CLA per 100 g of diet. CLA was added at the expense of the high oleic sunflower oil and 0.66 g of the Clarinol G-80 preparation was added per 100 g of diet. The CLA diet contained 122 mg *trans*-10, *cis*-12 CLA per 1000 kJ metabolizable energy.

‡ Composition of the vitamin and mineral premix has been described previously by Terpstra et al. [2].

The air-dried basal semipurified diet without the 2% HOSF was stored at 4°C. Every other day, the CLA and the HOSF preparations were added to the basal semipurified diet and two parts of diet were mixed with one part of water in a Kitchen Aid blending machine (model K5SS/PKM5, KitchenAid Europe, Brussels, Belgium). Animals were offered the diets for ad libitum consumption and had free access to tap water. Food intake was not measured, as the mice spilled large amounts of food in the cages.

### 2.2. Analytical methods

At the end of the 3-week and 12-week feeding periods, and at least 2 hours after removing food, the mice were bled

Table 2
Composition of the high oleic acid sunflower oil (HOSF) and the conjugated linoleic acid (CLA) preparation*

| | HOSF | CLA (Clarinol G-80) |
|---|---|---|
| 16:0 (Palmitic acid) | 3.3 | 4.8 |
| 16:1n-7 (Palmitoleic acid, cis-7) | 0.1 | |
| 18:0 (Stearic acid) | 2.9 | 1.6 |
| 18:1n-9 (Oleic acid, cis-9) | 83.5 | 11.6 |
| 18:2n-6 (Linoleic acid, cis-9, cis-12) | 7.9 | 1.3 |
| 18:2n-6 (trans-9, cis-12) | | 0.8 |
| 18:2 (CLA, rumenic acid, cis-9, trans-11 CLA) | | 36.9 |
| 18:2 (CLA, trans-10, cis-12) | | 38.2 |
| 18:2 (CLA, cis-9, cis-11) | | 0.8 |
| 18:2 (CLA, cis-10, cis-12) | | 0.8 |
| 18:2 (CLA, trans-9, trans-11, and trans-10, trans-12) | | 1.2 |
| 18:2 (CLA, 11, 13) | | 1.5 |
| 18:2 (Main CLA isomers (cis-9, trans-11 + trans-10, cis-12) | | 75.1 |
| Oxidized CLA | | 0.2 |
| 18:3n-3 (Linolenic acid or α-linoleic acid) | 0.3 | |
| 20:0 (Arachidonic acid) | 0.3 | 0.1 |
| 20:1n-11 (Gondoic acid) | 0.4 | 0.1 |
| 22:0 (Behenic acid) | 0.9 | 0.1 |
| 24:0 (Lignoceric acid) | 0.3 | |
| Other fatty acids | 0.1 | 0.0 |
| Total | 100 | 100 |
| Total CLA | | 79.4 |
| Free fatty acids as oleic acid | | 0.72 |
| Saturated fatty acids | 7.7 | 6.7 |
| Peroxide number mEq $O_2$/Kg) | 0.8 | 0.7 |

* Data as provided by the manufacturer (Loders Croklaan B.V., Wormerveer, The Netherlands) and expressed in % of fatty acid methyl esters. The Clarinol G-80 contained 79.4% total CLA.

by cardiac puncture into ethylenediamenetetraacetate-treated mini-tubes under a light anesthesia with ether and then killed by cervical dislocation. Plasma samples were stored at −20°C until assayed for plasma insulin, glucose, nonesterified fatty acids (NEFA), and cholesterol. Livers were removed, placed in ice-cold saline, divided into portions for the different assays, frozen in liquid nitrogen, and stored at −70°C until analyses of 3-HAD and CS activities.

For the assays of ACC and FAS activities, fresh liver pieces were homogenized immediately with five strokes of a loosely-fitted Dounce homogenizer in three volumes of ice-cold 250 mmol/L mannitol, 50 mmol/L HEPES, 6.2 mmol/L Na-EDTA, 4 mmol/L potassium citrate, and 2.5 mmol/L β-mercaptoethanol, pH 7.5. The crude homogenate was centrifuged at 12,000 × *g* for 5 minutes. The supernatant was stored at −70°C until analyzed for the activities of ACC and FAS as described [14].

For the assay of CS and 3-HAD activities, about one half of the frozen liver was placed in 9 volumes of 25 ice-cold mmol/L HEPES, 5 mmol/L β-mercaptoethanol, pH 8.0, and homogenized with an IKA-Ultra Turrax T5-FU tissue homogenizer (Janke and Knukel GmbH and Co. KG, Staufen, Germany). The CS and 3-HAD activities in the homogenate were assayed as described [14,15]. Aliquots of this homogenate were also used to measure liver glycogen levels [16]. Hepatic lipids were extracted from the latter homogenate by the method of Bligh and Dyer [17]. Part of the extract was used for determination of the TAG and part for the determination of the fatty acid composition of total hepatic lipids. Total lipids were saponified and methylated according to Metcalfe et al. [18] and fatty acid composition was determined by gas liquid chromatography. Protein was determined by using the Lowry method [19]) with bovine serum albumin as a standard.

Body composition was determined as described previously [2]. Insulin was measured with a kit supplied by Linco Research Inc. (St. Charles, MO) and purified rat insulin was used as a standard. Cholesterol (CHOD-PAP method) and TAG (Triglycerides/GB) were measured with kits supplied by Roche Diagnostics (Mannheim, Germany). NEFA (NEFA C, kit number 994-75409; Instruchemie B.V., Hilversum, The Netherlands) and glucose (HK-method; ABX, Montpellier, France) were measured with test kits as specified in parentheses. Cholesterol, TAG, NEFA, and glucose were measured on a COBAS BIO autoanalyzer (Roche, Basel, Switzerland).

### 2.3. Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) with diet (HOSF diet and CLA diet) and feeding period (3 and 12 weeks) as independent variables. When ANOVA indicated a significant effect for a certain variable, the following groups were compared pairwise with correction for multiple comparisons (*t* test with the Bonferroni adaptation): 1) HOSF vs CLA diet within each feeding period; and 2) 3 weeks vs 12 weeks of feeding within each diet. Each group was used for two comparisons, and therefore the level of significance for these multiple comparisons was preset at $P < 0.025$ (= 0.05/2). The SigmaStat statistical software package (version 2.0; Jandel Corp., San Rafael, CA) was used for all statistical analyses.

## 3. Results

### 3.1. Body weight and body composition

Body weight gain was significantly lower in the mice on the CLA diet than those on the HOSF diet after 3 and 12 weeks of feeding (Table 3). The proportion of body fat in the CLA-fed group was significantly lower than in the control group after 3 weeks on the diet (Table 3, Fig. 1). The same was true after 12 weeks on the diet, but the proportion of body fat in the control group was considerably higher after 12 weeks on the diet than after 3 weeks. The proportion of body fat in the CLA group was the same after 3 and

Table 3
Body composition and plasma concentrations of insulin, glucose, NEFA, and cholesterol in mice fed semi-purified diets containing either high oleic sunflower oil (HOSF) (control) or conjugated linoleic acid (CLA) for periods of 3 and 12 weeks*

| | 3 Weeks | | 12 Weeks | | Two-way ANOVA† |
|---|---|---|---|---|---|
| | HOSF | CLA | HOSF | CLA | |
| Initial body weight (g) | 20.75 ± 1.33 | 21.23 ± 1.59 | 20.75 ± 1.22 | 21.12 ± 1.18 | |
| Final body weight (g) | 24.27 ± 1.72 | 23.82 ± 1.42 | 31.95 ± 3.55† | 27.50 ± 1.93*† | D,T, DxT |
| Weight gain (g) | 3.52 ± 0.63 | 2.58 ± 0.47* | 11.20 ± 3.97† | 6.38 ± 1.28*† | D,T, DxT |
| Liver weight (g) | 1.12 ± 0.09 | 1.23 ± 0.15 | 1.27 ± 0.14 | 1.58 ± 0.19*† | D,T |
| Liver weight (%) | 4.61 ± 0.35 | 5.16 ± 0.36* | 3.98 ± 0.39† | 5.72 ± 0.39* | D, DxT |
| Body composition | | | | | |
| Fat (g) | 3.14 ± 0.49 | 1.96 ± 0.31* | 7.31 ± 2.09† | 2.36 ± 0.26*† | D,T, DxT |
| Water (g) | 13.60 ± 0.69 | 13.93 ± 0.73 | 15.92 ± 1.40† | 16.04 ± 1.18† | T |
| Protein (g) | 4.10 ± 0.26 | 4.16 ± 0.21 | 5.22 ± 0.30† | 4.98 ± 0.31† | T |
| Ash (g) | 0.83 ± 0.04 | 0.83 ± 0.06 | 1.05 ± 0.07† | 0.98 ± 0.04† | T |
| Recovery (%) | 98.67 ± 0.44 | 98.94 ± 0.09 | 99.68 ± 1.05 | 99.19 ± 0.32 | |
| Grams water/gram protein | 3.32 ± 0.05 | 3.35 ± 0.02 | 3.05 ± 0.16† | 3.22 ± 0.05*† | D,T |
| Fat (%) | 14.44 ± 1.55 | 9.33 ± 0.89* | 24.4 ± 4.22† | 9.66 ± 0.57* | D,T,DxT |
| Water (%) | 62.79 ± 1.25 | 66.75 ± 0.68* | 54.19 ± 2.96† | 65.84 ± 0.45* | D,T,DxT |
| Protein (%) | 18.93 ± 0.22 | 19.95 ± 0.24* | 17.81 ± 1.29 | 20.47 ± 0.25* | D,DxT |
| Ash (%) | 3.84 ± 0.26 | 3.97 ± 0.50 | 3.60 ± 0.31 | 4.03 ± 0.24* | D |
| Insulin (pmol/L) | 143 ± 181 | 136 ± 51 | 271 ± 252 | 528 ± 315† | T |
| Glucose (mmol/L) | 13.34 ± 1.37 | 13.52 ± 0.74 | 12.51 ± 2.14 | 11.53 ± 1.16 | |
| NEFA (mmol/L) | 0.69 ± 0.08 | 0.83 ± 0.06* | 0.85 ± 0.13 | 0.77 ± 0.14 | DxT |
| Cholesterol (mmol/L) | 4.42 ± 023 | 4.31 ± 0.47 | 4.74 ± 0.35 | 4.39 ± 0.41 | |

* Values are means ± SD, $n = 6$.

† The data were analyzed with two-way (diet and feeding period as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with $t$ tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. D, effect of diet (CLA vs HOSF); T, effect of feeding period (3 wk vs 12 wk); DxT, interaction between diet and feeding period. *Significant effect of diet within each feeding period (3 or 12 wk); †Significant effect of time (HOSF vs CLA) between feeding period (3 or 12 wk).

12 weeks on the diet, which suggests that feeding CLA for 3 weeks had already resulted in a maximum body fat–lowering effect.

The proportion of body water was significantly higher in the CLA-fed mice after both 3 weeks and 12 weeks on the diet. Furthermore, the proportion of body water in the control group was lower after 12 weeks than after 3 weeks on the diets, whereas the proportion of body water in the CLA-fed mice was the same after 3 and 12 weeks on the diet.

The proportion of body ash and body protein was higher in the CLA-fed group than in the control group both after 3 and 12 weeks on the diet. This effect, however, was not statistically significant for the proportion of ash in the mice fed the diet for 3 weeks. The absolute amounts of body water, ash, and protein were not significantly different between the CLA-fed group and the control group after 3 and 12 weeks of feeding.

### 3.2. Liver weight and liver lipids

Absolute liver weights of the control and the CLA-fed groups were similar after 3 weeks of feeding (Table 3) but were significantly higher in the CLA-fed mice after 12 weeks of feeding. Relative liver weights were significantly higher in the CLA-fed mice both after 3 and 12 weeks on the diets. There were no significant differences between the

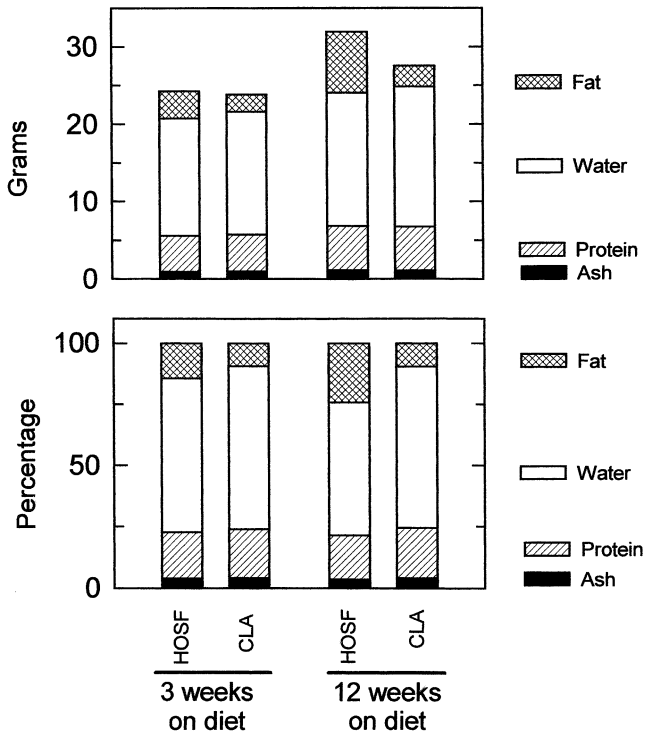


Fig. 1. Absolute body composition (*upper panel*) and relative body composition (*lower panel*) of mice fed semi-purified diets containing high oleic sunflower oil (HOSF) or conjugated linoleic acid (CLA) for 3 and 12 weeks. Values are means, $n = 6$ per group. Results of the statistical analyses of the data are given in Table 3.

Table 4
Concentrations of liver glycogen, triacylglycerol, cholesterol and enzyme activities in mice fed semi-purified diets containing either high oleic sunflower oil (HOSF) (control) or conjugated linoleic acid (CLA) for periods of 3 and 12 weeks*

| | 3 Weeks | | 12 Weeks | | Two-Way ANOVA |
|---|---|---|---|---|---|
| | HOSF | CLA | HOSF | CLA | |
| *Liver* | | | | | |
| Glycogen (μmol/g liver) | 90.5 ± 44.2 | 88.9 ± 33.4 | 94.6 ± 39.8 | 88.5 ± 36.0 | |
| TAG (μmol/g liver) | 17.9 ± 5.5 | 20.2 ± 3.8 | 31.2 ± 4.5 | 46.0 ± 14.3*† | D,T |
| Cholesterol (μmol/g liver) | 7.2 ± 1.0 | 7.6 ± 0.4 | 8.7 ± 1.2 | 9.4 ± 2.6 | T |
| *Key enzymes for fatty acid oxidation* | | | | | |
| 3-HAD (nmol/min · mg protein) | 2.47 ± 0.20 | 2.28 ± 0.22 | 2.23 ± 0.29 | 2.25 ± 0.60 | |
| 3-HAD (nmol/min · total liver) | 595.5 ± 52.8 | 610.9 ± 127.3 | 609.2 ± 110.7 | 765.7 ± 94.1* | D,T |
| CS (nmol/min · mg protein) | 33.0 ± 5.3 | 32.6 ± 4.6 | 38.9 ± 4.3 | 39.8 ± 5.4 | T |
| CS (nmol/min · total liver) | 8.00 ± 1.54 | 8.75 ± 2.08 | 10.58 ± 0.93‡ | 13.61 ± 2.72† | T |
| *Key enzymes for fatty acid synthesis* | | | | | |
| ACC (nmol/min · mg protein) | 0.33 ± 0.05 | 0.41 ± 0.09 | 0.50 ± 0.08‡ | 0.56 ± 0.11 | D,T |
| ACC (nmol/min · total liver) | 80.1 ± 15.6 | 110.6 ± 36.8 | 136.2 ± 30.6 | 201.1 ± 52.7*† | D,T |
| FAS (nmol/min · mg protein) | 0.39 ± 0.06 | 0.44 ± 0.08 | 0.52 ± 0.08 | 0.64 ± 0.07*† | D,T |
| FAS (nmol/min · total liver) | 95.1 ± 16.5 | 117.4 ± 34.0 | 143.3 ± 31.3 | 230.3 ± 42.8*† | D,T,DxT |

Values are means ± SD, $n = 6$.

The data were analyzed with a two-way (diet and feeding period as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with $t$ tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. D, effect of diet (conjugated linoleic acid vs high oleic sunflower oil); T, effect of feeding period (3 weeks vs 12 weeks); DxT, interaction between diet and feeding period.

* Significant effect of diet (HOSF vs CLA) within each feeding period (3 or 12 weeks);

† significant effect of time (HOSF vs CLA) between feeding period (3 or 12 weeks);

‡ Significant effect of feeding period (3 vs 12 weeks) within each diet (HOSF or CLA).

ACC = acetyl-CoA carboxylase; CS = citrate synthase; FAS = fatty acid synthase; 3-HAD = 3-hydroxy-acyl-CoA dehydrogenase.

diets in the concentrations of liver cholesterol, TAG, and glycogen after 3 weeks of feeding (Table 4). Liver cholesterol and glycogen concentrations were also not different after feeding the diets for 12 weeks, but liver TAG level was significantly higher in the mice fed CLA compared with the control group.

Feeding CLA resulted in a significant decrease in the proportions of γ-linolenic (C18:3n-6), dihomo γ-linolenic (C20:3n-6) and arachidonic (C20:4n-6) acid (Table 5), the fatty acids which can be derived from linoleic acid (C18:2n-6) by elongation and desaturation. The proportion of docosahexaenoic (C22:6n-3) was also significantly decreased, whereas that of 6,9,12,15 octadecatetraenoic acid (C18:4n-3), which can be converted into docosahexanoic acid by desaturation and elongation, was significantly increased (Table 5). These findings may indicate a lower desaturation and elongation activity in the livers of the CLA-fed mice. The effects became apparent after 3 weeks of CLA feeding.

### 3.3. Plasma metabolites

Concentrations of plasma cholesterol, nonesterified fatty acids (NEFA), and glucose were not affected by feeding CLA. Plasma insulin levels were considerably higher after feeding CLA for 12 weeks, but this effect did not reach statistical significance because of the large variation (Table 3).

### 3.4. Liver enzyme activities

The hepatic specific activities of 3-HAD and CS, which are indicators of the capacity for fatty acid oxidation, were not affected by CLA both after 3 and 12 weeks on the diets (Table 4). The specific activity of FAS was also not significantly affected by CLA after 3 weeks on the diet but had significantly increased after 12 weeks of feeding. The 53% and 23% increase in the specific ACC activity after 3 and 12 weeks of CLA feeding, respectively, did not reach statistical significance. Because of the CLA-induced liver enlargement, the overall capacity for fatty acid oxidation and synthesis—as evidenced by the total activities of 3-HAD, CS, ACC, and FAS—was significantly greater in the CLA-fed group after 12 weeks on the experimental diet. However, the overall capacity of fatty acid synthesis increased more (67% for total ACC and 54% for total FAS) than that of fatty acid oxidation (26% for total 3-HAD and 29% for total CS). Feeding of mixed CLA isomers resulted in an increase in the capacity for fatty acid oxidation and synthesis, i.e., oxidation as a result of liver enlargement, and synthesis as a result of both liver enlargement and enhanced specific activity of FAS.

## 4. Discussion

Our study indicates that the specific activity of FAS increased in the livers of CLA-fed mice which may help to

Table 5
Fatty acid composition of liver lipids in mice fed semi-purified diets containing either high oleic sunflower oil (HOSF) (control) or conjugated linoleic acid (CLA) for periods of 3 and 12 weeks

| | 3 Weeks | | 12 Weeks | | Two Way ANOVA |
|---|---|---|---|---|---|
| | HOSF | CLA | HOSF | CLA | |
| 14:0 (Myristic) | 0.57 ± 0.13 | 0.53 ± 0.05 | 0.51 ± 0.07 | 0.47 ± 0.05 | |
| 16:0 (Palmitic) | 21.39 ± 0.73 | 22.30 ± 0.80 | 21.51 ± 0.77 | 23.49 ± 1.31* | D |
| 16:1n-7 (Palmitoleic) | 1.24 ± 0.32 | 1.29 ± 0.23 | 1.72 ± 0.36† | 1.53 ± 0.12 | T |
| 18:0 (stearic) | 10.78 ± 0.79 | 9.67 ± 0.91† | 8.93 ± 0.78† | 8.06 ± 1.03 | T |
| 18:1n-9 (Oleic) | 25.61 ± 2.50 | 27.55 ± 2.50 | 31.77 ± 2.99 | 28.39 ± 1.25† | T |
| 18:1n-7 (Vaccenic) | 1.78 ± 0.13 | 2.00 ± 0.43 | 2.37 ± 0.46† | 2.74 ± 0.28† | T |
| 18:2n-6 (Linoleic) | 13.43 ± 0.36† | 14.19 ± 1.18† | 12.39 ± 0.76 | 11.64 ± 1.33 | T |
| 18:2n-6 (Conjugated Linoleic) | 0.00 ± 0.00 | 0.18 ± 0.06* | 0.00 ± 0.00 | 0.19 ± 0.03* | D |
| 18:3n-6 (γ-linolenic) | 0.22 ± 0.11 | 0.08 ± 0.09* | 0.23 ± 0.05 | 0.00 ± 0.00* | D |
| 18:3n-3 (α-linolenic) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.03 ± 0.05 | 0.00 ± 0.00 | |
| 18:4n-3 (octadecatetraenoic) | 0.00 ± 0.00 | 0.48 ± 0.04* | 0.03 ± 0.05 | 0.54 ± 0.03*† | D,T |
| 20:0 (Arachidic) | 0.29 ± 0.03 | 0.33 ± 0.05 | 0.33 ± 0.08 | 0.37 ± 0.02 | |
| 20:1n-9 (Gondoic) | 0.63 ± 0.05 | 0.80 ± 0.19* | 0.81 ± 0.09† | 1.05 ± 0.59 | D,T |
| 20:2n-6 (Eicosadienoic) | 0.23 ± 0.02 | 0.29 ± 0.01* | 0.25 ± 0.03 | 0.31 ± 0.04* | D |
| 20:3n-6 (dihomogammalinoleic) | 1.09 ± 0.12 | 0.90 ± 0.08* | 1.05 ± 0.12† | 0.85 ± 0.13* | D,T |
| 20:4n-6 (Arachidonic) | 14.07 ± 1.34 | 11.66 ± 1.13* | 12.58 ± 0.78 | 9.44 ± 1.70*† | D,T |
| 20:5n-3 (Eicosapentaenoic, EPA) | 0.00 ± 0.00 | 0.03 ± 0.08* | 0.00 ± 0.00 | 0.18 ± 0.09*† | D,T,DxT |
| 22:0 (Behenic) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.01 ± 0.03 | 0.00 ± 0.00 | |
| 22:1n-9 (Erucic) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.04 ± 0.06 | 0.00 ± 0.00 | |
| 22:4n-6 (Adrenic) | 0.35 ± 0.02 | 0.31 ± 0.04 | 0.42 ± 0.02† | 0.25 ± 0.13* | D,T |
| 22:5n-3 (docosapentaenoic) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.06 ± 0.07 | 0.00 ± 0.00* | D |
| 22:6n-3 (Docosahexaenoic, DHA) | 3.80 ± 0.30† | 3.20 ± 0.31*† | 3.26 ± 0.24 | 2.51 ± 0.50* | D,T |
| Other | 4.52 ± 0.42 | 4.38 ± 0.55 | 5.08 ± 0.62 | 4.81 ± 0.44 | |
| Total | 100 | 100 | 100 | 100 | |
| ΣSaturates | 33.03 ± 1.36 | 32.84 ± 1.41 | 31.29 ± 1.09 | 32.39 ± 1.16 | |
| ΣMUFA | 29.25 ± 2.88 | 31.63 ± 3.16 | 33.33 ± 1.73† | 37.08 ± 3.63† | T |
| ΣPUFA | 35.54 ± 1.88 | 33.59 ± 2.41 | 33.05 ± 1.27† | 28.21 ± 3.68*† | D,T |

Values are means ± SD, $n = 6$.

The data were analyzed with a two-way (diet and feeding period as factors) analysis of variance (ANOVA) and the significance level was preset at $P < 0.05$. Subsequently, multiple comparisons were made with $t$ tests, and the level of significance was preset at $P < 0.025$ according to the Bonferroni adaptation. D, effect of diet (conjugated linoleic acid vs high oleic sunflower oil); T, effect of feeding period (3 weeks vs 12 weeks);

* Significant effect of diet within each feeding period (3 or 12 weeks);

† significant effect of time (HOSF vs CLA) between feeding period (3 or 12 weeks).

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; Conjugated linoleic = *trans* - 10, *cis* - 12 and *cis* - 9, *trans* - 11 octadecaenoic acids.

explain the accumulation of lipids in the liver. Similarly, Clement et al. [10] found that liver mRNA levels of FAS were also increased in CLA-fed mice. In this study the specific activity of hepatic fatty acid oxidizing enzymes were not changed; this is in line with the studies of Park et al. [4], who reported no change in the activity of CPT-I (a key enzyme involved in fatty acid oxidation) in the livers of CLA-fed mice. In nonhepatic tissues, on the other hand, reduced activity of fatty acid synthesizing enzymes and increased activity of fatty acid oxidizing enzymes have previously been observed. Tsuboyama-Kasaoka et al. [3] found considerably lower mRNA levels of FAS and ACC in white adipose tissue, and Clement et al. [10] also reported lower mRNA levels for FAS in adipose tissue of CLA-fed mice. In addition, Park et al. [4] reported increased CPT-I activity in adipose tissue. Thus, CLA appears to decrease the synthesis and to increase the oxidation of fatty acids in nonhepatic tissues of mice, but seems also to increase fatty acid synthesis in the liver. As a result, there is a decrease in the accumulation of lipids in adipose tissue and an increase in accumulation of lipids in the liver, similar to what is seen in lipodystrophic mice [12].

The effects of CLA on lipid metabolizing enzymes have also been studied in the rat. Dietary CLA, however, has only a minor or no effect on body fat and liver weight in rats [20,21], which may explain why the results for the lipid metabolizing enzymes found in rats are not always consistent with or similar to those observed in mice. Feeding CLA to rats increased CPT-I activity [5,7] and decreased fatty acid synthesis [7,22] in adipose tissue, which is in agreement with results found in mice. However, Azain et al. [22] reported a decrease in hepatic FAS activity in CLA-fed rats and Rahman et al. [6] found an increase in hepatic CPT-I activity, opposite to what we and others found in mice. Other studies did not find any effect on lipid metabolizing enzymes in liver of CLA-fed rats [23]. In addition, studies in rabbits [24] showed a lower activity of ACC in both liver and adipose tissue after feeding 0.5% CLA (a 1:1 mixture of

the 9 *cis*,11 *trans* and 10 *trans*,12 *cis*) for 90 days. Thus, the effects of CLA on body composition and enzyme activity appear to be species dependent.

Several studies have indicated that CLA (10 *trans*,12 *cis*) inhibits the activity [25] and the expression of stearoyl-CoA or Δ-9 desaturase mRNA [26,27] in the liver of mice. Inhibition of Δ-9 desaturase will result in a reduced conversion of stearic acid oleic acid and of palmitic acid palmitoleic acid. Studies in mice [27], chickens [28], and perch [29] have indeed shown that the ratio of palmitoleic acid to palmitic acid and of oleic acid to stearic acid in the liver was decreased when fed CLA. We, however, did not find consistent and significant changes in these ratios in the liver and similar results in mice have been reported by Belury et al. [9]. Furthermore, CLA (10 *trans*,12 *cis*) has also been reported to inhibit the Δ-6 and Δ-5 desaturase activities in HepG2 cells [30], and there is also evidence that CLA may inhibit elongase activity [31]. We found that feeding mice mixed CLA isomers resulted in significant reductions of the proportions of arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6n-3), the elongation and Δ-6 and Δ-5 desaturation products of linoleic acid and α-linolenic acid, respectively. Similar results have been reported in other studies with mice [9], chickens [28], and perch [29].

Feeding mixed CLA isomers for 12 weeks resulted in an increase in plasma insulin levels, although this effect did not reach statistical significance. Other studies have also shown increased levels of plasma insulin levels after feeding mice with CLA (9 *cis*,11 *trans* and 9 *trans*,11 *cis* and 10 *trans*,12 *cis*) [3,8,10,32,33] and similar results have been reported in humans fed the *trans* -10, *cis* -12 octadecanoic acid isomer of CLA [34]. On the other hand, feeding CLA to diabetic Zucker rats resulted in a lowering of insulin levels [35,36]. Thus, the response to feeding CLA may depend on the species studied (inasmuch as different species have a different enzymatic make up and metabolic rate), or it may depend on whether the animals are diabetic.

In the present study, CLA caused hepatomegaly accompanying the accumulation of lipids in the liver of mice. Although fatty acid oxidation and synthesis are both up-regulated by the mixed CLA isomers diet, the synthesis of fatty acids is up-regulated more as evidenced by higher increases in the activities of ACC and FAS as compared to those of 3-HAD and CS, and by the accumulation of hepatic TAG. The results of the present study are in agreement with those of a recent study by Takahashi et al. [13] in which mice were fed for 3 weeks with a CLA-containing diet. Our results demonstrated that after 12 weeks of feeding CLA the initial CLA-induced effects are still present, illustrating the absence of a long-term mechanism neutralizing the effects. Quite different from our results is the 10-fold increase in hepatic TAG level found in the study by Takahashi et al. [13]. The basal rate of hepatic fatty acid oxidation may be different between the two experiments, as we used 0.5% CLA instead of the 1.5% CLA in the experimental diet used by Takahashi et al. [13]. In addition, we used a mixture of saturated, monounsaturated, and polyunsaturated fats, whereas Takahashi et al. [13] used only a saturated fat, which may also be a factor in determining the difference in hepatic TAG accumulation.

In conclusion, feeding CLA to mice resulted in lower body fat but increased liver weight and induced liver steatosis. Based on previous studies, the reduction in body fat in CLA-fed mice is a result of an enhanced fatty acid oxidation and reduced fatty acid synthesis in nonhepatic tissues. However, this study suggests that the increase in liver TAG may be mediated by increased hepatic synthesis of fatty acids relative to oxidation. Definite proof of this suggestion can be obtained only by investigating a more detailed time course of events.

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