Desaturation Indices in Liver, Muscle, and Bone of Growing Male and Female Mice Fed *trans*-10,*cis*-12 Conjugated Linoleic Acid

Srikant Viswanadha, Michael L. McGilliard, and Joseph H. Herbein*

Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

ABSTRACT: *trans*-10,*cis*-12-CLA (*t*10,*c*12-CLA) inhibits lipid deposition in adipose tissue of many species, but it also enhances lipid deposition in liver. We evaluated effects of dietary *t*10,*c*12-CLA content and gender on carcass composition, FA profile of selected tissues, and expression of FA synthase (FAS) and stearoyl-CoA desaturase-1 (SCD) mRNA in adipose tissue. Male and female (63 of each) CD-1 mice were assigned a diet containing 0.0, 0.15, or 0.30% *t*10,*c*12-CLA at 4 wk of age. Seven mice per dietary group within gender were sacrificed after 2, 4, or 6 wk. The CLA isomer caused dose-dependent reductions in dry carcass weight and fat content, without altering protein content, but carcass fat and epididymal fat pad weights of males were reduced to a greater extent than carcass fat and inguinal fat pad weights of females. FAS and SCD mRNA in adipose tissue was more abundant in females than males, but expression in both genders decreased as the *t*10,*c*12-CLA content of the diet increased. Although the weight of gastrocnemius muscle was not influenced by diet, total FA content of the muscle of both genders decreased in response to dietary *t*10,*c*12-CLA content. Femur weight of male mice increased as the *t*10,*c*12-CLA content of the diet increased, but the weight increase was associated with a reduction in total FA content. The Δ9 desaturation indices for muscle and femur suggested a linear reduction in SCD activity, whereas Δ9 indices for liver indicated linear enhancement of SCD activity. Overall, results suggested that growing male mice were more susceptible than females to *t*10,*c*12-CLA inhibition of lipid deposition.

Paper no. L9970 in *Lipids 41*, 763–770 (August 2006).

Alterations in lipid metabolism attributed to *trans*-10,*cis*-12-CLA (*t*10,*c*12-CLA) include reduced deposition of lipid in body fat depots, increased deposition of lipid in liver, and reduced monounsaturated FA content of tissues, blood plasma, milk, and eggs due to inhibition of stearoyl-CoA desaturase-1 (SCD) activity or mRNA expression in tissues (1–7). FA concentrations in plasma lipid fractions of humans were used to calculate Δ5, Δ6, and Δ9 desaturase indices in response to a diet containing a mixture of equal amounts of *cis*-9,*trans*-11-CLA (*c*9,*t*11-CLA) and *t*10,*c*12-CLA (8) or diets containing purified *c*9,*t*11-CLA or *t*10,*c*12-CLA (9). Both reports suggested a reduction in overall capacity for Δ6 and Δ9 desaturation and an increase in Δ5 desaturation capacity due to the CLA mixture or the individual isomers in the diet. Desaturation indices derived from FA concentrations in blood lipid fractions, however, probably were confounded by differential effects of CLA isomers on liver, muscle, adipose tissue, bone, and other tissues. Other confounding factors may include gender (3), metabolic status (1), genetic potential for body fat accretion (1,4), dietary CLA content (6,10), or overall fat content and FA profile of the diet (11,12).

Excess hepatic lipid deposition in response to *t*10,*c*12-CLA may be a consequence of the pronounced inhibition of lipid accumulation and cell development in adipose tissue. In adipocytes, *t*10,*c*12-CLA alters expression of several transcription factors that target genes, including SCD and FA synthase (FAS), required for lipid synthesis and accumulation during cell differentiation and maturation (13). Quantification of cell number and diameter indicated reduced adipocyte cell volume accounted for reduced epididymal fat pad weight of lean rats fed a CLA mixture for 35 d (4). Although subcutaneous adipocyte cell number and diameter in male pigs fed a CLA mixture for 35 d were not altered, the Δ9 desaturation index and SCD activity were reduced (14). Reduced SCD expression in adipose tissue may be linked to enhanced lipid oxidation, as well as reduced triacylglycerol synthesis and storage (15).

An initial evaluation of hepatic SCD response to a dietary CLA mixture or the purified *c*9,*t*11-CLA isomer indirectly indicated that reduced hepatic SCD mRNA expression and reduced hepatic Δ9 desaturation index (ratio of oleic acid to stearic acid) in growing male mice (16) was most likely due to the *t*10,*c*12-CLA in the CLA mixture. A subsequent study with HepG2 cells indicated reduced SCD activity and Δ9 desaturation index (ratio of oleic to stearic) due to *t*10,*c*12-CLA, but SCD mRNA expression was not altered (17). In agreement with the *in vitro* study, hepatic SCD activity, but not SCD mRNA expression, in lactating mice (18) was reduced by *t*10,*c*12-CLA (1% of the diet). In contrast, dietary *t*10,*c*12-CLA (0.5% of the diet) increased the ratio of oleic acid to stearic acid in liver lipids of nonlactating mice, indicating an increase in hepatic SCD activity (19).

Due to these inconsistencies, the present study evaluated carcass composition, FA profiles and total FA in selected tissues, and SCD expression in liver and adipose tissue of growing male

*To whom correspondence should be addressed at Dairy Science Department (0315), Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. E-mail: herbeinj@vt.edu

Current address of first author: 50 South Drive, Building 50, National Institute of Diabetes, Digestive, and Kidney Diseases, Bethesda, MD 20892.

Abbreviations: CP, crude protein; EE, ether extract; FAS, FA synthase; SCD, stearoyl-CoA-desaturase; *c*9,*t*11-CLA, *cis*-9,*trans*-11-CLA; *t*10,*c*12-CLA, *trans*-10,*cis*-12-CLA.

and female mice fed diets containing 0.0, 0.15, or 0.30% purified *t*10,*c*12-CLA for 6 wk. FA profiles for muscle and bone were used to calculate Δ9 and Δ5 plus Δ6 desaturation indices, which served as estimates of CLA-induced alterations in FA metabolism in lean body mass, for comparison with alterations in desaturation indices in liver. Because a dietary concentration of *t*10,*c*12-CLA as low as 0.1% may be adequate to obtain some of the desirable metabolic responses to this CLA isomer, such as inhibition of tumor metastasis (11), the 0.15% level was included in this study. The 0.30% level approximated the amount of *t*10,*c*12-CLA in a diet containing 0.5–1.0% CLA mixture.

EXPERIMENTAL PROCEDURES

Animals, diets, and sampling. All procedures involving animals were approved by the Virginia Polytechnic Institute and State University Animal Care Committee. Sixty-three male and 63 female, 3- to 4-wk-old CD-1 mice (Harlan, Madison, WI) were housed individually with access to food and water at all times. A 12-h light/12-h dark cycle was maintained throughout the study. For 7 d before the start of the study, all mice were fed Harlan Teklad (Harlan, Madison, WI) Global Rodent Diet (2018) (18% protein, 5% fat) with 3% (wt/wt) high-oleic sunflower oil added. On day 1 of the study, male and female mice were randomly assigned to receive diets containing 3.0% high-oleic sunflower (control), 2.85% high-oleic sunflower oil + 0.15% *t*10,*c*12-CLA, or 2.70% high-oleic sunflower oil + 0.30% *t*10,*c*12-CLA. The free FA form of *t*10,*c*12-CLA (>95% purity) was obtained from Natural Lipids (Hovdebygda, Norway). Mice were fed daily at 1600 h, and food refusals were weighed to estimate intake during the previous 24 h. Samples of each diet were obtained each week and stored at 4°C prior to FA analysis (Table 1).

Body weights were determined twice weekly and the day of sacrifice. At the end of weeks 2, 4, and 6, seven mice per dietary treatment within gender were anesthetized with Metofane® (Pitman-Moore, Inc, Washington Crossing, NJ) prior to cervical dislocation. The liver was removed, rinsed with diethyl pyrocarbonate (Sigma, St. Louis, MO) in distilled water (1:1000, vol/vol), and weighed. A portion of liver was frozen in liquid nitrogen and stored at –80°C prior to mRNA analysis. The remainder of the liver was stored at –20°C prior to FA analysis. Adipose tissue from the inguinal region of females or the epididymal region of males was excised, weighed, frozen in liquid nitrogen, and stored at –80°C prior to mRNA analysis. After evisceration, the head, skin, feet, and tail were removed. Gastrocnemius muscle and femur in the left hind leg of the carcass were excised and stored at –20°C prior to FA analysis. The remainder of the carcass was stored at –20°C prior to drying at 55°C, grinding, and analyses (20) for crude protein (CP) and ether extract (EE) content.

TABLE 1
FA Composition of Diets Fed to Growing Male and Female Mice for 6 Wk

	Dietary *t*10,*c*12-CLA (% total FA)		
FA	0%	0.15%	0.30%
10:0	0.02	0.02	0.02
12:0	0.04	0.05	0.05
14:0	0.12	0.13	0.12
16:0	9.26	9.41	9.18
cis-9-16:1	0.18	0.20	0.19
18:0	2.49	2.65	2.52
trans-11-18:1	0.02	0.02	0.02
cis-9-18:1	34.41	33.09	31.77
18:2n-6	47.34	46.54	46.28
cis-9,*trans*-11-18:2	0.02	0.18	0.32
trans-10,*cis*-12-18:2	0.00	1.37	2.67
18:3n-3	3.72	3.57	3.77
20:0	0.39	0.39	0.38
20:4n-6	0.02	0.02	0.03
Total (μg/mg of diet)	44.07	44.76	46.14

Lipids in diets, liver, femur, and muscle were extracted using the Folch procedure (21). Undecenoic acid (Nu-Check Prep, Inc., Elysian, MN), added to extracted lipids prior to formation of FAME (22), served as an internal standard. Using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (Agilent Technologies, Palo Alto, CA), FAME were separated by a 100 m × 0.25 mm i.d. (0.2 μm film thickness) CP-Sil 88 column (Varian, Lake Forest, CA) protected by a 2.5 m × 0.25 mm i.d. fused silica retention gap. Ultrapure hydrogen at constant pressure was the carrier gas. Pure methyl ester standards (Nu-Check Prep, Inc.) were used for identification and quantification of sample FA (23). Quantities of substrate and product FA (μg/mg of tissue) for selected FA desaturation reactions were used to calculate desaturation indices for liver, muscle, and femur, where desaturation index = substrate/(substrate + product).

Northern blotting. A pkk160 plasmid containing the mouse SCD fragment was donated by Dr. James Ntambi (Department of Biochemistry, University of Wisconsin—Madison), and a pmFAS/CR11 plasmid containing the mouse FAS fragment was obtained from Dr. Hitoshi Shimano (Department of Internal Medicine, University of Tsukuba, Japan). The pDrive cloning vector containing rat β-actin was obtained from Dr. William Huckle (Department of Biomedical Sciences and Pathobiology, Virginia Tech). Plasmids were used to prepare cDNA probes as previously described (18). Total adipose tissue and liver RNA were extracted using TRI REAGENT® (MRC, Cincinnati, OH), separated by electrophoresis on 1% agarose gel containing 0.66 M formaldehyde, and transferred to a Magna Charge nylon membrane (MSI, Westborough, MA) by downward capillary blotting and UV crosslinking. Specific mRNA bands on the membrane were detected using the DIG High Prime DNA Labeling and Detection Starter Kit 2 (Roche Diagnostics Corporation, Indianapolis, IN). Intensity of bands was determined by GelWorks® 1D Intermediate software, version 4.01 (UVP, Inc., Upland, CA). The β-actin band was used for normalization of SCD and FAS mRNA abundance.

Statistical analysis. Data were analyzed as a completely randomized design with a factorial arrangement of treatments using the MIXED procedure of SAS® (Windows version 9.1, Cary, NC). Interactions in the initial model were dietary treat-

ment × gender, treatment × week, gender × week, and treatment × gender × week. Because gender had a significant ($P < 0.05$) effect on most variables in the initial model, data for each gender were analyzed separately. The separate analyses for males and females included a treatment × week interaction. Least-square means ± SEM (combined observations for weeks 2, 4, and 6) for variables within gender are presented in tables and figures. Within each gender, contrasts were performed to test the linear (dose-dependent) effects of the amount of dietary *t*10,*c*12-CLA on each variable.

RESULTS

Table 2 lists overall (mean of weeks 2, 4, and 6) effects of dietary *t*10,*c*12-CLA content on body weight, carcass composition, tissue weights, and total FA in tissues in male and female mice. Dietary *t*10,*c*12-CLA did not alter body weight or food intake (data not shown) during the 6-wk study, regardless of gender, when compared with the control groups. Dry carcass weight, however, was reduced by *t*10,*c*12-CLA. In the initial statistical analysis, which included both genders, a gender by

TABLE 2
Mean[a] Body Weight, Carcass Composition, Tissue Weights, and Tissue FA Content for Male and Female Mice Fed *t*10,*c*12-CLA

	Dietary *t*10,*c*12-CLA				
	0%	0.15%	0.30%	SEM	P <[b]
Body weight (g)					
Male	32.2	32.3	31.8	0.5	0.71
Female	24.7	24.7	25.2	0.3	0.48
Dry carcass					
Weight (g)					
Male	5.88	4.32	4.10	0.20	0.01
Female	3.62	3.16	3.14	0.12	0.01
CP (g)					
Male	2.55	2.42	2.55	0.09	0.51
Female	1.92	1.83	1.91	0.05	0.46
CP (%)					
Male	44.8	56.2	62.2	0.9	0.01
Female	53.6	58.4	61.2	1.0	0.01
EE (g)					
Male	2.10	1.01	0.81	0.12	0.01
Female	1.07	0.79	0.60	0.07	0.01
EE (%)					
Male	35.0	23.7	19.8	1.5	0.01
Female	29.3	24.0	18.7	1.8	0.01
Liver					
Weight (g)					
Male	2.04	2.30	2.36	0.06	0.01
Female	1.50	1.60	1.77	0.05	0.01
Total FA (Ìg/mg)					
Male	38.8	45.0	59.0	3.0	0.01
Female	34.4	43.3	49.7	2.4	0.01
Epididymal adipose (g)	0.73	0.26	0.14	0.04	0.01
Inguinal adipose (g)	0.34	0.20	0.16	0.03	0.01
Muscle					
Weight (g)					
Male	0.19	0.20	0.20	0.006	0.20
Female	0.15	0.14	0.15	0.004	0.20
Total FA (Ìg/mg)					
Male	23.7	14.0	10.7	1.3	0.01
Female	19.7	13.0	11.9	1.2	0.01
Femur					
Weight (mg)					
Male	66.6	73.8	70.0	1.6	0.01
Female	54.6	58.5	58.6	1.5	0.11
Total FA (Ìg/mg)					
Male	4.18	3.47	2.32	0.25	0.01
Female	3.34	2.95	2.65	0.24	0.13

[a]Values are an overall least-square mean for 21 observations, where seven mice within each gender were sacrificed after consuming their assigned diet for 2, 4, or 6 wk.
[b]Probability of a linear effect due to dietary *t*10,*c*12-CLA content.

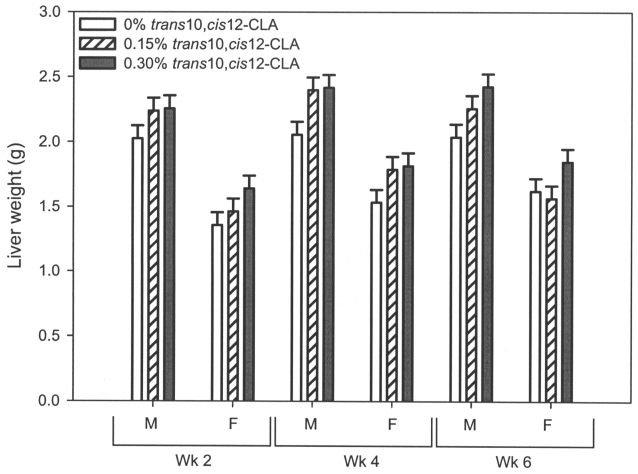

FIG. 1. Biweekly changes in liver weight for male (M) and female (F) mice fed 0.15% or 0.30% *t*10,*c*12-CLA, where $n = 7$. The overall (combined data for weeks 2, 4, and 6) increase in liver weight due to dietary CLA content was linear ($P < 0.01$) for male and female mice, as indicated in Table 2.

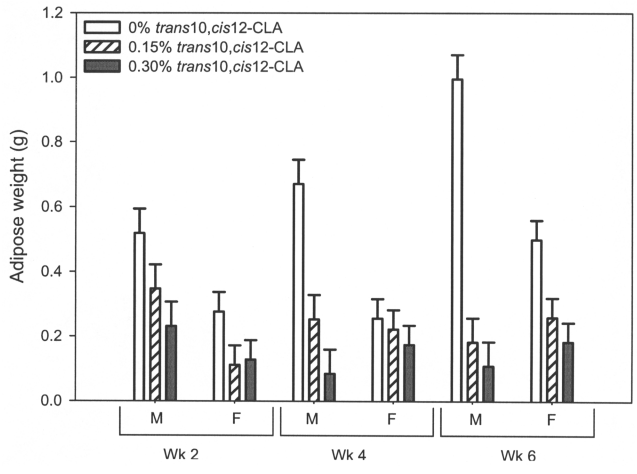

FIG. 2. Biweekly changes in epididymal (M) or inguinal (F) fat pad weight of mice fed 0.15% or 0.30% *t*10,*c*12-CLA, where $n = 7$. The overall (combined data for weeks 2, 4, and 6) reduction in fat pad weight due to dietary CLA content was linear ($P < 0.01$) for male and female mice, as indicated in Table 2.

treatment interaction ($P < 0.05$) resulted due to females responding to the dietary treatments to a lesser extent than males. At 6 wk, carcass weight was 30% lower for males fed 0.15% or 0.30% *t*10,*c*12-CLA, compared with the control, but female dry carcass weight was reduced by only 13%. The reduced carcass weight of males and females was due primarily to a dose-dependent reduction in the amount (g) of EE, whereas amount of carcass CP was similar for all dietary treatments. Thus, CP became a greater percentage of carcass weight as dietary *t*10,*c*12-CLA content increased.

Overall, liver weight of male and female mice increased in response to dietary *t*10,*c*12-CLA in a dose-dependent manner, and the increase was accompanied by an increase in total FA content (Table 2). With the exception of females at 6 wk, liver weight was consistently greater for mice fed *t*10,*c*12-CLA at 2, 4, and 6 wk (Fig. 1). There were overall reductions in inguinal (female) and epididymal (male) fat pad weights in response to increasing dietary *t*10,*c*12-CLA content. The CLA-induced reductions in epididymal adipose tissue weight in males were greater than the reductions in inguinal adipose tissue weight in females due primarily to a biweekly increase in epididymal adipose tissue weight for control-fed male mice compared with a decline in weight for those fed 0.15% or 0.30% *t*10,*c*12-CLA (Fig. 2). Gastrocnemius muscle weight was not altered by dietary treatments, but total FA content of muscle of male and female mice decreased in a dose-dependent manner in response to dietary *t*10,*c*12-CLA. Although femur weight was numerically greater for male and female mice fed 0.15% or 0.30% *t*10,*c*12-CLA, compared with the control groups, the response was significant ($P < 0.01$) only for males. Likewise, the reduction in femur total FA content in response to increasing dietary *t*10,*c*12-CLA content was significant ($P < 0.01$) only for males.

When expressed as a percentage of total FA (data not shown), concentration of *t*10,*c*12-CLA was highest in muscle (0.36% and 0.59% for mice fed 0.15% and 0.30% *t*10,*c*12- CLA, respectively), intermediate in femur (0.20% and 0.39%), lowest in liver (0.15% and 0.31%), and not detectable in these tissues of mice fed the control diet. Palmitic acid (19–24% of total FA), stearic acid (6–10%), oleic acid (29–36%), and linoleic acid (15–21%) were the primary FA in muscle and femur. Palmitic acid (22–23% of total FA) and linoleic acid (17–21%) concentrations in liver were similar to those in muscle and femur. However, stearic acid (17–23%) was greater and oleic acid (20–23%) was lower in liver compared with muscle or femur.

Desaturation indices for liver, gastrocnemius muscle, and femur are listed in Table 3. Potential substrates for the Δ9 desaturation reaction include palmitic acid, stearic acid, and vaccenic acid (*trans*-11-18:1). Desaturation of vaccenic acid was included in Table 3 because it accounted for a small portion of total FA in all diets (Table 1). The desaturation indices for 18:0 and *trans*-11-18:1 in liver of males and females were reduced by dietary *t*10,*c*12-CLA. In contrast, the desaturation indices for 16:0 and 18:0 in muscle (males and females) and femur (males only) were elevated by *t*10,*c*12-CLA. The desaturation index for 18:2n-6, which includes Δ5 and Δ6 desaturases, in liver was not influenced by dietary *t*10,*c*12-CLA content, but it was reduced in muscle (males and females) and femur (males only) of mice fed *t*10,*c*12-CLA. The 18:3n-3 desaturation index in liver of male mice was elevated in response to dietary *t*10,*c*12- CLA, but was not altered in muscle or femur of either gender.

FA analysis of adipose tissue was not possible, because all available tissue was needed for RNA isolation. Evaluation of adipose tissue RNA revealed linear reductions in SCD (Fig. 3) and FAS (Fig. 4) mRNA, regardless of gender, due to increasing dietary *t*10,*c*12-CLA content. In addition, SCD and FAS expression in adipose tissue of females was greater ($P < 0.05$) than in males. Unlike adipose tissue, overall hepatic SCD mRNA expression was not affected by dietary *t*10,*c*12-CLA content or gender (data not shown).

TABLE 3
Desaturation Indices[a] for Liver, Gastrocnemius Muscle, and Femur of Male and Female Mice Fed *t*10,*c*12-CLA

	Dietary *t*10,*c*12-CLA				
	0%	0.15%	0.30%	SEM	P <[b]
Liver					
16:0 to 16:1					
Male	0.97	0.97	0.97	0.003	0.58
Female	0.99	0.98	0.98	0.001	0.13
18:0 to 18:1					
Male	0.49	0.45	0.37	0.018	0.01
Female	0.55	0.51	0.48	0.017	0.02
trans-11-18:1 to *c*9,*t*11-CLA					
Male	0.89	0.75	0.66	0.040	0.01
Female	0.84	0.67	0.59	0.048	0.01
18:2n-6 to 20:4n-6					
Male	0.74	0.76	0.79	0.017	0.16
Female	0.75	0.77	0.77	0.019	0.85
18:3n-3 to 20:5n-3					
Male	0.83	0.87	0.90	0.016	0.01
Female	0.80	0.83	0.86	0.019	0.14
Muscle					
16:0 to 16:1					
Male	0.80	0.87	0.90	0.006	0.01
Female	0.85	0.89	0.91	0.008	0.01
18:0 to 18:1					
Male	0.13	0.17	0.21	0.010	0.01
Female	0.15	0.17	0.22	0.014	0.01
trans-11-18:1 to *c*9,*t*11-CLA					
Male	0.45	0.52	0.53	0.034	0.22
Female	0.43	0.38	0.38	0.031	0.33
18:2n-6 to 20:4n-6					
Male	0.90	0.83	0.80	0.013	0.01
Female	0.87	0.84	0.79	0.010	0.01
18:3n-3 to 20:5n-3					
Male	0.90	0.89	0.86	0.017	0.29
Female	0.86	0.80	0.83	0.020	0.07
Femur					
16:0 to 16:1					
Male	0.79	0.81	0.86	0.011	0.01
Female	0.84	0.85	0.86	0.011	0.52
18:0 to 18:1					
Male	0.18	0.20	0.26	0.013	0.01
Female	0.22	0.23	0.25	0.014	0.44
trans-11-18:1 to *c*9,*t*11-CLA					
Male	0.38	0.40	0.51	0.042	0.06
Female	0.41	0.35	0.40	0.038	0.40
18:2n-6 to 20:4n-6					
Male	0.85	0.83	0.77	0.013	0.01
Female	0.82	0.82	0.82	0.013	0.94
18:3n-3 to 20:5n-3					
Male	0.81	0.80	0.79	0.008	0.31
Female	0.80	0.78	0.79	0.010	0.55

[a]Desaturation index = substrate/(substrate + product). Values are an overall least-square mean for 21 observations, where seven mice within each gender were sacrificed after consuming their assigned diet for 2, 4, or 6 wk.
[b]Probability of a linear effect due to dietary *t*10,*c*12-CLA content.

DISCUSSION

Despite reductions in weight of the dry carcass and carcass fat content in this study, total body weight and food intake were not influenced by 0.15% or 0.30% dietary *t*10,*c*12-CLA. Previous dose-response studies using male mice indicated diets containing 0.5% or more CLA mixture were needed for reductions of body weight or energy intake (10,24), and these reductions eventually were attributed to dietary *t*10,*c*12-CLA rather than *c*9,*t*11-CLA (25).

A dry carcass without head, skin, feet, and tail was used in the present study to obtain estimates of changes in lipid and protein content of structural body components in response to dietary *t*10,*c*12-CLA. Carcass weight, carcass lipid content,

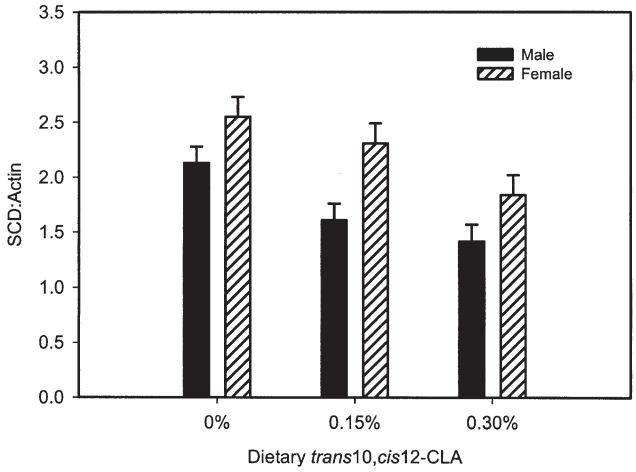

FIG. 3. SCD mRNA in epididymal adipose tissue of male mice and inguinal adipose tissue of female mice fed 0.15% or 0.30% *t*10,*c*12-CLA. Each bar represents the mean for 21 mice, where seven mice were sacrificed at 2, 4, or 6 wk within the 6-wk study. The decline in SCD mRNA due to dietary *t*10,*c*12-CLA content was linear ($P < 0.05$) for male and female mice, and SCD mRNA abundance was greater ($P < 0.05$) for females compared with males.

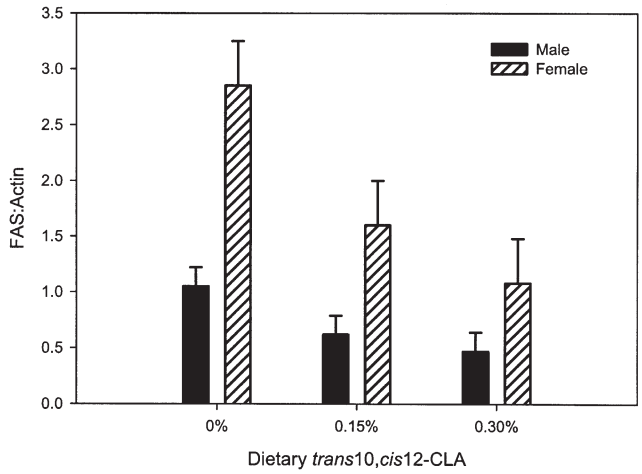

FIG. 4. FAS mRNA in adipose tissue of male and female mice fed *t*10,*c*12-CLA. Each bar represents the mean for 21 mice, where seven mice were sacrificed at 2, 4, or 6 wk within the 6-wk study. The decline in FAS mRNA due to dietary *t*10,*c*12-CLA content was linear ($P < 0.05$) for male and female mice, and FAS mRNA abundance was greater ($P < 0.05$) for females compared with males.

and weight of the epididymal or inguinal fat pad were reduced in a dose-dependent manner. Reduction in carcass fat content accounted for a majority of the reduction in dry carcass weight, because protein content of the carcass was not altered. Although there was a dose-dependent decrease in total FA in muscle of both genders, femur FA content was reduced to a greater extent in males (45%) than in females (21%). In association with reduced deposition of lipids in peripheral tissues, however, was a 15–18% increase in weight of the liver. Liver enlargement was reported for male mice fed a diet containing 1.0% CLA mixture (40% *t*10,*c*12-CLA) (24), male mice fed 1.5% CLA mixture (48% *t*10,*c*12-CLA) (26), female mice fed 1% CLA mixture (36% *t*10,*c*12-CLA) (27), female mice fed 0.4% purified *t*10,*c*12-CLA (28), and male and female mice fed 1.0% purified *t*10,*c*12-CLA (29). Hepatic enlargement was associated with a 3- to 7-fold increase in triacylglycerol content (26,28). The dose-dependent increase in liver weight and total FA content noted in this study suggested the lower threshold for these hepatic responses may be 0.15% *t*10,*c*12-CLA or less in a diet.

Gastrocnemius muscle weight of male and female mice was not affected by dietary treatments. However, femur weight increased due to 0.15% and 0.30% dietary *t*10,*c*12-CLA. Increased bone weight may be due to a decrease in 20:4n-6 concentration in bone (30), which also was observed in the current study (data not shown). Inadequate 20:4n-6 may reduce production of eicosanoids, including prostaglandin E2 (1), essential for bone resorption and osteoclastic activity (31). In response to reduced osteoclastic activity, net bone turnover may be low and lead to increased bone forming rate.

Previous studies evaluated potential causes of reduced body fat deposition, including adipocyte apoptosis and insulin resistance, due to CLA mixtures or purified *t*10,*c*12-CLA (13,27,28,29). Female mice fed a diet with 1.0% CLA mixture for 5 mon had reduced FAS mRNA expression in white adipose tissue (27). In contrast, SCD mRNA expression was reduced in mouse 3T3-L1 pre-adipocytes in response to *t*10,*c*12-CLA (13). In the current study, there was a linear decrease in expression of FAS and SCD mRNA in response to dietary *t*10,*c*12-CLA in male and female mice. Males, however, had lower FAS and SCD mRNA in adipose tissue than females. Lower expression of FAS and SCD in males suggested that their adipose tissue depots may be more susceptible to the inhibitory effects of *t*10,*c*12-CLA, which appeared to be confirmed by the greater dose-dependent reduction in epididymal fat pad weight of males compared with inguinal fat pad weight reduction of females at 4 and 6 wk (Fig. 2).

FA desaturation indices as indicators of Δ9, Δ5, and Δ6 desaturation in this study were based on the concentration (μg/mg) of a selected FA in a tissue and calculated as a ratio of substrate to (substrate plus product). In the liver and most other tissues, SCD catalyzes the conversion of 16:0 to *cis*-9-16:1 and 18:0 to *cis*-9-18:1 by insertion of a double bond at the Δ9 position (32). Likewise, SCD desaturates *trans*-11-18:1 to *c*9,*t*11-18:2 (33). Highly unsaturated FA, such as 20:4n-6 and 20:5n-3, are formed from 18:2n-6 or 18:3n-3 by Δ5 and Δ6 desaturation. Thus, an increase in a desaturation index (Table 3) suggested a reduction in activity of a corresponding desaturase enzyme. Increases in 16:0 and 18:0 desaturation indices for muscle (male and female) and femur (males only) were observed for mice fed *t*10,*c*12-CLA, indicating a corresponding reduction in SCD activity as previously reported for mouse mammary tissue (18) and pre-adipocytes (13). In contrast, reductions in 18:0 and *trans*-11-18:1 desaturation indices for liver of male and female mice were observed in the present study, and suggested enhanced hepatic SCD activity. Expression of SCD mRNA in the liver of male and female mice in the present study, however, was not altered by dietary *t*10,*c*12-

CLA. Hepatic SCD mRNA in lactating mice also was not altered by dietary *t*10,*c*12-CLA, but SCD activity was reduced (18). The *t*10,*c*12 CLA isomer most likely altered hepatic SCD activity by post-translational mechanisms rather than gene transcription (17).

The hepatic 18:2n-6 desaturation index was not altered by dietary treatments in this study, but the index was reduced in muscle (males and females) and femur (males only). The proportion of 20:4n-6, the product of 18:2n-6 desaturation and elongation, in bone and muscle of male and female mice was increased (data not shown). An increase in activity of Δ5 or Δ6 desaturase in muscle and femur may be a metabolic response to compensate for the apparent decrease in Δ9 desaturase activity noted above. In contrast, dietary *t*10,*c*12-CLA caused an increase in the hepatic 18:3n-3 desaturation index of male mice, suggesting inhibition of Δ5 or Δ6 desaturation. There was a reduction in Δ6 desaturation in Hep2G cells in response to *t*10,*c*12-CLA when the ratio 20:3n-6 to18:2n-6 was used as an index (34). Likewise, the ratio of (18:3n-6 + 20:3n-6) to 18:2n-6 in plasma phospholipids suggested potential inhibition of Δ6 desaturation in response to daily consumption of purified *t*10,*c*12-CLA by overweight men and women (9). In addition, hepatic Δ6 desaturase activity was reduced by *c*9,*t*11-CLA and *t*10,*c*12-CLA added to microsomal incubations (35). Thus, alterations in 18:2n-6 or 18:3n-3 desaturation indices in this study may have resulted primarily from changes in activity of Δ6 desaturases.

Our results indicated gender accounted for minor differences in the capacity of adipose tissue, via FAS and SCD, to respond to the inhibitory actions of dietary *t*10,*c*12-CLA on lipid synthesis and storage. However, results also indicated that evaluations of lower concentrations of dietary *t*10,*c*12-CLA are needed to establish the appropriate amount of this CLA isomer required for acceptable long-term reductions in body fat content with minimal negative effects on liver lipid content and essential FA desaturation in muscle, bone, and other tissues. For example, conjugated EPA (36) apparently has fewer of the undesirable side effects associated with *t*10,*c*12-CLA. Thus, both conjugated isomers could be compared at similar dietary concentrations in future studies to determine their desirable and undesirable dose-dependent characteristics in male and female animals.

ACKNOWLEDGMENTS

This work was supported by a fellowship and research funds from the John Lee Pratt Animal Nutrition Program. We thank Dr. Hitoshi Shimano (Department of Internal Medicine, University of Tsukuba, Japan), Dr. James Ntambi (Department of Biochemistry, University of Wisconsin—Madison), and Dr. William Huckle (Department of Biomedical Sciences and Pathobiology, Virginia Tech) for the plasmids containing FAS, SCD, and actin cDNA respectively.

REFERENCES

1. Belury, M.A. (2002) Dietary Conjugated Linoleic Acid in Health: Physiological Effects and Mechanisms of Action, *Annu. Rev. Nutr. 22*, 505–531.
2. Ostrowska, E., Muralitharan, M., Cross, R.F., Bauman, D.E., and Dunshea, F.R. (1999) Dietary Conjugated Linoleic Acids Increase Lean Tissue and Decrease Fat Deposition in Growing Pigs, *J. Nutr. 129*, 2037–2042.
3. Park, Y., Albright, K.J., Liu, W., Storkson, J.M., Cook, M.E., and Pariza, M.W. (1997) Effect of Conjugated Linoleic Acid on Body Composition in Mice, *Lipids 32*, 853–858.
4. Sisk, M.B., Hausman, D.B., Martin, R.J., and Azain, M.J. (2001) Dietary Conjugated Linoleic Acid Reduces Adiposity in Lean but Not Obese Zucker Rats, *J. Nutr. 131*, 1668–1674.
5. Macarulla, M.T., Fernandez-Quintela, A., Zabala, A., Navarro, V., Echevarria, E., Churruca, I., Rodriguez, V.M., and Portillo, M.P. (2005) Effects of Conjugated Linoleic Acid on Liver Composition and Fatty Acid Oxidation Are Isomer-Dependent in the Hamster, *Nutrition 21*, 512–519.
6. Shang, X.G., Wang, F.L., Li, D.F., Yin, J.D., Li, X.J., and Yi, G.F. (2005) Effect of Dietary Conjugated Linoleic Acid on the Fatty Acid Composition of Egg Yolk, Plasma and Liver as Well as Stearoyl-Coenzyme A Desaturase Activity and Gene Expression in Laying Hens, *Poultry Sci. 84*, 1886–1892.
7. Park, Y., Storkson, J.M., Albright, K.J., Liu, W., and Pariza, M.W. (1999) Evidence That the *trans*-10,*cis*-12 Isomer of Conjugated Linoleic Acid Induces Body Composition Changes in Mice, *Lipids 34*, 235–241.
8. Smedman, A., and Vessby, B. (2001) Conjugated Linoleic Acid Supplementation in Humans—Metabolic Effects, *Lipids 36*, 773–781.
9. Thijssen, M.A., Malpuech-Brugere, C., Gregoire, S., Chardigny, J.M., Sebedio, J.L., and Mensink, R.P. (2005) Effects of Specific CLA Isomers on Plasma Fatty Acid Profile and Expression of Desaturases in Humans, *Lipids 40*, 137–145.
10. Hayman, A., MacGibbon, A., Pack, R.J., Rutherfurd, K., and Green, J.H. (2002) High Intake, but Not Low Intake, of CLA Impairs Weight Gain in Growing Mice, *Lipids 37*, 689–692.
11. Hubbard, N.E., Lim, D., and Erickson, K.L. (2006) Beef Tallow Increases the Potency of Conjugated Linoleic Acid in the Reduction of Mouse Mammary Tumor Metastasis, *J. Nutr. 136*, 88–93.
12. Yanagita, T., Wang, Y.-M., Nagao, K., Ujino, Y., and Inoue, N. (2005) Conjugated Linoleic Acid–Induced Fatty Liver Can Be Attenuated by Combination with Docosahexaenoic Acid in C57BL/6N Mice, *J. Agric. Food Chem. 53*, 9629–9633.
13. Granlund, L., Pederson, J.I., and Nebb, H.I. (2005) Impaired Lipid Accumulation by *trans*-10,*cis*-12 CLA During Adipocyte Differentiation Is Dependent on Timing and Length of Treatment, *Biochim. Biophys. Acta 1687*, 11–22.
14. Smith, S.B., Hively, T.S., Cortese, G.M., Han, J.J., Chung, K.Y., Castenada, P., Gilbert, C.D., Adams, V.L., and Mersmann, H.J. (2002) Conjugated Linoleic Acid Depresses the D9 Desaturase Index and Stearoyl Coenzyme A Desaturase Enzyme Activity in Porcine Subcutaneous Adipose Tissue, *J. Anim. Sci. 80*, 2110–2115.
15. Loor, J.J., Lin, X., and Herbein, J.H. (2002) Dietary *trans*-Vaccenic Acid (*trans*-11-18:1) Increases Concentration of *cis*-9,*trans*-11-Conjugated Linoleic Acid (Rumenic Acid) in Tissues of Lactating Mice and Suckling Pups, *Reprod. Nutr. Dev. 42*, 85–99.
16. Lee, K.N., Pariza, M.W., and Ntambi, J.M. (1998) Conjugated Linoleic Acid Decreases Hepatic Stearoyl-CoA Desaturase mRNA Expression, *Biochem. Biophys. Res. Commun. 248*, 817–821.
17. Choi, Y., Park, P., Pariza, N.M., and Ntambi, J.M. (2001) Regulation of Stearoyl-CoA Desaturase Activity by the *trans*-10,*cis*-

12 Isomer of Conjugated Linoleic Acid in HepG2 Cells, *Biochem. Biophys. Res. Commun. 284*, 689–693.
18. Lin, X., Loor, J.J., and Herbein, J.H. (2004) *trans*-10,*cis*-12-18:2 Is a More Potent Inhibitor of *de novo* Fatty Acid Synthesis and Desaturation Than *cis*-9,*trans*-11-18:2 in the Mammary Gland of Lactating Mice, *J. Nutr. 134*, 1362–1368.
19. Kelley, D.S., Bartolini, G.L., Warren, J.M., Simon, V.A., Mackey, B.E., and Erickson, K.L. (2004) Contrasting Effects of *t*10,*c*12- and *c*9,*t*11-Conjugated Linoleic Acid Isomers on Fatty Acid Profiles of Mouse Liver Lipids, *Lipids 39*, 135–141.
20. Helrich, K. (1997) *Official Methods of Analysis*, 15th edn., pp. 931 and 935, Association of Official Analytical Chemists, Inc., Arlington, VA.
21. Folch, J., Lees, M., and Sloane Stanley, G.A. (1957) A Simple Method for Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem. 226*, 497–502.
22. Park, P.W., and Goins, R.E. (1994) *In situ* Preparation of Fatty Acid Methyl Esters for Analysis of Fatty Acid Composition in Foods, *J. Food Sci. 59*, 1262–1266.
23. Loor, J.J., Lin, X., and Herbein, J.H. (2003) Effects of Dietary *cis*-9,*trans*-11-18:2, *trans*-10,*cis*-12-18:2, or Vaccenic Acid (*trans*-11-18:1) During Lactation on Body Composition, Tissue Fatty Acid Profiles, and Litter Growth in Mice, *Br. J. Nutr. 90*, 1039–1048.
24. DeLany, J.P., Blohm, F., Truett, A.A., Scimeca, J.A., and West, D.B. (1999) Conjugated Linoleic Acid Rapidly Reduces Body Fat Content in Mice Without Affecting Energy Intake, *Am. J. Physiol. 276*, R1172–R1179.
25. Hargrave, K.M., Li, C., Meyer, B.J., Kachman, S.D., Hartzell, D.L., Della-Fera, M.A., Miner, J.L., and Baile, C.A. (2002) Adipose Depletion and Apoptosis Induced by *trans*-10,*cis*-12 Conjugated Linoleic Acid in Mice, *Obes. Res. 10*, 1284–1290.
26. Takahashi, Y., Kushiro, M., Shinohara, K., and Ide, T. (2003) Activity and mRNA Levels of Enzymes Involved in Hepatic Fatty Acid Synthesis and Oxidation in Mice Fed Conjugated Linoleic Acid, *Biochim. Biophys. Acta 1631*, 265–273.
27. Tsuboyama-Kasaoka, N., Takahashi, M., Tanemura, K., Kim, H.J., Tange, T., Okuyama, H., Kasai, M., Ikemoto, S., and Ezaki, O. (2000) Conjugated Linoleic Acid Supplementation Reduces Adipose Tissue by Apoptosis and Develops Lipodystrophy in Mice, *Diabetes 49*, 1534–1542.
28. Clement, L., Poirier, H., Niot, I., Bocher, V., Guerre-Millo, M., Krief, S., Staels, B., and Besnard, P. (2002) Dietary *trans*-10,*cis*-12 Conjugated Linoleic Acid Induces Hyperinsulinemia and Fatty Liver in the Mouse, *J. Lipid Res. 43*, 1400–1409.
29. Chardigny, J.M., Hasselwander, O., Genty, M., Kraemer, Ptock, A., and Sébédio, J.L. (2003) Effect of Conjugated FA on Feed Intake, Body Composition, and Liver FA in Mice, *Lipids 38*, 895–902.
30. Watkins, B.A., and Seifert, M.F. (2000) Conjugated Linoleic Acid and Bone Biology, *J. Am. Coll. Nutr. 19*, 478S–486S.
31. Yoshida, K., Oida, H., Kobayashi, T., Maruyama, T., Tanaka, M., Katayama, T., Yamaguchi, K., Segi, E., Tsuboyama, T., Matsushita, M., Ito, K., Ito, Y., Sugimoto, Y., Ushikubi, F., Ohuchida, S., Kondo, K., Nakamura, T., and Narumiya, S. (2002) Stimulation of Bone Formation and Prevention of Bone Loss by Prostaglandin E EP4 Receptor Activation, *Proc. Natl. Acad. Sci. 99*, 4580–4585.
32. Ntambi, J.M., Miyazaki, M., Stoehr, J.P., Lan, H., Kendziorski, C.M., Yandell, B.S., Song, Y., Cohen, P., Friedman, J.M., and Attie, A.D. (2002) Loss of Stearoyl-CoA Desaturase-1 Function Protects Mice Against Adiposity, *Proc. Natl. Acad. Sci. 99*, 11482–11486.
33. Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V., and Bauman, D.E. (2000) Conjugated Linoleic Acid Is Synthesized Endogenously in Lactating Dairy Cows by Delta(9)-Desaturase, *J. Nutr. 130*, 2285–2291.
34. Eder, K., Slomma, N., and Becker, K. (2002) *trans*-10,*cis*-12 Conjugated Linoleic Acid Suppresses the Desaturation of Linoleic and alpha-Linolenic Acids in HepG2 Cells, *J. Nutr. 132*, 1115–1121.
35. Bretillon, L., Chardigny, J.M., Grégoire, S., Berdeaux, O., and Sébédio, J.L. (1999) Effects of Conjugated Linoleic Acid Isomers on Hepatic Microsomal Desaturation Activities *in vitro, Lipids 34*, 965–969.
36. Tsuzuki, T., Kawakami, Y., Suzuki, Y., Abe, R., Nakagawa, K., and Miyazawa, T. (2005) Intake of Conjugated Eicosapentaenoic Acid Suppresses Lipid Accumulation in Liver and Epididymal Adipose Tissue in Rats, *Lipids 40*, 1117–1123.

[Received March 23, 2006; accepted August 17, 2006]