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Fatty acid composition of liver, adipose tissue, spleen, and heart of mice fed diets containing *t*10, *c*12-, and *c*9, *t*11-conjugated linoleic acid

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Abstract

Conjugated linoleic acid (CLA) isomers have unique effects on tissue lipids. Here we investigated the influence of individual CLA isomers on the lipid weight and fatty acid composition of lipid metabolizing (i.e. liver and retroperitoneal adipose) and lipid sensitive (i.e. spleen and heart) tissues. Female mice (8 week old; $n = 6$/group) were fed either a control or one of the two CLA isomer supplemented (0.5%) diets for 8 weeks. The *cis*-9, *trans*-11-CLA diet reduced the 18:1n-9 wt% by 20–50% in liver, adipose tissue, and spleen, reduced the spleen n-3 polyunsaturated fatty acid (PUFA) by 90%, and increased the n-6 PUFA wt% by 20–50% in all tissues except heart. The *trans*-10, *cis*-12-CLA reduced both the n-6 and n-3 PUFA wt% in liver (>50%), reduced the heart n-3 PUFA wt% by 25%, and increased the wt% of spleen n-3 PUFA by 700%. The functional consequences of such changes in tissue fatty acid composition need to be investigated.

1. Introduction

Conjugated linoleic acid (CLA) is the collective term for a group of linoleic acid isomers having conjugated double bonds. Several isomers of CLA have been reported with unique double-bond position and geometry [1]. Most studies exploring the biological consequences of CLA feeding have used isomeric mixtures comprised of the two major forms, *cis*-9, *trans*-11-CLA (*c*9, *t*11-CLA) and *trans*-10, *cis*-12-CLA (*t*10, *c*12-CLA), and a number of minor isomers. The major dietary sources of *c*9, *t*11-CLA are dairy products and ruminant meat, while that of *t*10, *c*12-CLA are partially hydrogenated vegetable oils from margarines and shortenings [2]. Feeding CLA isomer mixtures has been reported to alter tissue fatty acid profiles in various animal models including rats [3], mice [4,5], hamsters [6], fish [7], chickens [8,9], and pigs [10–12]. However, a few studies have examined the effects of individual CLA isomers on tissue fatty acid profiles [13–18].

From the studies feeding individual CLA isomers, it can be seen that *c*9, *t*11-CLA and *t*10, *c*12-CLA produced different effects on the fatty acid profiles of rat liver and adipose tissue [13,18], mouse liver [15,16], mouse carcass, mammary gland, and milk [17], as well as bovine milk fat [14]. However, the effects of these CLA isomers on the fatty acid profiles of other mouse tissues have not been examined. Therefore, to enhance our understanding of these CLA isomer effects, we investigated lipid content, fatty acid profiles, and CLA isomer incorporation in the liver, retroperitoneal (RP) adipose tissue, spleen, and heart of mice fed either CLA-free diets (Control), or diets containing highly enriched *c*9,

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*t*11-CLA or *t*10, *c*12-CLA. Liver and adipose tissue were selected for their major role in fatty acid metabolism, while the spleen and heart were selected because fatty acids significantly modulate their functions. Our results show that the two CLA isomers differed in their incorporation into tissue lipids and in their effects on tissue fatty acid composition.

2. Materials and methods

2.1. CLA isomers and diets

The *c*9, *t*11-CLA, and *t*10, *c*12-CLA isomer free acids (85–88% pure) were a kind gift from Natural ASA, Hovdebygda, Norway. The concentration of CLA used in this study was 0.5 wt% of the diet, which is comparable to the concentration range of 0.1–1.5 wt% of a CLA isomer mixture used in previous studies with rodent models. The basal diet was AIN-93G high carbohydrate mouse diet. The nutrient and fatty acid composition of this diet has been previously reported [19,20]. The diet used in this study was low in 18:3n-3, with an 18:2n-6:18:3n-3 ratio of 25:1, as compared to a 7:1 ratio for the AIN-93 diet prepared with soybean oil. However, since *t*10, *c*12-CLA caused the development of fatty livers and the reduction in adipose tissue weights in mice fed diets based on either soybean oil [21] or sunflower plus linseed oil-based diets [22], the CLA isomer effects do not appear linked to a low 18:3n-3 intake. All animal protocols were approved by the Animal Use Committee at the University of California, Davis.

2.2. Animals, feeding, and tissue collection

Eighteen, 8-week-old, pathogen-free C57BL/6N female mice were purchased from Charles River (Raleigh, NC). They were maintained in a sterile air curtain isolator at the animal facility of the University of California, Medical School with controlled temperature (25 °C) and light and dark cycle (12 h each). They were fed the laboratory Chow diet for first 7 days and experimental diets for the last 56 days. Animals were divided into three groups at the start of experimental diets (study day 1), 6 per group. Details regarding animal handling, sacrifice, tissue collection and storage have been published [16,19,20].

2.3. Lipid extraction and fatty acid analysis

Livers, RP adipose tissue, spleens and hearts were removed, blotted dry with tissue paper, weighed, placed in liquid nitrogen, and stored frozen at −80 °C until processed. Extraction of lipids and fatty acid analysis was performed according to the previously published methods from our laboratory [23], with the exception that mass spectral, rather than flame ionization detection was used to record fatty acid responses. Isolated fatty acid methyl esters were analyzed on an Agilent 6890 gas chromatograph equipped with a 100 m × 0.25 mm × 0.2 μm film SP-2380 (Sigma-Aldrich; St. Louis, MO) and interfaced with an Agilent 5973N Mass Spectral Detector with electron impact ionization scanning from m/z 50–436, All data analysis and quantification was performed using Agilent Chemstation Software. Methylated fatty acid standards were used to calibrate instrumental retention times and responses. These along with the acquired full scan mass spectra were used to identify and quantify sample fatty acids. All fatty acid results are shown as the means ± SEM ($n = 6$) for the weight percent (wt%) of individual fatty acids. Fatty acids with concentrations of less than 0.5 wt%, were considered minor, and are not shown unless they were CLA isomers or other fatty acids whose concentration exceeded 0.5 wt% in at least one tissue.

2.4. Statistical analysis

The SAS proc glm was used for a one-way analysis of variance between treatments and for Levene's test for heterogeneity of variance. Dunnett's test was used to compare the two treatment means with the control. When there was evidence of heterogeneity of variance, the SAS proc mixed was used to incorporate the heterogeneity in the model. When the control data is entirely zero, t tests were used to test for treatment means significantly different from zero [24]. Treatment effects with $P < 0.05$ are considered significant.

3. Results

3.1. Effect of CLA isomers on tissue wet and lipid weights

The *c*9, *t*11-CLA diet did not alter either the tissue wet or lipid weights for the four tissues examined when compared to the controls (Table 1). In contrast, while *t*10, *c*12-CLA feeding did not alter the wet or lipid weights of the spleen or heart, this isomer influenced the liver and RP adipose tissue (Table 1). The *t*10, *c*12-CLA diet increased the liver wet weight and concentration of lipid by 2-folds, while increasing the total tissue lipid mass 4-fold. The *t*10, *c*12-CLA diet also caused a 50% reduction in RP adipose tissue weight and 75% reduction in its total lipid content. Thus, neither isomer affected the total lipid content of the spleen and heart, while the *t*10, *c*12-CLA altered the lipid contents of liver and adipose tissue.

Table 1
Effect of CLA isomers on tissue weight and lipid content

Group	Control	*c*9,*t*11-CLA	*t*10,*c*12-CLA
Liver			
Wet weight (g)	1.28±0.030	1.46±0.060	2.54±0.070*
Lipid weight (mg)	107±13	95.2±9.7	430±39*
Lipid (mg/g)	79.5±8.6	65.1±5.0	171±8.2*
Retroperitoneal adipose tissue			
Wet weight (g)	0.49±0.04	0.48±0.05	0.20±0.02*
Lipid weight (mg)	224±29	253±35	63.0±10*
Lipid (mg/g)	423±15	521±46	360±21
Spleen			
Wet weight (g)	0.10±0.01	0.12±0.02	0.12±0.01
Lipid weight (mg)	4.4±0.2	5.5±0.4	5.0±0.3
Lipid (mg/g)	41.8±1.4	47.6±2.4	44.5±3.3
Heart			
Wet weight (g)	0.17±0.03	0.17±0.03	0.16±0.02
Lipid weight (mg)	8.8±1.3	8.3±0.7	8.6±0.6
Lipid (mg/g)	52.0±4.9	53.1±3.0	51.4±2.9

Data shown are mean±SEM ($n = 6$). Numbers with an * are significantly different ($P < 0.05$) from control.

3.2. CLA isomers incorporation into tissue lipids

The mean wt% of *c*9, *t*11-CLA in RP adipose tissue, liver, and heart lipids were 3.9, 1.0, and 0.6, respectively; corresponding values for *t*10, *c*12-CLA were 1.2, 0.2, and 0.4, respectively (Table 2). Incorporation of both isomers into spleen lipids was below the detection limit. The wt% of *c*9, *t*11-CLA in liver and adipose tissue lipids was approximately 4-fold greater than those of the corresponding concentrations of the *t*10, *c*12-CLA.

3.3. Effect of CLA isomers on tissue fatty acid composition

As expected, each control tissue showed unique fatty acid compositions (Fig. 1 and Table 2). The liver and adipose lipid composition was rather similar. Relative to these tissues, the spleen was enriched in saturated fatty acids (SFAs) and low in monounsaturated fatty acids (MUFAs), while the heart was greatly enriched in polyunsaturated fatty acids (PUFAs) at the expense of MUFAs.

As shown in Section 3.1 above, neither CLA isomer changed the total lipid content of the spleen and heart (Table 1); however, they both altered the fatty acid wt% in all examined tissues (Fig. 1 and Table 2). As seen in Fig. 1, the *c*9, *t*11-CLA diet caused a reduction in the wt% of MUFAs in all tissues except the heart, showing 16–50% reductions in the wt% of 18:1n-9 and a corresponding increase in the wt% of total n-6 PUFA in lipids from all tissues except heart. This increase in n-6 PUFA was due to a 1.2-fold increase in the wt% of 18:2n-6 in liver (from 12 to 15 wt%) and RP adipose tissue (from 19 to 23 wt%), and a 1.7-fold increase (from 16 to 27 wt%) in 20:4n-6 in spleen lipids (Table 2). The lipids of the spleen also showed the only effect on n3-PUFAs by the *c*9, *t*11-CLA diet, a reduction in DHA from 2.8 to 0.3 wt%. Representative chromatograms of spleen lipids are shown in Fig. 2 to highlighted peak identifications.

While the relative abundance of the total SFAs were not altered by either CLA isomers in any tissue (Fig. 1), small yet significant changes in SFA composition were observed (Table 2). Most notably, the *t*10, *c*12-CLA diet reduced the 18:0 contribution to the total SFA by 10, 9, and 5% in the liver, spleen, and adipose tissue, respectively with a concomitant increase in 16:0. Moreover, the *t*10, *c*12-CLA diet increased the wt% of MUFAs in the liver and heart and decreased them in spleen, but did not change their wt% in adipose tissue (Fig. 1). From Table 2, it can be clearly seen that these changes are primarily being driven by shifts in 18:1n-9.

Similar to the *c*9, *t*11-CLA diet, feeding the *t*10, *c*12-CLA isomer had dramatic, yet distinct effects on tissue PUFA compositions. The *t*10, *c*12-CLA diet decreased the wt% of n-6 PUFAs in the liver and spleen, but not the heart or RP adipose tissue (Fig. 1). In the liver, the reduction in n6-PUFA wt% was due to a 50% reduction in 18:2n-6 and a 66% reduction in 20:4n-6. No changes in the wt% of 18:2n-6 was observed in the other three tissues, and the 20:4n-6 wt% was also unchanged in the RP adipose tissue and heart lipids. In the spleen, however, 20:4n-6 was reduced from 16.4 to 0.6 wt% (Table 2). The 22:5n-6 comprised less than 1 wt% of the isolated lipids in all tissues except the heart, where the control level of 9.3 wt% was reduced to 6.0 wt% in the *t*10, *c*12-CLA group.

With respect to the n3-PUFAs, the *t*10, *c*12-CLA diet reduced the wt% of these fatty acids in both the liver and heart, while increasing them in the spleen (Figs. 1 and 2). The n-3 PUFA were below the method detection limit in the RP adipose tissue. Changes in liver, heart, and spleen n-3 PUFA were largely due to changes in the concentration of DHA, which decreased in liver (1.2–0.4 wt%) and heart (13.1–9.7 wt%) and increased in spleen (2.8–21.0 wt%). The wt% of EPA increased from undetectable to 2.8% in the spleen in the *t*10, *c*12-CLA fed group (Table 2).

Therefore, in terms of alterations of fatty acid wt%, the *c*9, *t*10-CLA diet affected the spleen $\gg$ liver $\approx$ RP adipose while the heart changed the least. In contrast the *t*10, *c*12-CLA diet affected the spleen > liver $\gg$ heart and the RP adipose was minimally changed. Moreover changes produced by the *t*10, *c*12-CLA diet were generally much larger than those produced by the *c*9, *t*11-CLA diet in all tissues.

Table 2
Effect of dietary CLA isomers on wt% fatty acid composition of mouse tissues

FAME	Liver			RP adipose tissue			Spleen			Heart		
	Control	*c*9, *t*11	*t*10, *c*12	Control	*c*9, *t*11	*t*10, *c*12	Control	*c*9, *t*11	*t*10, *c*12	Control	*c*9, *t*11	*t*10, *c*12
Saturated fatty acids												
14:0	0.3±0.1	0.3±0.1	0.4±0.1	1.5±0.1	1.8±0.0*	1.4±0.1	1.2±0.2	1.1±0.1	2.8±0.3*	1.0±0.2	0.9±0.2	1.2±0.3
16:0	23.9±0.6	25.4±1.3	25.3±0.6	21.7±0.2	21.9±0.5	23.1±0.3*	26.4±1.0	26.5±0.7	29.1±0.6*	13.4±1.5	14.1±0.6	14.3±0.9
18:0	7.6±0.5	9.5±0.5	4.3±0.3*	4.2±0.2	2.5±0.3*	2.8±0.3*	15.4±0.6	16.4±1.1	11.6±0.6*	17.5±0.5	17.1±0.3	16.5±0.5
Monounsaturated fatty acids												
16:1n-9	0.8	0.6±0.2	1.7±0.1*	0.6±0	0.6±0.0	1.1±0.0*	0.4±0.1	0.2±0.0	0.4±0.1	0.2±0.0	0.2±0.0	0.4±0.1
16:1n-7	3.0±0.2	1.7±0.3 *	2.5±0.1	7.0±0.2	7.4±0.5	3.5±0.1*	1.7±0.3	1.8±0.4	1.8±0.1	0.6±0.1	0.6±0.2	0.5±0.2
18:1n-9	38.7±1.6	34.1±1.3*	48.8±0.8*	39.2±0.8	32.8±0.8*	40.7±0.7	21.4±1.8	10.5±2.4*	17.0±2.0*	10.2±0.7	9.6±0.6	13.9±1.3*
18:1n-7	4.1±0.3	3.3±0.3*	5.0±0.1*	2.7±0.5	2.1±0.1	3.0±0.1	2.8±0.1	1.3±0.1*	1.8±0.1*	2.4±0.2	2.0±0.1*	2.6±0.1
Polyunsaturated fatty acids												
18:2n-6	12.0±0.9	14.7±1.5*	6.4±0.2*	19.1±0.5	22.7±0.6*	18.2±0.3	4.4±0.2	2.6±0.4*	5.6±0.2	16.0±0.9	17.5±0.7	16.9±0.6
*c*9, *t*11-CLA	ND	1.0±0.1*	0.1±0.0	ND	3.9±0.1*	0.2±0.0	ND	ND	ND	ND	0.6±0.1*	ND
*t*10, *c*12-CLA	ND	0.1±0.0*	0.2±0.0*	ND	0.3±0.0*	1.2±0.0*	ND	ND	ND	ND	ND	0.4±0.1*
20:3n-6	0.4±0.1	0.4±0.1	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.6±0.1	0.9±0.1*	0.2±0.0*	0.5±0.1	0.6±0.1	0.6±0.0
20:4n-6	6.2±0.4	6.2±0.5	2.1±0.2*	0.6±0.1	0.5±0.1	0.7±0.1	16.4±1.0	27.3±0.8*	0.6±0.1*	10.2±0.6	9.9±0.1	11.3±0.2
22:4n-6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	2.1±0.2	6.3±0.3*	ND	0.9±0.1	1.0±0.1	0.9±0.1
22:5n-6	0.6±0.1	0.5±0.1	0.3±0.1	0.1±0.0	0.1±0.0	0.1±0.0	0.5±0.0	0.5±0.1	ND	9.3±0.6	8.4±0.5	6.0±0.8*
20:5n-3	ND	ND	ND	ND	ND	ND	ND	ND	2.8±0.3*	ND	ND	ND
22:5n-3	ND	ND	ND	ND	ND	ND	ND	ND	0.5±0.0	0.2±0.0	0.3±0.0	0.1±0.0
22:6n-3	1.2±0.1	1.1±0.1	0.4±0.0*	ND	ND	ND	2.8±0.3	0.3±0.0*	21.0±1.4*	13.1±0.9	13.2±0.4	9.7±0.5*
Minor/unknown	1.1	1	2.2	3	3.1	3.8	3.9	4.3	4.8	4.5	4	4.7
Ratios												
n-3/n-6	0.06±0.0	0.05±0.0	0.04±0.0	—	—	—	0.12±0.0	0.01±0.0*	3.80±0.2*	0.36±0.04	0.36±0.02	0.27±0.04*
MUFA:SFA	1.47±0.1	1.13±0.1*	1.93±0.1*	1.81±0.1	1.65±0.1	1.77±0.1	0.61±0.1	0.31±0.07*	0.48±0.06	0.42±0.02	0.39±0.02	0.54±0.05*

Data are mean±SEM ($n = 6$). Numbers with *are significantly different ($P<0.05$) from corresponding numbers in control group, FAME = fatty acid methyl esters; ND = not detected; RP = retroperitoneal; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. The ratio between n-3 and n-6 PUFA for adipose tissues is not calculated because the concentration of n-3PUFA was below the detection limit. Minor fatty acids include those with concentrations less than 0.5 wt% in all tissues.

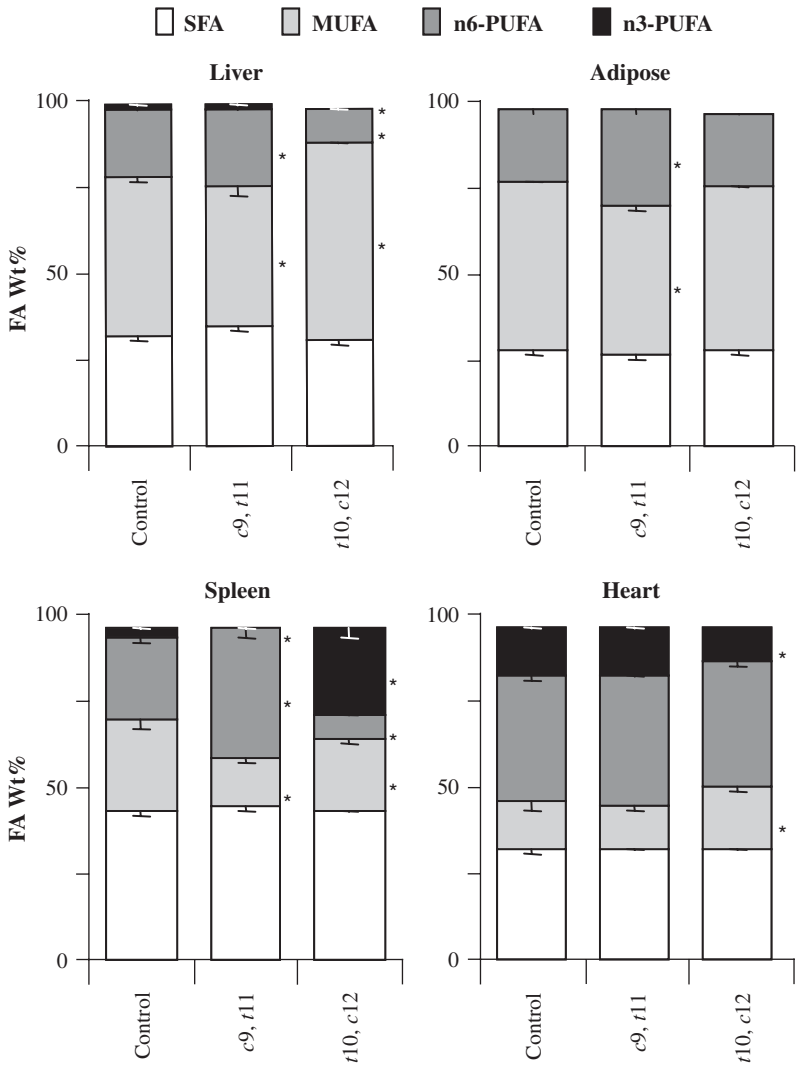

Fig. 1. Tissue fatty acid class composition is differentially altered by CLA isomers. Total saturated fatty acids (SFA) were unaffected in all tissues. The *c*9, *t*11-CLA diet reduced total monounsaturated fatty acids (MUFAs) in liver, adipose, and spleen and led to corresponding increases in total polyunsaturated fatty acids (PUFAs). Conversely, feeding a *t*10, *c*12-CLA enriched diet produced more variable tissue responses: RP adipose tissue no effect; liver increased MUFAs and decreased n-6 PUFAs; spleen increased MUFA and n3-PUFA, and decreased n6-PUFA; heart increased MUFA and decreased n-3 PUFAs. An astrix (*) indicates group differences from control means ($P<0.05$).

4. Discussion

It has become evident that the primary isomers of CLA (i.e. *c*9, *t*11- and *t*10, *c*12-CLA) offer another model system of structurally similar lipids which produce distinct effects on tissue lipid metabolism. In this study we have added to the characterization of these differences by investigating the effects of diets supplemented with *c*9, *t*11- or *t*10, *c*12-CLA isomers on the weight, lipid content and fatty acid composition of mouse liver, RP adipose tissue, spleen, and heart.

Our findings generally fell into two areas: (1) changes in lipid mass, (2) changes in lipid composition. Since lipid masses were only determined for the gross lipid extract, the discussion of alterations in lipid metabolism in tissues with altered lipid mass are limited. However, changes in tissue masses were only observed for the liver and RP adipose tissues of the *t*10, *c*12-CLA fed group and our results regarding the effects of purified CLA isomers on liver and adipose tissue lipids are consistent with those previously reported by us and others [15–17]. Moreover, this study extended the previous findings by showing that neither of the isomers altered the lipid content of heart and spleen, and both isomers caused specific changes in the fatty acid composition of three additional tissue lipids (RP adipose tissue, spleen, and heart).

The administered CLA isomers were incorporated into all tissues, except the spleen, with a maximum wt% of 3.9% for *c*9, *t*11-CLA appearing in the RP adipose tissue. The higher relative abundance of *c*9, *t*11-CLA in tissues, as compared with *t*10, *c*12-CLA fed at the same

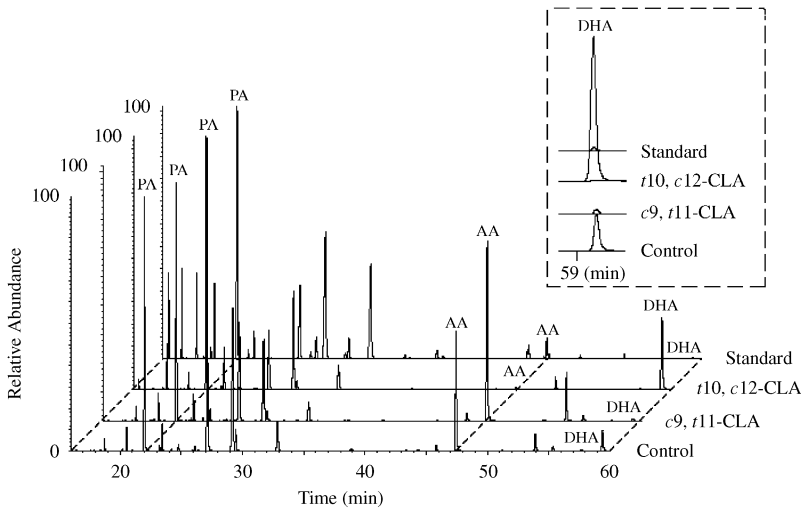

Fig. 2. Spleen PUFA profiles were differentially altered by dietary CLA isomers. This figure shows representative chromatograms from the analysis of spleen tissue (~35 mg) from control, *c*9, *t*11-CLA, *t*10,*c*12-CLA fed groups superimposed with a reference standard 87 (each with a 5 min time offset). As seen in this figure, palmitic acid (PA) was unchanged while arachidonic acid (AA) and docosahexenoic acid (DHA) were influenced by CLA feeding. In particular *c*9, *t*11-CLA increased AA and decreased DHA amounts, while *t*10, *c*12-CLA decreased AA and increased DHA amounts. The inset shows the superposition of the DHA peaks without time offset. Signal to noise calculation for the peaks were 190:1, 40:1, and 750:1 for the control, *c*9,*t*11-CLA, *t*10, *c*12-CLA fed groups, respectively. The mass spectra of the peak co-eluting with DHA in the spleens of *t*10, *c*12-CLA fed animals match the published DHA spectra (data not shown).

dose, may be due to either increased absorption of *c*9, *t*11-CLA or increased degradation of *t*10, *c*12-CLA. The present study cannot discriminate among these possibilities.

While traces of CLA isomers are likely to be present in the spleen, we were unable to detect them even by lowering the threshold for peak detection to 0.05% of the total peak area, and close visual inspection indicated no perturbation of the chromatographic baseline at retention times corresponding to these peaks. We have previously reported that for liver lipids, concentration of *c*9, *t*11-CLA in cholesteryl esters, triglycerides, and phospholipids was 3.1, 1.3, and 0.1%, respectively [16]. Failure to detect CLA in spleen lipids may be due to higher proportion of phospholipids in spleen lipids. In addition, since spleens were not perfused before lipid extraction, the RBC lipids may have also contributed significantly to the lipids in these extracts. In a previous human study, we found that CLA isomers were not detectable in RBC lipids when healthy women supplemented their diets with a CLA mixture (3.8 g/d) for 63 days (Nelson et al., unpublished). Even after 24 weeks of supplementing CLA to healthy men, incorporation of *c*9, *t*11-CLA and *t*10, *c*12-CLA into RBC lipids was only 0.31 and 0.19 wt% [25].

In animals fed with the control diet, concentrations of several fatty acids differed among the tissues studied (Table 2). Liver and RP adipose fatty acid profiles were quite similar with the exception of 20:4n-6 and 22:6n-3, which appeared only at trace levels in the adipose tissues. Spleen and heart lipids contained even higher concentrations of long chain PUFA as compared with those in liver lipids. Previous studies have shown that the fatty acid composition of the tissues varied with the concentrations of n-3 and n-6 PUFA in the diet [26–34]. In animals fed the control diet, tissue fatty acid profiles are generally comparable to those previously reported for mice fed moderate amount of n-6 PUFA (Table 2) and [26,27,31,34].

Changes in the liver fatty acid profiles caused by both *c*9, *t*11- and *t*10, *c*12-CLA are consistent with those we previously reported for mouse liver [16]. Most noticeable effects of *c*9, *t*11-CLA was a reduction in wt% 18:1n-9 in liver, adipose tissue, and spleen. It also increased the wt% of n-6 PUFA (20:4n-6 and 22:4n-6) and decreased n-3:n-6 PUFA (0.01 vs. 0.12 in control) in spleen lipids. This isomer had only marginal effects on the fatty acid profile of heart lipids.

Feeding of *t*10, *c*12-CLA decreased the wt% of both 18:2n-6 and 20:4n-6 in liver and that of 20:4n-6 in spleen, while it did not alter these fatty acids in the RP adipose tissue or heart lipids. In contrast to *c*9, *t*11-CLA, *t*10, *c*12-CLA increased the wt% of 18:1n-9 in liver and heart lipids, but did not significantly alter the wt% of this fatty acid in the other two tissues. Failure of both CLA isomers to alter the wt% of 20:4n-6 in heart lipids in our study is consistent with the conservation of heart 20:4n-6 in mice fed an essential fatty acid deficient diet [32]. In control mice, the wt% of 22:5n-6 and 22:6n-3 were less than 1 in all tissues except the heart, where their concentrations were 9.3 and 13.1 wt% respectively. However, in the *t*10, *c*12-CLA fed group, these long

chain PUFAs were reduced 25–30% in the heart. This isomer also reduced the spleen 20:4n-6 by 95% (from 16.6 to 0.60 wt%) and caused a 7.5-fold increase in the wt% (from 2.80 to 21.0 wt%) of DHA in the lipids isolated from spleens. This *t*10, *c*12-CLA-induced shift in n-3 and n-6 PUFAs increased the n-3:n-6 PUFA ratio in the spleen ~30-fold (from 0.12 to 3.8). To the best of our knowledge, these are the most dramatic changes in the fatty acid composition of any tissue caused by a dietary intervention for just 8 weeks.

In summary, our results show that *c*9, *t*11-CLA did not alter the weights of lipids in the examined tissues, and that *t*10, *c*12-CLA did not alter lipid weights in heart and spleen, but altered those in liver and adipose tissue. Both isomers altered the fatty acid composition of all tissues, and differed in their effects on the fatty acid composition. The major effect of *t*10, *c*12-CLA was an increase in wt% of DHA, and a decrease in arachidonic acid in spleen lipids; also highly significant were the reductions in 22:6n-3 and 22:5n-6 in heart lipids. Since changes in total lipid mass were not detected in the spleen and heart, this data suggests that a high *t*10, *c*12-CLA diet can produce quantitative changes in these long chain PUFAs of the spleen and heart. While the underlying mechanisms for the changes in the fatty acid composition are unclear, these may reflect a tissue specific shift in fatty acid distribution. Thus, the increase in spleen DHA may be a compensatory response to the reduction in DHA concentration in liver and heart. If in fact the RBC lipids are a significant portion of those isolated from the spleen, the circulatory system may provide a reservoir large enough to account for these changes. Regardless, this study suggests that further investigations of the effect of *t*10, *c*12-CLA on the fatty acid profiles of heart and spleen lipids in other species and the mechanisms driving these changes are needed. The changes in fatty acid composition observed in this relatively short-term study may in fact have adverse health consequences.

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