Effects of Specific Conjugated Linoleic Acid Isomers on Growth Characteristics in Obese Zucker Rats

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ABSTRACT: Growing female obese Zucker (fa/fa) rats were treated (*via* intragastric gavage) for 21 d with either a (i) vehicle [corn oil; 0.9 g/kg body weight (BW)], (ii) CLA mixture [50:50; *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA], (iii) *cis*-9,*trans*-11 CLA, or (iv) *trans*-10,*cis*-12 CLA (CLA treatments at 1.5 g CLA/kg BW). Compared with controls, average daily gain (g/d) was reduced 24 and 44% by the CLA mixture and *trans*-10,*cis*-12 CLA, respectively. There was no treatment effect on average whole-body (minus heart and liver) composition (dry matter basis): fat (70.2%), protein (21.0%), and ash (4.3%). Compared with animals treated with *cis*-9,*trans*-11 CLA, obese Zucker rats treated with *trans*-10,*cis*-12 and the CLA mixture had 7.8% more carcass water. Treatment had no effect on heart or liver weights or on heart or liver weights as a percentage of body weight, but compared with the other treatments *trans*-10,*cis*-12 CLA increased liver lipid content by 33%. Hepatic lipid ratios of 16:1/16:0 and 18:1/18:0 (a proxy for Δ^9-desaturase capability) were not affected by treatment (0.1 and 0.6, respectively). Similar to previous reports, CLA increased hepatic lipid content and altered both liver and carcass FA composition (i.e., reduced arachidonic acid content), but the ability of CLA to manipulate body composition in obese Zucker rats remains questionable.

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CLA represent a mixture of geometric and positional isomers of octadecadienoic acid without a methylene group between double bonds. CLA are found naturally in dairy products and ruminant meat as a result of PUFA biohydrogenation by rumen bacteria (1). Several CLA isomers that differ in the position of the double bond pairs have been identified, each having a potentially different and unique biological or biochemical effect. Dietary CLA is associated with a number of potential human health benefits, including a decrease in the incidence and severity of mutagenesis, carcinogenesis, atherosclerosis, cachexia, and obesity (2,3). Identifying the specific CLA isomer responsible for the aforementioned biological effects has been difficult, as most investigations have utilized a supplement containing a variety of isomers. However, anticancer properties are associated with the *cis*-9,*trans*-11 CLA isomer (4), whereas the *trans*-10,*cis*-12 isomer markedly alters adipocyte and mammary lipid metabolism in a number of species (5–9).

Although dietary CLA has been shown to affect glucose parameters adversely in some animal models (10), CLA supplements actually improve metabolic parameters of type 2 diabetes (11,12) in the Zucker diabetic fatty rat. Based on differences in CLA isomer composition between treatments, it was speculated that the *trans*-10,*cis*-12 isomer was responsible for improving type 2 diabetes (13), and this has now been confirmed, as *trans*-10,*cis*-12 CLA dramatically improved whole-body and skeletal muscle insulin action, whereas *cis*-9,*trans*-11 CLA was ineffective at altering these metabolic variables (14). It is unclear whether these improvements are a direct result of CLA mediating a specific aspect of glucose homeostasis or an indirect result of altered body composition. High body fat content or obesity has long been tightly linked with adult-onset type 2 diabetes (15). The mechanism by which obesity either causes or contributes to this disorder is not clear but may include adipocyte-derived cytokines (16) and dyslipidemia (17).

As stated earlier, dietary CLA has been demonstrated to be extremely effective at decreasing the fat content (≥60%) in a number of rodent models (3) and pigs (8). However, a CLA supplement was ineffective at decreasing the fat content of lean Sprague-Dawley rats (18) and genetically lean pigs (19), and CLA actually increased fat pad weight in the obese Zucker rat (20). Inconsistent effects on carcass composition studies may be due to differences in CLA isomer composition, CLA dose, feeding duration, physiological age and state of experimental animals, and animal genotype.

The objectives of this study were to compare two specific CLA isomers (*cis*-9,*trans*-11 and *trans*-10,*cis*-12) on growth characteristics, whole body composition, organ weight, and FA profiles in the obese Zucker (fa/fa) rat. Furthermore, we were interested in determining whether the improvements in defective metabolic parameters observed in the obese Zucker rat were associated with decreases in carcass fat content.

EXPERIMENTAL PROCEDURES

All protocols and procedures were approved by The University of Arizona Institutional Animal Care and Use Committee. Female obese Zucker rats (Hsd/Ola: ZUCKER-fa; Harlan, Indianapolis, IN) were used to determine the effects of a CLA supplement and specific CLA isomers (relatively pure *cis*-9,

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Abbreviations: ADG, average daily body weight gain; BW, body weight; N, nitrogen.

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trans-11 and *trans*-10,*cis*-12) on glucose homeostasis parameters (glucose tolerance tests, skeletal muscle glucose transport activity, muscle glucose transporter-4 (GLUT-4) protein level, muscle citrate synthase activity, muscle carbonyl levels, and muscular TG concentration), and these results have been presented elsewhere (14).

Animals and experimental design. The study protocol has been described in detail previously (14). Briefly, growing female obese Zucker (fa/fa) rats [*n* = 19; mean initial body weight (BW) was 279 ± 11 g, mean ± SD] were fed a standard common diet (Table 1) and treated, *via* intragastric gavage, for 21 d with either a (i) control/carrier (*n* = 5; corn oil; 0.9 g/kg BW), (ii) CLA mixture (*n* = 4; 45% *trans*-10,*cis*-12; 45% *cis*-9,*trans*-11 CLA; 8% 18:1; <1% 18:0 + 16:0; 1.5 g/kg BW), (iii) *cis*-9,*trans*-11 CLA (*n* = 5; 53% *cis*-9,*trans*-11; 27% 18:1; 17% *trans*-10,*cis*-12 CLA; 3.3% 18:2; <0.5% 16:0 + 18:0; 1.5 g/kg BW), or (iv) *trans*-10,*cis*-12 CLA (*n* = 5; 72% *trans*-10,*cis*-12; 9% 18:1; 8% *cis*-9,*trans*-11 CLA; 7% 16:0; 2% 18:0; 1.5 g/kg BW). All CLA were kindly provided by BASF (Ludwigshafen, Germany) and were delivered as ethyl esters.

Following an overnight fast, animals were anesthetized with pentobarbital sodium (50 mg/kg BW ip), and final carcass weights were determined. The heart, kidney, liver, and soleus and plantaris muscles were dissected out and weighed. Kidneys were added back to the carcass for whole-body composition analysis, but livers were kept separate for additional analyses. Hearts and aforementioned muscles (~100–200 mg) were used as previously described (14) and were not included in the body composition analyses. Animals were quartered and freeze-dried (Virtis, Gardiner, NY) for 7 d to ensure complete removal of water. Freeze-dried carcasses were homogenized, along with dry ice, using a commercial food processor (Robot Coupe, Jackson, MS), and the homogenate was used to determine dry matter, ash, protein, and fat percentage.

TABLE 1
Diet Chemical Composition[a]

Nutrient[b]	Units	Composition[b]
Water[c]	%	10.00
Protein[c]	%	25.03
Fat[c,d]	%	4.25
Fiber[c]	%	4.67
Ash[c]	%	10.09
Nitrogen-free extract	%	46.16
Gross energy	kcal/g	3.82
Digestible energy	kcal/g	3.23
Metabolizable energy	kcal/g	2.94

[a]Teklad 7001 4% Mouse/Rat Diet; Harlan Teklad (Madison, WI).
[b]Ingredients include: soybean meal, ground corn, meat and bone meal, ground wheat, ground barley, ground oats, dehydrated alfalfa meal, ground limestone, dried skim milk, animal fat (lard), iodized salt, dicalcium phosphate, choline chloride, vitamin A acetate, vitamin D-activated animal sterol, vitamin E supplement, niacin, calcium pantothenate, riboflavin, thiamine mononitrate, pyridoxine hydrochloride, menadione sodium bisulfite complex, folic acid, biotin, vitamin B_{12} supplement, calcium carbonate, manganous oxide, ferrous sulfate, copper sulfate, zinc oxide, calcium iodate, and cobalt carbonate.
[c]On an as-fed basis.
[d]FA composition is as follows: 14:0 2%, 16:0 23%, 18:0 9%, 18:1 37%, 18:2 26%, 18:3 3%, 20:4 0.5%.

Body composition analysis. For ashing, tissue (2 g) was dried overnight in a 100°C oven, weighed, placed in a muffle furnace for 6 h at 550°C, and then reweighed. Protein analysis was performed using a FP-528 Nitrogen Determinator (LECO Corp., St. Joseph, MI) using 0.2 g of tissue. Total N content was multiplied by a correction factor of 6.25 to obtain protein concentration. Both ash and protein analyses were performed in triplicate. Percent fat was determined using a modified Folch *et al.* method (21). Briefly, a 2:1 chloroform/methanol solution was added to tissue (2 g), vortexed for 5 min, and centrifuged (400 × *g*). Supernatant was filtered through a Buchner funnel using #1 Whatman paper. To the filtrate, 0.58% NaCl solution was added; this mixture was mixed, recentrifuged (400 × *g*), and the top layer was removed and discarded. The lower layer was dried under N_2 until less than 8 mL remained and then transferred to a preweighed extraction tube and dried completely. Fat extractions were performed in duplicate. A separate extraction was performed for FA analyses.

FA analysis. FAME from carcass and liver lipids were prepared by the transmethylation procedure described by Christie (22) with modifications (23). Briefly, hexane (2 mL, HPLC grade) was added to 40 mg of lipid followed by 40 µL of methyl acetate. After vortexing, 40 µL of methylation reagent (1.75 mL methanol and 0.4 mL of 5.4 mol/L sodium methylate) was added, the mixture was revortexed and then allowed to react for 10 min and then 60 µL of termination reagent (1 g oxalic acid in 30 mL diethyl ether) and ~200 mg of calcium chloride were added and allowed to stand for 60 min. Samples were centrifuged at 2,400 × *g* at 4°C for 5 min. Following centrifugation, the liquid portion was transferred to a labeled GC vial and stored at –20°C. FAME were quantified using a gas chromatograph (Hewlett-Packard GC system 6890; Wilmington, DE) equipped with an FID and a CP-7420 fused-silica capillary column (100 m × 0.25 mm i.d. with 0.2-µm film thickness; Varian, Walnut Creek, CA). Initial oven temperature (160°C) was held for 28 min then ramped at 5°C/min to 220°C, where it was held for 10 min. Inlet and detector temperatures were maintained at 250°C, and the split ratio was 100:1. Hydrogen carrier gas flow rate through the column was 1 mL/min. Hydrogen flow to the detector was 30 mL/min, air flow was 400 mL/min, and the nitrogen makeup gas flow was 25 mL/min. Peaks in the chromatogram were identified and quantified using pure methyl ester standards (GLC60; Nu-Chek-Prep, Elysian, MN; GLC60, Matreya, Inc., Pleasant Gap, PA; *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA; Nu-Chek-Prep).

Statistical analyses. Data were statistically analyzed using the 1992 PROC-MIXED procedure of SAS (Cary, NC). Data are presented as least square means ± SEM and considered significant when main effects were less than $P < 0.05$.

RESULTS

Average daily body weight gain (ADG) was reduced ($P < 0.01$) 24 and 44% by the CLA mixture and *trans*-10,*cis*-12 CLA, respectively (Table 2). Compared to control and *cis*-9,*trans*-11 CLA treated animals, obese Zucker rats treated with

TABLE 2
Growth Rates and Body Composition of Obese Zucker Rats Treated with Isomers of CLA

Variable	Treatment[a]				SEM	P
	Control	CLA mix[b]	c9,t11 CLA	t10,c12 CLA		
ADG[c] (g/d)	2.5[b,c]	1.9[a,b]	2.7[c]	1.4[a]	0.2	<0.01
Body composition						
Water %	38.0[a,b]	40.8[c]	37.0[a]	39.0[b,c]	0.7	<0.01
Protein[d] %	20.4	22.2	20.3	20.9	0.7	0.32
Fat[d] %	70.9	68.6	71.4	70.2	1.1	0.36
Ash[d] %	4.1	4.5	4.5	4.1	0.4	0.76

[a]Rows with different roman superscripts indicate difference, $P < 0.05$.
[b]Contains 50:50 *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA.
[c]Average daily body weight gain.
[d]Values are on a dry matter basis.

trans-10,*cis*-12 CLA and the CLA mixture had 7.8% more ($P < 0.01$) carcass water (Table 2). There was no treatment effect on whole-body composition (average dry matter basis): fat (70.2%), protein (21.0%), and ash (4.3%). There was no treatment effect on soleus and plantaris muscle weights (13) or on wet heart, kidney, or liver weights (Table 3). Compared with the other treatments, Zucker rats treated with *trans*-10, *cis*-12 CLA had increased (33%; $P < 0.01$) liver lipid content (Table 3). There was no treatment effect on hepatic moisture content (29.2%; data not shown).

Analysis of carcass FA indicated *cis*-9,*trans*-11 CLA content was increased ($P < 0.01$) more than 10-fold in both the *cis*-9,*trans*-11- and CLA mix-treated animals (Table 4). Similarly, *trans*-10,*cis*-12 CLA content was increased (>ninefold; $P < 0.01$) in both the *trans*-10,*cis*-12- and mixed CLA-treated animals (Table 4). Similar to carcass FA composition, hepatic FA analysis indicated higher ($P < 0.01$) levels of both *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA due to specific CLA treatments and the CLA mix administration (Table 5). Ratios of 16:1/16:0 and 18:1/18:0 (a proxy for Δ^9-desaturase capability) were unaffected in hepatic lipids (0.10 and 0.60, respectively). Both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA and the CLA mixture decreased ($P < 0.01$) the arachidonic acid (20:4n-6) concentration in liver lipids by ~15% (Table 5).

DISCUSSION

Although dietary CLA have been demonstrated to have a wide range of beneficial effects in animal models (2), it has been shown to cause mild insulin resistance in nondiabetic rodent models and human individuals susceptible to type 2 diabetes (10,24). However, dietary CLA improves defective metabolic parameters associated with type 2 diabetes (10) in some species, and it is thought that *trans*-10,*cis*-12 CLA is an isomer responsible for this phenomenon (13). Henriksen and colleagues (14) recently directly demonstrated that *trans*-10,*cis*-12 CLA improves glucose disposal and reduces the insulin resistance of skeletal muscle glucose transport in obese Zucker rats. The *cis*-9,*trans*-11 CLA isomer had little or no effect on the aforementioned glucose homeostatic parameters (14).

Animals receiving both the CLA mixture and the purified *trans*-10,*cis*-12 CLA had reduced ADG compared with controls and the *cis*-9,*trans*-11 CLA-treated groups (Table 2), and we assume that a reduction in caloric intake can at least partially explain this (unfortunately, feed intake was not measured). Although reduced weight gain cannot be ruled out as a partial mechanism by which CLA improves type 2 diabetes, the ability of CLA to improve glucose metabolic parameters in the obese Zucker diabetic rat model is not due to improved body composition because we demonstrated that neither *trans*-10,*cis*-12 nor *cis*-9,*trans*-11 CLA reduced carcass fat percentage or increased body protein content (Table 2). Although these findings are in agreement with previous work using the obese Zucker model (20), the lack of effect on tissue composition in the current investigation is surprising as CLA, specifically, *trans*-10,*cis*-12 CLA, has been shown to be very effective at reducing body fat (i.e., >50%) in a number of rodent models (25–27). A small sample size (n = 4–5/treatment) may have limited our ability to detect statistical differences, but there were no numerical trends even hinting

TABLE 3
Wet Organ Weights of Obese Zucker Rats Treated with Isomers of CLA

Organ (g)	Treatment[a]				SEM	P
	Control	CLA mix[b]	c9,t11 CLA	t10,c12 CLA		
Heart	0.7	0.6	0.7	0.7	<0.1	0.37
Kidney	1.8	1.7	1.8	1.7	<0.1	0.85
Liver	10.1	10.2	9.9	9.6	0.5	0.80
Lipid[c] %	23.6[a]	22.0[a]	24.4[a]	31.0[b]	1.1	<0.01

[a]Rows with different roman superscripts indicate difference, $P < 0.05$.
[b]Contains 50:50 *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA.
[c]On a dry matter basis.

TABLE 4
Carcass FA Profile of Obese Zucker Rats Treated with Isomers of CLA

FA	Treatment[a]				SEM	*P*
	Control	CLA mix[b]	*c*9,*t*11 CLA	*t*10,*c*12 CLA		
			g/100 g FA			
12:0	0.29	0.28	0.23	0.38	0.08	0.58
14:0	1.80[a]	1.71[a]	2.08[b]	2.07[b]	0.07	<0.01
14:1	0.14	0.13	0.15	0.13	0.01	0.37
16:0	29.95[a]	25.27[b]	30.66[a]	31.19[a]	1.06	<0.01
16:1	6.68	6.61	7.34	6.53	0.23	0.19
18:0	4.81[a]	3.96[b]	4.70[b]	5.18[a]	0.18	<0.01
18:1*c*9	33.39	32.14	34.39	31.95	0.79	0.21
18:1*c*11	2.29	2.27	2.29	2.30	0.09	0.99
18:2*c*9,*c*12	15.56[a,b]	18.99[a]	11.66[b]	12.81[b]	1.39	0.01
18:2*c*9,*t*11	0.16[a]	2.15[b]	1.96[b]	0.46[a]	0.24	<0.01
18:2*t*10,*c*12	0.14[a]	1.35[b]	0.49[a]	1.37[b]	0.26	0.02
18:3	0.93[a]	1.52[b]	1.07[a,b]	0.97[a]	0.14	0.03
20:0	0.10	0.13	0.20	0.24	0.09	0.66
20:1n-9	0.34	0.37	0.55	0.36	0.05	0.16
20:2n-6	0.23	0.32	0.29	0.21	0.06	0.49
20:4n-6	0.60[a,b]	0.82[a]	0.66[a,b]	0.42[b]	0.09	0.04
Unknown	2.73	1.98	1.27	3.62	1.13	0.53
Totals						
Saturated	36.92[a]	31.35[b]	37.87[a]	38.96[a]	1.30	<0.01
MUFA[c]	42.84	41.52	44.72	41.27	1.03	0.19
PUFA	20.23[a]	27.13[b]	17.41[a]	19.77[a]	1.79	<0.01
16:1/16:0	0.22[b]	0.26[a]	0.24[a,b]	0.21[b]	0.01	0.04
18:1/18:0	6.98[a]	8.12[b]	7.33[a,b]	6.22[a]	0.36	0.01

[a]Rows with different roman superscripts indicate difference, $P < 0.05$.
[b]Contains 50:50 *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA.
[c]Monounsaturated FA.

at treatment effects. In addition, others have demonstrated effects with a similar sample size (6). An insufficient CLA dose can likely be ruled out, as the animals utilized in this study were fed relatively purified CLA isomers at ~1.5% of the diet or ~1,100 mg pure CLA/kg $BW^{0.75}$ [based on feed intake of obese Zucker rats at a similar stage and weight (28)]. The percentage of CLA both in the diet and on a metabolic BW basis are higher than typically utilized in experiments in which marked improvements in body composition are observed (29, 30), and this is especially pertinent as we utilized semipurified (>70%) CLA isomers (*cis*-9,*trans*-11 and *trans*-10,*cis*-12).

The length of time (3 wk) that rats were treated with purified CLA isomers may not have been long enough to elicit changes in nutrient partitioning. Previous CLA rodent trials generally supplied dietary CLA for 4–5 wk or longer (6,18). Rats used in this study were at the approximate age (9 wk) when obese Zucker rats are typically depositing a large amount of adipose tissue (28) *via* hepatic-derived preformed FA (31). There are many reports that suggest that one mechanism by which CLA, and specifically the *trans*-10,*cis*-12 isomer, reduces adiposity is *via* reducing lipoprotein lipase expression and activity (32,33), thus reducing cellular FA uptake. Therefore, because these obese Zucker rats were depositing large amounts of adipose tissue at the time of CLA treatment, it is likely that 3 wk should have been adequate to detect differences in body fatness.

A more likely explanation for the lack of an effect of CLA on body fat content in this study is species or genotypic variation. For example, CLA appears to be more effective in mice than in rats (6,7,18). Furthermore, CLA has been less effective or ineffective at decreasing the fat content of Sprague-Dawley rats [generally a lean rodent model (18)], fish (34), genetically lean pigs (19,35), and humans (36,37). In fact, even in the same species there are large differences in CLA effect on lipid metabolism as CLA decreased the adiposity of lean Zucker rats but actually increased fat pad weights in the obese Zucker rats (20). Similar inconsistencies have been demonstrated in different strains of mice (38). The mechanism by which these species differences exist is not clear, but the rate of intracellular adipocyte TG turnover (26) and basal metabolic rate (30) have been identified as probable causes.

Neither the CLA mixture nor specific isomers changed the carcass percentage of protein or ash (Table 2). Dietary CLA has been demonstrated to modestly increase carcass protein percentage in a number of rodent models (6,26,39), although there appears to be little or no effect of CLA on yield or amount of carcass protein in these rodent trials.

A more consistent observation made in CLA-treated animals is the increase in carcass moisture, and this was confirmed in the current study (Table 2). Obese Zucker rats treated with the CLA mixture and *trans*-10,*cis*-12 CLA had enhanced carcass water compared with controls and *cis*-9,*trans*-

TABLE 5
Liver FA Profile of Obese Zucker Rats Treated with Isomers of CLA

FA	Treatment[a]				SEM	*P*
	Control	CLA mix[b]	*c*9,*t*11 CLA	*t*10,*c*12 CLA		
			g/100 g FA			
14:0	0.32	0.50	0.41	0.41	0.04	0.09
15:0	0.08	0.10	0.08	0.09	0.01	0.32
16:0	17.72^{a}	18.54^{a,b}	19.76^{b}	20.16^{b}	0.62	0.04
16:1	1.74	2.12	2.08	1.90	0.18	0.46
17:0	0.28	0.27	0.26	0.24	0.02	0.40
18:0	23.52	21.93	22.62	21.55	1.04	0.56
18:1*c*9	13.33	12.49	13.47	13.47	0.70	0.75
18:1*c*11	1.70	1.76	1.69	1.71	0.03	0.56
18:2*c*9,*c*12	9.62	10.39	8.49	10.53	0.58	0.08
18:2*c*9,*t*11	0.05^{a}	1.06^{b}	1.11^{b}	0.37^{c}	0.09	<0.01
18:2*t*10,*c*12	0.05^{a,b}	0.57^{b}	0.16^{a}	1.06^{c}	0.14	<0.01
18:3	0.27	0.39	0.31	0.48	0.06	0.07
20:0	0.26	0.19	0.18	0.17	0.03	0.17
20:1n-9	0.12	0.11	0.11	0.12	0.16	0.87
20:2n-6	0.21^{b,c}	0.25^{a}	0.19^{c}	0.22^{b}	0.01	<0.01
20:4n-6	19.61^{a}	17.09^{b}	17.36^{b}	15.87^{b}	0.70	0.01
22:0	0.72^{a}	0.67^{a}	0.66^{a}	0.51^{b}	0.04	0.01
22:5n-3	0.77^{a}	1.21^{b}	1.01^{c}	1.00^{c}	0.05	<0.01
22:6n-3	7.00	7.45	7.22	7.06	0.37	0.84
Unknown	2.70	2.91	2.82	3.08	0.15	0.36
Totals						
Saturated	42.89	42.19	43.98	43.13	0.59	0.26
MUFA[c]	16.90	16.47	17.36	17.20	0.89	0.91
PUFA	37.51	38.42	35.84	36.58	0.72	0.12
16:1/16:0	0.10	0.11	0.10	0.10	0.01	0.43
18:1/18:0	0.57	0.57	0.60	0.64	0.06	0.77

[a]Rows with different roman superscripts indicate difference, $P < 0.05$.
[b]Contains 50:50 *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA.
[c]Monounsaturated FA.

11 CLA-supplemented animals, respectively (Table 2). The increase in carcass water content is in agreement with other rodent trials (6,40) and finishing pig experiments (8,41). It is unknown why CLA, specifically the *trans*-10,*cis*-12 isomer, increases carcass moisture. A large portion of body fluid is associated with lean tissue, which is composed of water and protein, and CLA has been demonstrated to increase water accretion rates while having very little effect on protein deposition in pigs (8,42). It is possible, because water represents such a large proportion of muscle (>70%), that minor increases in muscle synthesis would be more easily detected in water content changes rather than protein. This may explain the enhanced carcass water content in the CLA mixture and *trans*-10,*cis*-12-treated Zucker rats without an effect on whole-body protein levels (Table 2).

There was no effect of either CLA isomer on heart or kidney weight (Table 3), which is in agreement with other rodent models (7,40,43). Likewise there was no effect of CLA on liver wet weight (g or percentage of BW), which is consistent with previous studies using rat models (18,39,44). However, this is in contrast to several studies using mice and hamster models that indicate CLA, and specifically *trans*-10,*cis*-12, induces marked hepatomegaly (7,30,45). CLA effect on hepatic lipid metabolism is clearly species dependent, as dietary CLA actually decreased liver weight in the obese and lean Zucker rats (20) and fish (34). Although actual liver weight did not differ between treatments, hepatic lipid content increased (31% compared to controls) in obese Zucker rats supplemented with *trans*-10,*cis*-12 CLA (Table 3). The increase in hepatic steatosis without an increase in liver weight agrees with another study using a rat model (46), and the increased liver lipid content agrees with other rodent (mainly mice) trials (5,38,43,47). The mechanism by which *trans*-10,*cis*-12 CLA mediates hepatocyte lipid filling is not clear, but reduced VLDL export *via* decreasing apoprotein B secretion has been demonstrated (48). In addition, the CLA-induced increase in plasma insulin that is sometimes observed (2) is thought to increase hepatic fat synthesis *via* up-regulating FA synthetase and acetyl CoA carboxylase, resulting in liver fat accumulation (38) Our results do not support this hypothesis, as the *trans*-10,*cis*-12 CLA-treated animals had reduced insulin levels (14) but had enhanced liver lipid content (Table 3), thus suggesting the increase in hepatic fat content is insulin-independent. Furthermore, contrary to previous suggestions (38,49), enhanced liver lipid is not necessarily adverse, as there are some physiological circumstances that will naturally cause fatty liver (i.e., liver regeneration) and not impair hepat-ocyte function (50). To our knowledge, there is no evidence suggesting

CLA or CLA-induced liver steatosis is cytotoxic to healthy hepatocytes.

As anticipated, the incorporation of specific CLA isomers into whole carcass lipids (Table 4) and total liver lipids (Table 5) reflected the CLA profile of specific treatments. The increase in liver and carcass *cis*-9,*trans*-11 and *trans*-10,*cis*-12 content in the CLA-treated groups corresponds to the proportionate impurities of the CLA supplements. In addition to the increase in respective CLA isomers, both CLA isomers and the CLA mixture decreased the content of hepatic arachidonic acid (Table 5). This is not surprising, as both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA have been shown to decrease Δ^5-desaturase (51) and Δ^6-desaturase (the rate-limiting step of arachidonic acid synthesis) systems (52), and CLA is thought to displace arachidonic acid in phospholipids (2). A decrease in arachidonic acid and thus arachidonate-derived eicosanoids (prostaglandins E_2 and $F_{2\alpha}$ etc.) has been suggested to be a mediator of many, if not most, of CLA' s biological effects (2,27).

In addition, although hepatic nonesterified FA (NEFA) uptake is proportionate to plasma NEFA levels (53), *trans*-10,*cis*-12 CLA enhanced liver lipid content (Table 3), even though plasma NEFA concentrations were actually decreased by this specific CLA isomer (14).

In conclusion, although dietary *trans*-10,*cis*-12 CLA improves defective glucose homeostatic parameters in the obese Zucker rat, neither this specific isomer nor *cis*-9,*trans*-11 CLA altered whole body composition. This suggests that the mechanism by which CLA alleviates insulin resistance is independent of changes in body protein and lipid content.

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