Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1601 – 1611
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Dietary conjugated linoleic acid decreases adipocyte size and favorably modifies adipokine status and insulin sensitivity in obese, insulin-resistant rats

Amy Noto[a], Peter Zahradka[a,b], Natalia Yurkova[b], Xueping Xie[b], Han Truong[b], Evan Nitschmann[c], Malcolm R. Ogborn[a,c], Carla G. Taylor[a,*]

[a]*Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, Canada R3T 2N2*
[b]*Department of Physiology, University of Manitoba, Winnipeg, MB, Canada R3E 3J7*
[c]*Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB, Canada R3E 3P4*

Received 22 February 2007; accepted 5 June 2007

Abstract

Conjugated linoleic acids (CLA) have been shown to alter adiposity in some species with varying effects on insulin resistance. The objective of this 8-week study was to investigate the effects of feeding a CLA mixture (1.5%, wt/wt) on adipocyte size, insulin sensitivity, adipokine status, and adipose lipid composition in *fa/fa* vs lean Zucker rats. The *fa/fa* CLA-fed rats had smaller adipocytes and improved insulin sensitivity compared with *fa/fa* rats fed the control diet. Conjugated linoleic acids did not affect select markers of adipose differentiation, lipid filling, lipid uptake, or oxidation. Dietary CLA, compared with the control diet, reduced circulating leptin and elevated fasting serum adiponectin concentrations in *fa/fa* rats. Adipose resistin messenger RNA levels were greater in *fa/fa* CLA-fed rats compared with *fa/fa* control rats. CLA did not markedly alter adipose phospholipid fatty acid composition, and the changes in the triacylglycerol fatty acid composition reflected a lower delta-9 desaturase index of CLA-fed vs control-fed rats. In conclusion, CLA reduced adipocyte size and favorably modified adipokine status and insulin sensitivity in *fa/fa* Zucker rats.

1. Introduction

The obesity trend among the youth is of great concern given the implications for early-onset causes of morbidity including cardiovascular disease and type 2 diabetes mellitus [1,2]. Adipose tissue is now viewed as more than an energy depot; it is a metabolically active tissue with endocrine functions. Adipokines, such as leptin, resistin, and adiponectin, are proteins synthesized and released by adipose tissue. Circulating leptin is positively correlated with obesity and development of insulin resistance [3]. Resistin seems to have an analogous relationship with insulin resistance based on studies in mouse models [4] but not humans [5,6]; and interestingly, mononuclear cells present in adipose tissue, not adipocytes, are the primary source of serum resistin [7,8]. Conversely, circulating adiponectin (also known as *acrp30*) is lower in individuals with obesity and type 2 diabetes mellitus compared with healthy individuals and is inversely associated with insulin resistance [9]. Although the mechanism is not clear, adipokine metabolism may be affected by individual fatty acids via membrane or intracellular fatty acid composition. For example, in 3T3-L1 cell culture, docosahexaenoic acid treatment lowers leptin messenger RNA (mRNA) levels [10] and arachidonic acid treatment lowers resistin mRNA levels [11].

Conjugated linoleic acid (CLA) is a term that describes a group of fatty acids that are positional (carbon 7 to 12) and geometric (*cis-cis*, *cis-trans*, *trans-cis*, and *trans-trans*) isomers of octadecadienoic acid (C18:2 [12]). CLA promotes fat loss and enhances lean body mass in certain species (eg, mice, hamsters [13,14]); and recently, the same effect has been reported in overweight or obese people [15]. In addition, CLA has been reported to modulate adipokine metabolism and tissue fatty acid composition. For example,

* Corresponding author. Tel.: +1 204 474 8079; fax: +1 204 474 7593.
E-mail address: ctaylor@cc.umanitoba.ca (C.G. Taylor).

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.06.025

circulating leptin is reduced in CLA-fed obese, insulin-resistant rats [16-18] and normal-weight rats and mice [19-21] in association with reduced fat pad mass. Human trials involving healthy women [22] and men with the metabolic syndrome [23] have shown that CLA supplementation does not affect circulating leptin, although Belury et al [24] found an inverse negative correlation between plasma t10,c12-CLA and serum leptin in people with type 2 diabetes mellitus. Dietary CLA supplementation increases circulating adiponectin in association with attenuated hyperinsulinemia in Zucker diabetic fatty (ZDF) rats [25], whereas worsening of insulin resistance with CLA feeding in mice is associated with reductions in serum adiponectin [26]. Several studies have reported that CLA alters adipose tissue fatty acid composition in vitro and in normal-weight animals by reducing the monounsaturated fatty acid (MUFA) content through inhibition of delta-9 desaturase (Δ9 DS)/stearoyl coenzyme A DS [27-29]. However, the relationships among CLA, adipose tissue fatty acid composition, insulin resistance, and adipokine status have not been examined together in obese, insulin-resistant models.

We hypothesized that CLA feeding would alter adipose tissue fatty acid composition, adipokine status, and adipose mass/adipocyte size and that each of these, directly or in combination, would improve insulin sensitivity. Thus, the objective of this 8-week study was to investigate the effects of feeding a CLA mixture (1.5%, wt/wt) on obesity, insulin resistance, adipokine status, and adipose tissue fatty acid composition in an obese, insulin-resistant model compared with lean controls.

2. Materials and methods

2.1. Animals and diets

After a 5- to 7-day acclimatization period, *fa/fa* (fa) and lean (ln) 6-week-old male Zucker rats (n = 20 per genotype, n = 40 rats total; Charles River, St Constant, Quebec, Canada) were randomly assigned to receive either the CLA (faCLA and lnCLA groups) or control (faCTL and lnCTL groups) diet for 8 weeks. The protocol for the animal care procedures was approved by the University of Manitoba Protocol Management and Review Committee. Other indices for these rats related to hepatic steatosis, pancreatic function, glucose disposal, and inflammatory markers have been reported elsewhere [30,31]. The dietary formulation and the fatty acid distributions of the CLA and CTL diets have been previously reported [30].

2.2. Tissue collection

At the end of the 8-week study, rats were fasted overnight and euthanized by CO_2 asphyxiation according to the Canadian Council on Animal Care Guidelines [32]. Trunk blood was collected and immediately placed on ice until centrifuged to separate the serum fraction that was stored at −80°C. Epididymal and perirenal adipose tissues were weighed, immediately frozen in liquid nitrogen, and subsequently stored at −80°C. A portion of epididymal adipose tissue was fixed in optimal cutting temperature (OCT) compound in a dry ice/ethanol bath.

2.3. Adipocyte size

Tissue sections (15 μm) were fixed in Streck solution for 10 minutes and stained with the Harris modified hematoxylin and eosin. Digital images were captured with a DAGE-MTI (Michigan City, IN) charge-coupled device camera, and cell area was measured with the open-source image analysis program ImageJ v1.34s (Rasband, WS, ImageJ, US National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/, 1997-2006).

2.4. Fasting serum biochemistry and insulin resistance calculation

Fasting serum leptin and adiponectin were determined using commercial radioimmunoassay kits (Linco Research, St Charles, MO).

Homeostasis model assessment (HOMA; Eq. (1) [33]) was used to calculate insulin resistance in the fasting state based on fasting serum glucose (in millimoles per liter) and insulin (in microunits per milliliter).

$$\frac{(\textit{fasting insulin})(\textit{fasting glucose})}{22.5} \quad (1)$$

2.5. Reverse transcriptase polymerase chain reaction for analysis of mRNA

Markers of adipose metabolism (peroxisome proliferator–activated receptor [PPAR] γ1 and γ2, leptin, adiponectin, resistin, lipoprotein lipase [LPL], and uncoupling protein [UCP] 2) and the housekeeping gene (glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) were assessed in epididymal adipose tissue by reverse transcriptase polymerase chain reaction (RT-PCR) using previously published procedures [34]. The GenBank accession numbers and sequences for the PCR primers (Invitrogen Life Technologies, Burlington, Ontario, Canada) are shown in Table 1. Briefly, total RNA was isolated using TRIzol. The RNA was resuspended in ribonuclease-free water, and concentration was determined by spectrophotometric absorbance at 260 nm. Reverse transcription of 1 μg RNA was conducted (after removal of possible genomic DNA contamination with deoxyribonuclease I) according to the protocol (25 cycles of amplification and 62°C annealing temperature) recommended for the Access RT-PCR system (Promega, Madison, WI). Vistra green (Amersham, Baie d'Urfe, Quebec, Canada) was used to visualize RT-PCR products after electrophoresis on 2% agarose gels. Relative band intensity was quantified by scanning the gel (Storm Fluorimager, Amersham), and results are expressed as arbitrary units.

Table 1
The PCR primers

Gene	GenBank accession nos.	Sense	Antisense
PPARγ1	AF156665	-acaagactacccttttactgaaaatacc	-gtcttcatagtgtggagcagaaatgctg
PPARγ2	Y12882	-tacagcaaatctctgttttatgctgtt	-gtcttcatagtgtggagcagaaatgctg
Leptin	NM013076	-atttcacacacgcagtcggt	-cttggagaaggccagcagat
Adiponectin	AY033885	-tgcccagtcatgaagggatt	-aatgggaacattggggacag
Resistin	NM144741	-ctgagctctctgccacgtact	-gctcagttctcaatcaaccgtcc
LPL	NM012598	-gctctgcctgagttgcagaa	-cgatgtccacctccgtgtaa
UCP-2	AB006613	-acagatgtggtaaaggtccgct	-ctgtcatgaggttggctttcag
GAPDH	NM017008	-cgctgtgaacggatttggccgtat	-agccttctccatggtggtgaagac

2.6. Western blot analysis

Frozen epididymal adipose tissue was ground with a mortar and pestle then suspended in 2× sodium dodecyl sulfate sample buffer. The solution was briefly sonicated and clarified by centrifugation (60 seconds at 12 000 rpm). Western blotting of cellular proteins separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis in a 7.5% gel and transferred to polyvinylidene difluoride membrane was conducted as previously described [35]. Horseradish peroxidase–conjugated secondary antibody was detected using the ECL chemiluminescent system (Amersham). Band intensity was quantified by scanning densitometry. Antibodies used included PPARγ, leptin (Santa Cruz Biotechnology, Santa Cruz, CA), resistin (Alpha Diagnostic International, San Antonio, TX), adiponectin (Calbiochem, San Diego, CA), and UCP-2, and adipophilin (Research Diagnostics, Concord, MA).

2.7. Fatty acid analysis

Lipids were extracted using a modified Bligh and Dyer extraction procedure [36]. Briefly, a 0.25-g sample of adipose was added to 10 mL of chloroform-methanol (2:1, by volume) with 0.01% butylated hydroxytoluene (Sigma-Aldrich, Oakville, Ontario, Canada) and extracted and separated by thin-layer chromatography as previously described [37]. All solvents were from Fisher Scientific (Nepean, Ontario, Canada).

Two lipid classes (triacylglycerol [TAG] and phospholipid [PL]) were methylated separately using a sodium methoxide ($NaOCH_3$)–based procedure (15 minutes at 50°C) to ensure that isomerization of double bonds in the conjugated polyunsaturated fatty acids (PUFAs) did not occur [38]. The PL fraction analysis required the use of 2 methylating agents because no single methylating agent was compatible with both CLA and the fatty acids found in sphingomyelin (ie, sphingomyelin fatty acids are not methylated by $NaOCH_3$, and CLA is isomerized by acid-based methylating agents [39]). Thus, a duplicate PL fraction was also methylated with methanolic hydrochloric acid for 2 hours at 80°C. The results from the 2 methylation procedures were combined according to the relative amounts of the internal standard 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (Avanti, Alabaster, AL) based on its addition in equal amounts to the duplicate samples. For gas chromatography, $NaOCH_3$-methylated samples were separated on a Chrompack CP-Select CB column (Varian Canada Inc, Mississauga, ON; diameter, 100 m × 0.25 mm; film thickness, 0.25 μm) using a Varian CP-3800 GC with flame ionization detector (Varian Canada Inc). The temperature program was 45°C hold × 4 minutes, 175°C at 13°C/min × 27 minutes, 190°C at 1°C/min × 38 minutes, 215°C at 4°C/min × 10 minutes, and 240°C at 4°C/min × 5 minutes. Total run time was 121.5 minutes, and samples were run with a 10:1 split ratio. The methanolic hydrochloric acid–methylated samples were separated on a DB225MS column (Hailent Technologies Canada Inc, Mississauga, ON; diameter, 30 m × 0.25 mm; film thickness, 0.25 μm) using a Varian 3400 GC with flame ionization detector. The temperature program was 70°C hold × 1 minute, 180°C at 20°C/min × 8.5 minutes, 220°C at 3°C/min × 15 minutes, and 240°C at 20°C/min × 10.5 minutes. Total run time was 55 minutes, and samples were run with a 20:1 split ratio. Individual fatty acid results are expressed as gram per 100 g total fatty acids. A Δ9 DS index ([16:1n-7 + 18:1-n9]/[16:0 + 18:0]) was calculated based on the fatty acid results.

2.8. Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine significant main effects (genotype, lipid, and genotype × lipid interaction), and Duncan multiple range test was used for means testing (SAS 6.04; SAS Institute, Cary, NC). The mRNA and protein data were assessed by contrasts for preplanned comparisons with the additional effect of gel treated as a block (4 treatments per block). For mRNA data,

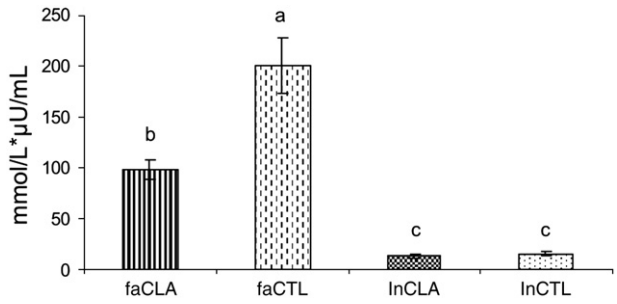

Fig. 1. The HOMA of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks. Values are means ± SEM for n = 10 rats per group. Means with different letters are significantly different ($P < .05$) by Duncan multiple range test. faCLA indicates *fa/fa* rats fed 1.5% CLA; faCTL, *fa/fa* rats fed 0% CLA; lnCLA, lean rats fed 1.5% CLA; lnCTL, lean rats fed 0% CLA.

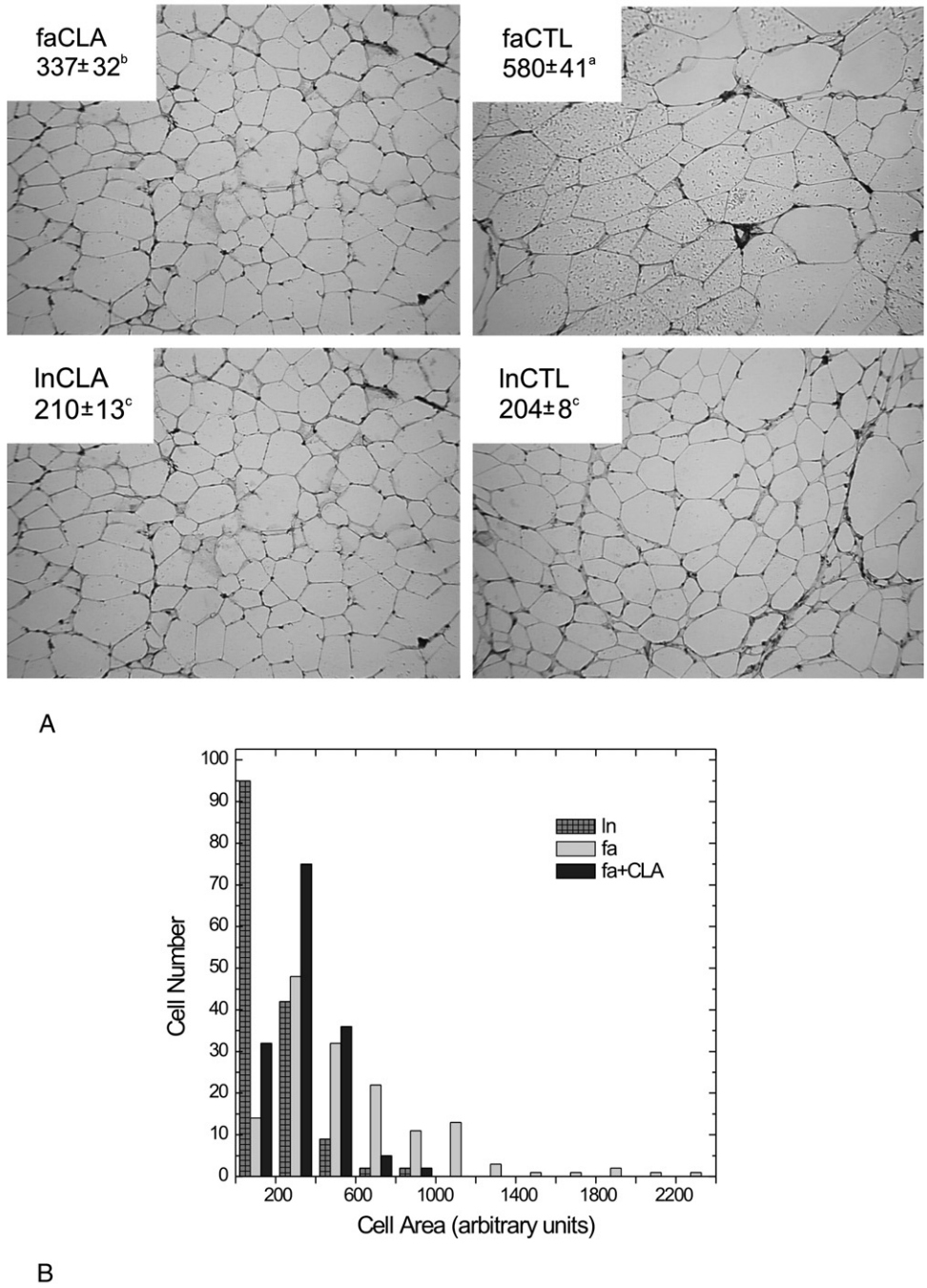

Fig. 2. Adipocyte size (A) and distribution (B) of *fa/fa* Zucker rats fed 1.5% CLA (faCLA) or 0% CLA (faCTL) and lean Zucker rats fed 1.5% CLA (lnCLA) and 0% CLA (lnCTL). Cell area is reported in pixels for the overall means ± SEM (A) and by size distribution categories (B) for n = 6 rats per group. Overall means (A) with different letters are significantly different ($P < .05$) by Duncan multiple range test.

the housekeeping gene (GAPDH) was analyzed as a covariate. The significance level was $P < .05$.

3. Results

3.1. Feed intake, body weight, and adiposity

We have previously reported that the *fa/fa* genotype had greater total feed intake, final body weight, and total weight gain than the lean genotype; but there was no difference due to dietary CLA supplementation. However, the faCLA rats had greater relative epididymal, perirenal, and visceral adipose tissue weights than faCTL rats [31].

3.2. Insulin resistance

The faCLA rats had a 50% reduction in HOMA compared with the faCTL rats, but the HOMA value was still elevated

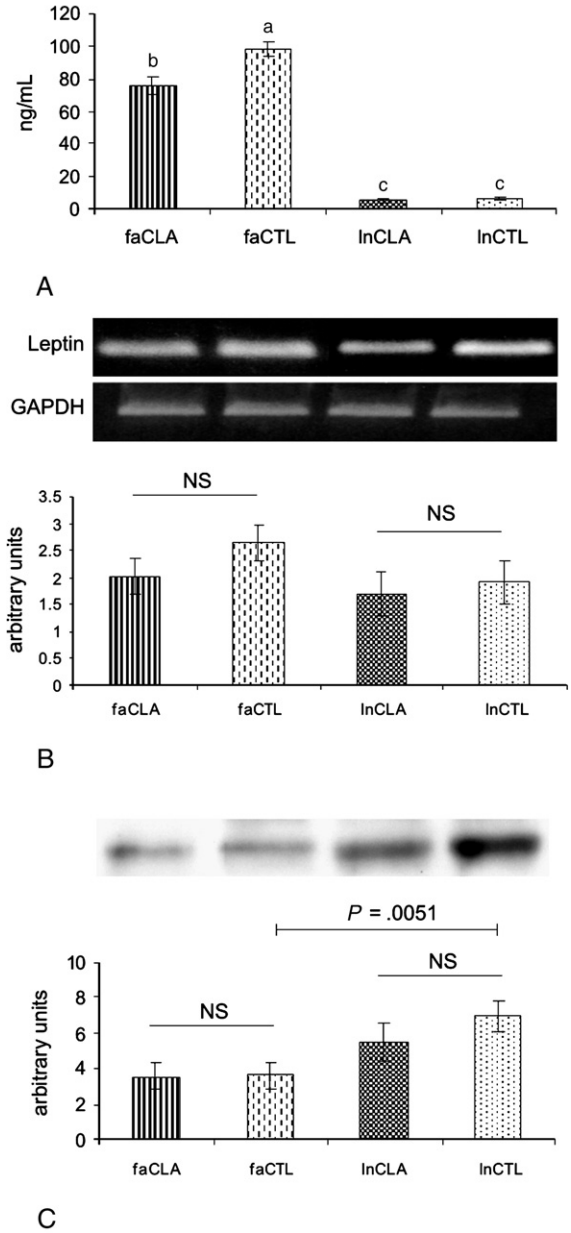

Fig. 3. Serum leptin (A), adipose leptin mRNA (B), and protein (C) levels of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks. Results for serum leptin (in nanograms per milliliter) are means ± SEM for n = 10 rats per group. Means with different letters are significantly different ($P < .05$). Results for mRNA and protein levels expressed as arbitrary units, means ± SEM, n = 9 rats per group for mRNA levels and n = 10 rats per group for protein levels. *P* values from preplanned comparisons. NS indicates not significantly different.

7-fold compared with that in lean rats (Fig. 1). In lean rats, there was no adverse effect of CLA supplementation on insulin sensitivity as assessed by HOMA.

3.3. Adipocyte size and adipokines

As was expected, adipocyte size was markedly higher in faCTL rats relative to lnCTL; however, the adipocytes were much smaller in faCLA compared with faCTL rats (Fig. 2A). Although reduced, adipocyte size in CLA-treated *fa/fa* rats was still significantly higher than that in the lnCTL rats (Fig. 2A) according to the median cell size (faCTL = 454, faCLA = 323, lnCTL = lnCLA = 169 pixels). The effect of CLA could be explained by the fact that the number of large adipocytes (>1000 pixels) present in the tissue was reduced from 14.7% to zero (Fig. 2B). Interestingly, there was no effect of genotype or CLA supplementation on markers of adipose differentiation (PPARγ1 and PPARγ2 mRNA levels or PPARγ protein levels), lipid filling (adipophilin protein levels), lipid uptake (LPL mRNA), and nonoxidative phosphorylation (UCP-2 mRNA and protein) (data not shown).

The faCLA rats had a 23% reduction in serum leptin compared with faCTL rats (Fig. 3A). Although no differences were detected in adipose leptin mRNA or protein levels between faCLA and faCTL rats, leptin protein levels were 48% lower in faCTL compared with lnCTL rats (Fig. 3B and C).

The *fa/fa* rats had higher circulating adiponectin than lean rats (12.5 ± 0.6 vs 10.7 ± 0.6 μg/mL). The CLA-fed rats had a 23% higher fasting serum adiponectin than CTL-fed rats (13.1 ± 0.5 vs 10.1 ± 0.5). There was no interaction of genotype and diet for fasting serum adiponectin (Fig. 4A). The faCLA rats had greater adipose adiponectin mRNA levels compared with faCTL rats (Fig. 4B). The faCLA rats also had greater adipose resistin mRNA levels (Fig. 4C) compared with faCTL rats; however, resistin protein levels were not different among groups (Fig. 4D).

3.4. Adipose fatty acid composition

The faCLA rats had one half the amount of CLA present in adipose TAG compared with lnCLA rats, as a percentage of total fatty acids (Fig. 5). The faCLA rats had less c9,t11/t8, c10 CLA; c11,t13 CLA; and t10,c12 CLA (expressed as a percentage) in adipose TAG compared with lnCLA rats. The dominant isomers in adipose TAG and PL were c9,t11/t8, c10. The CLA isomer incorporation in adipose PL was not different between genotypes.

The adipose TAG of CLA-fed rats had more total saturated fatty acids (SFAs; eg, C14:0, C16:0; percentage of composition) and less total MUFAs (eg, C18:1n-9, C18:1n-7, C16:1n-7; percentage of composition) compared with CTL-fed rats (Table 2). These observations were paralleled by a reduction in the Δ9 DS index, demonstrating an effect of CLA on Δ9 DS independent of the metabolic derangements present in the *fa/fa* rats. In addition, certain PUFAs (eg, C18:2n-6, C18:3n-6, C18:3n-3, C20:4n-6) were present in lower amounts in CLA-fed compared with CTL-fed rats, which may be accounted for by the greater SFA composition because these results are relative. In addition, the n6/n3 ratio was higher in lean rats fed CLA compared with that in lean rats fed CTL diet. In adipose PL (Table 3), CLA did not affect fatty acid composition, except that the percentages of C18:1n-7 and C24:1n-9 were lower in CLA-fed rats compared with those fed CTL diets.

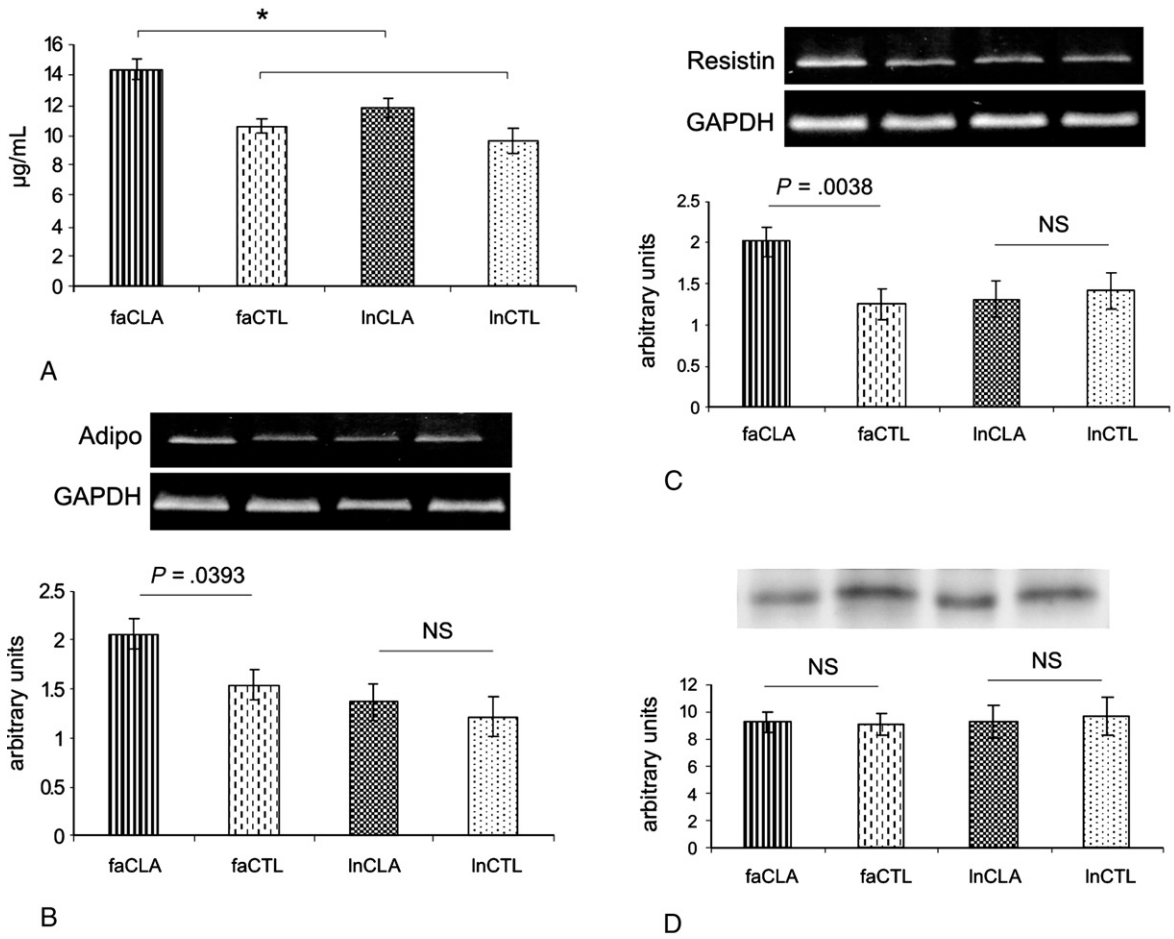

Fig. 4. Serum adiponectin (A), adipose adiponectin mRNA (B), and adipose resistin mRNA (C) and protein (D) levels of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks. Results for serum adiponectin (in micrograms per milliliter) are means ± SEM for n = 10 rats per group. *CLA-fed rats significantly different from CTL-fed rats ($P < .05$). Results for mRNA and protein levels expressed as means for arbitrary units for n = 4 rats per group for adiponectin mRNA levels and n = 10 rats per group for resistin mRNA and protein levels. *P* values from preplanned comparisons. NS indicates not significantly different; Adipo, adiponectin.

There were some interesting genotype differences in both the TAG and PL lipid classes in adipose tissue. In particular, the *fa/fa* genotype had a greater proportion of fatty acids that are saturated (eg, C14:0, C16:0, but not C18:0) and monounsaturated (eg, C16:1n-7 in TAG and PL and C16:1n-9, C18:1n-9, and C18:1n-1 in TAG only) and had less proportion of fatty acids that are polyunsaturated (attributable to C18:2n-6 but not other longer-chain fatty acids such as C20:4n-6) compared with the lean genotype.

4. Discussion

The current study showed that dietary CLA reduced peripheral insulin resistance in *fa/fa* Zucker rats in the fasting (Fig. 1) and fed [31] states despite the fact that these rats had greater visceral adipose mass [31]. Interestingly, these changes occurred in conjunction with a decrease in adipocyte size (Fig. 2) and a parallel increase in serum adiponectin levels (Fig. 4). Other CLA studies using insulin-resistant rats have shown that improved insulin sensitivity was achieved concurrently with reduced adipose mass (eg, ZDF rats [18,25], Otsuka Long-Evans Tokushima fatty rats [16]), but these studies did not assess adipocyte size. Even so, it has been previously shown that CLA treatment in rat models leads to an increase in serum adiponectin associated with reductions in hyperinsulinemia and hypertension [25] and hepatic steatosis [40]. In fact, adiponectin may directly decrease cell size by inhibiting differentiation, as demonstrated in mice overexpressing this gene [41]. It is also important to note that there were no adverse effects on insulin sensitivity in the lean Zucker rat in fasting (Fig. 1) or fed states [31], along with no effect on body weight or adiposity [31]. The adverse effects of CLA on insulin sensitivity have generally been reported in lean mice along with fat ablation and depletion of circulating adiponectin [20,21,26,42].

The current study is limited in that adipocyte size was measured in only one adipose depot. Others have shown that troglitazone, a known PPARγ agonist, corrects the hyperinsulinemia present in the *fa/fa* Zucker rat and leads to smaller adipocytes in both retroperitoneal and subcutaneous adipose depots [43]. Thus, it is plausible that CLA, similarly

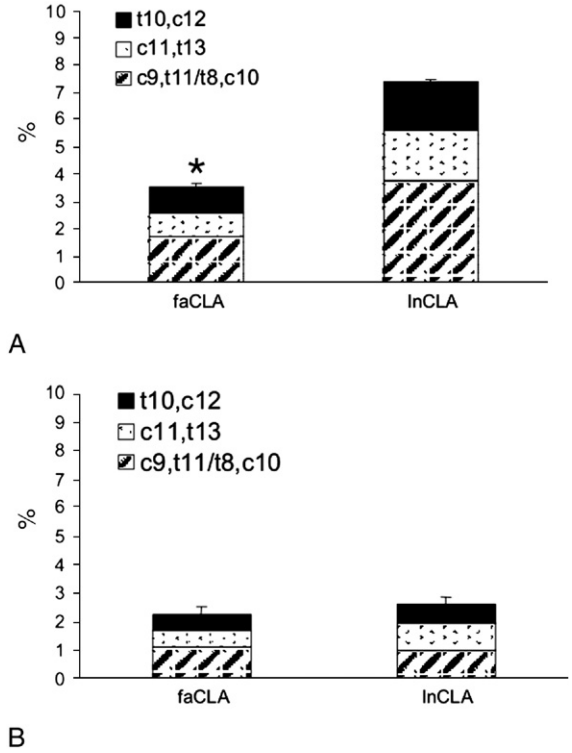

Fig. 5. The CLA isomers present in adipose tissue: TAG (A) and PL (B). Results (percentage = grams per 100 g total fatty acids) expressed as means for separate CLA isomers and means ± SEM for total CLA for n = 5 rats per group; c9,t11/t8,c10 = sum of c9,t11 and t8,c10 because these isomers coelute when analyzed by gas chromatography; however, c9,t11 is the predicted dominant isomer (17). *faCLA different from lnCLA ($P < .05$) for total CLA (TAG).

to troglitazone, affects PPAR-regulated adipocyte differentiation, metabolism, and/or lipid filling. However, CLA did not alter adipose PPARγ at either the gene or protein level of 2 of its target genes involved in lipid uptake and oxidation, LPL and UCP-2, respectively, in either genotype (data not shown). Adipophilin is a member of the perilipin-adipophilin-TIP47 family of proteins involved in the packaging of intracellular lipid droplets and is a marker of lipid filling [44,45]. Although others have reported more abundant adipose adipophilin mRNA levels in *fa/fa* compared with lean Zucker rats [46], there were no differences detected in adipose adipophilin protein levels due to dietary treatment or genotype. Other mechanisms independent of PPARγ and/or other markers of adipocyte differentiation and lipid filling (eg, proliferation) need to be explored to explain the smaller adipocytes present in the CLA-fed *fa/fa* rats.

The results of the current study indicate that CLA can dissociate adiposity from insulin resistance. Adipocyte size is closely linked to function. Normal adipocytes are capable of serving as a storage depot under conditions where lipid levels are high and can mobilize the stored lipids when they are required. In parallel, the adipocytes operate to control appetite (via leptin) and fatty acid oxidation in peripheral tissues such as liver and skeletal muscle (via adiponectin) [47]. Adiponectin can in turn influence glucose metabolism and insulin sensitivity [48]. In contrast, large (hypertrophied) adipocytes tend to exhibit characteristics that are markedly altered [49]. Consequently, a decrease in the number of large adipocytes in CLA-fed *fa/fa* rats (Fig. 2) suggests that adipose tissue function in this group is more normal; and this view is supported by the concomitant changes in adiponectin and leptin expression (Figs. 3 and 4). Thus, we have identified a plausible mechanism of action wherein CLA promotes a reduction in adipocyte size and consequently normalizes adipose function. Given that the lipid storage capacity remains unchanged, we speculate that the improvement produced by CLA is mediated solely through its effect on the endocrine actions of adipose tissue. Addressing this issue will not only provide information regarding the mechanism of action of CLA, it will provide additional insight into the relationship between adipokine and obesity-dependent morbidity.

In addition, the improved insulin sensitivity due to CLA in insulin-resistant rat models may be attributable to reduced lipid accumulation in other insulin-sensitive tissues such as liver [30] and muscle [18], thus demonstrating that examination of the multiorgan response to CLA is critical for explaining its effects. Changes in skeletal muscle metabolism, including improved insulin-mediated glucose transport [18,50], greater glycogen synthase activity [50], and increased fatty acid oxidation [17,51-53], have been associated with improved glucose tolerance in obese and normal-weight rats supplemented with CLA. In the present study, it is unclear whether dietary CLA affected muscle mass (ie, increased visceral adipose mass without changes in body weight may imply reduced muscle mass); however, CLA significantly reduces liver weight in this model [30]. Future studies should examine the effects of CLA on changes in whole-body composition in the insulin-resistant, obese state because muscle, adipose, and liver are all intricately involved in whole-body insulin sensitivity.

Although it has been generally accepted that circulating leptin concentrations correlate with adipose mass, the CLA-fed *fa/fa* rats in the current study had more adipose mass and less circulating leptin. These changes may be related to the decrease in adipocyte size because leptin secretion is markedly decreased from large, dysfunctional adipocytes [54]. Although it is unclear whether the reduction in serum leptin in CLA-fed *fa/fa* rats is physiologically significant, others have reported that CLA feeding reduces circulating leptin in insulin-resistant rat models [16-18] and white adipose tissue leptin mRNA levels in obese mice [55]. The reduction in serum leptin levels in CLA-fed vs CTL-fed *fa/fa* rats in the current study was not paralleled by changes in leptin mRNA or protein levels measured in whole adipose tissue and expressed relative to a housekeeping gene or total protein, respectively. The adipose leptin protein levels were actually lower in *fa/fa* vs lean CTL-fed rats (Fig. 3), suggesting less translation of leptin in the obese model. Our results show that the *fa/fa* CLA-fed rats had higher fasting

Table 2
Adipose TAG fatty acid composition of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks

Fatty acid	faCLA	faCTL	lnCLA	lnCTL	Pr > F		
% Composition *					Geno	Lipid	Geno × Lipid
C14:0	2.33 ± 0.03[a]	1.61 ± 0.03[b]	1.28 ± 0.10[c]	0.97 ± 0.05[d]	<.0001	<.0001	<.01
C16:0	33.44 ± 0.84[a]	27.36 ± 0.30[b]	21.86 ± 1.22[c]	19.47 ± 0.59[c]	<.0001	<.0001	<.01
C16:1n-9	0.44 ± 0.03	0.40 ± 0.02	0.31 ± 0.03	0.35 ± 0.01	<.01	NS	NS
C16:1n-7	6.81 ± 0.21	8.01 ± 0.24	1.92 ± 0.20	3.23 ± 0.20	<.0001	<.0001	NS
C18:0	2.78 ± 0.13	2.85 ± 0.11	2.87 ± 0.08	2.64 ± 0.03	NS	NS	NS
C18:1n-9	25.56 ± 0.34[b]	31.20 ± 0.30[a]	21.51 ± 0.21[c]	24.86 ± 0.56[b]	<.0001	<.0001	<.01
C18:1n-7	2.15 ± 0.04[bc]	2.93 ± 0.09[a]	1.95 ± 0.06[c]	2.33 ± 0.08[b]	<.0001	<.0001	<.05
C18:2n-6	17.70 ± 0.22	20.34 ± 0.28	34.43 ± 0.83	38.77 ± 0.50	<.0001	<.0001	NS
C18:3n-6	0.13 ± 0.03	0.19 ± 0.01	0.30 ± 0.01	0.36 ± 0.01	<.0001	<.01	NS
C18:3n-3	1.59 ± 0.03[d]	1.85 ± 0.05[c]	2.52 ± 0.07[b]	3.30 ± 0.13[a]	<.0001	<.0001	<.01
C20:4n-6	0.17 ± 0.04	0.56 ± 0.02	0.17 ± 0.05	0.68 ± 0.08	NS	<.0001	NS
Totals							
mg TG/g adipose	610.55 ± 25.70	648.47 ± 25.52	622.79 ± 35.03	639.15 ± 21.04	NS	NS	NS
SFA	38.73 ± 0.90[a]	31.87 ± 0.42[b]	26.24 ± 1.35[c]	23.25 ± 0.60[d]	<.0001	<.0001	<.05
MUFA	35.37 ± 0.59[b]	43.07 ± 0.45[a]	25.87 ± 0.40[d]	30.99 ± 0.60[c]	<.0001	<.0001	<.05
PUFA	24.35 ± 0.36	23.93 ± 0.38	46.25 ± 1.18	44.29 ± 0.67	<.0001	NS	NS
SAT + MUFA + PUFA	98.45 ± 0.15	98.87 ± 0.17	98.35 ± 0.25[a]	98.53 ± 0.23	NS	NS	NS
n-9	19.06 ± 0.29	21.65 ± 0.29	36.93 ± 0.90	40.09 ± 0.52	.0001	<.0001	NS
n-6	26.14 ± 0.37[b]	31.79 ± 0.31[a]	21.90 ± 0.20[c]	25.32 ± 0.57[b]	<.0001	<.0001	<.01
n-3	1.94 ± 0.05[c]	2.45 ± 0.011[b]	2.84 ± 0.19[b]	4.28 ± 0.23[a]	<.0001	<.0001	<.05
Ratios							
MUFA/SFA	0.90 ± 0.06	1.40 ± 0.07	1.02 ± 0.06	1.44 ± 0.04	NS	<.0001	NS
PUFA/SFA	0.62 ± 0.03	0.76 ± 0.04	1.91 ± 0.16	2.02 ± 0.07	<.0001	NS	NS
n-9/n-6	1.36 ± 0.01	1.51 ± 0.03	0.58 ± 0.01[c]	0.65 ± 0.01	<.0001	<.01	NS
n-6/n-3	9.55 ± 0.53[c]	9.51 ± 0.20[c]	13.13 ± 0.89[a]	10.10 ± 0.67[b]	<.01	<.0001	<.01
Δ9 DS index **	0.90 ± 0.03	1.30 ± 0.03	0.96 ± 0.05	1.28 ± 0.05	NS	<.0001	NS

Means ± SEM for n = 5 rats per group; main effects from ANOVA: genotype (*fa/fa* vs lean rats), lipid (0% vs 1.5% CLA), and lipid × genotype interaction. Means with different superscript letters are significantly different ($P < .05$) by Duncan multiple range test. faCLA indicates *fa/fa* rats fed 1.5% CLA; faCTL, *fa/fa* rats fed 0% CLA; lnCLA, lean rats fed 1.5% CLA; lnCTL, lean rats fed 0% CLA; Geno, genotype; Pr, probability; NS, not significant.

* % Composition = grams per 100 g total fatty acids. Only fatty acids >0.25% are reported; see Fig. 5 for CLA isomer distribution.

** Δ9 DS index = [(16:1n-7 + 18:1-n9)/(16:0 + 18:0)].

serum adiponectin and adipose tissue adiponectin mRNA levels compared with the *fa/fa* CTL-fed rats (Fig. 4), agreeing with data from ZDF rats [25]. The lean CLA-fed rats also had higher fasting serum adiponectin concentrations compared with lean CTL-fed rats, suggesting that CLA influences adiponectin independently of adipose mass and the other metabolic derangements of the *fa/fa* rat. CLA treatment in various mouse models reduces resistin mRNA levels in adipose tissue [55,56]. Reduced resistin levels have been associated with improved insulin sensitivity in mice, largely attributed again to inhibition of hepatic gluconeogenesis [7]. In the present study, the CLA-fed *fa/fa* rats had greater adipose tissue resistin mRNA levels compared with CTL-fed *fa/fa* rats; but this did not translate into differences in resistin at the protein level. The adipose tissue resistin levels did not explain the level of insulin resistance because *fa/fa* and lean CTL-fed rats had similar mRNA and protein expression. Further study should examine the effects of resistin on hepatic insulin resistance in this model. The adipokines reported in this study—leptin, adiponectin, and resistin—are only 3 of many factors (eg, tumor necrosis factor α, interleukins) that may alter insulin sensitivity.

Others have shown that the lipid environment of plasma and nuclear membranes may have a direct effect on insulin binding and/or signaling. For example, a greater PUFA/SFA ratio present in lipid membrane PLs may result in greater membrane fluidity and improved insulin binding [57]. Because we did not observe an effect of CLA on the adipose tissue PL fatty acid composition (Table 3), the positive effect that CLA had on HOMA in *fa/fa* rats cannot be explained by these factors. In the adipose tissue TAG of both genotypes, there were more SFAs and less MUFAs and PUFAs present in rats fed CLA (Table 2). This suggests reduced Δ9 DS activity in adipose tissue. Azain et al [58] also reported less MUFA content of adipose and smaller adipocyte cell size in CLA-fed rats, but the relationship with insulin sensitivity was not addressed. An effect of Δ9 DS on adiposity is not apparent in this study because both *fa/fa* and lean rats had this change independent of fat mass. The 20:4n-6 composition of adipose TAG was lower in both genotypes fed CLA (Table 2); however, there was no change apparent in the PL fraction (Table 3), which may be more relevant to a discussion regarding eicosanoid production. There may have been differences in the PL fatty acid composition of

Table 3
Adipose PL fatty acid composition of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks

Fatty acid	faCLA	faCTL	lnCLA	lnCTL	Pr > F		
% Composition *					Geno	Lipid	Geno × Lipid
C14:0	0.84 ± 0.23	0.51 ± 0.10	0.32 ± 0.08	0.23 ± 0.07	<.05	NS	NS
C16:0	23.38 ± 1.48	21.87 ± 0.58	16.81 ± 0.42	17.80 ± 0.54	<.0001	NS	NS
C16:1n-7	2.32 ± 0.51	2.03 ± 0.35	0.72 ± 0.20	0.91 ± 0.14	<.01	NS	NS
C18:0	17.39 ± 2.78	19.80 ± 2.02	18.48 ± 2.13	18.96 ± 1.88	NS	NS	NS
C18:1n-9	11.94 ± 2.17	12.50 ± 1.84	9.29 ± 1.53	9.63 ± 1.41	NS	NS	NS
C18:1n-7	1.37 ± 0.11	1.90 ± 0.15	1.27 ± 0.17	1.58 ± 0.06	NS	<.01	NS
C18:2n-6	15.03 ± 0.96	13.51 ± 1.03	22.10 ± 1.73	21.24 ± 2.06	<.01	NS	NS
C18:3n-3	0.67 ± 0.22	0.44 ± 0.12	0.63 ± 0.28	0.69 ± 0.25	NS	NS	NS
C20:0	0.20 ± 0.02	0.23 ± 0.02	0.30 ± 0.04	0.37 ± 0.05	<.01	NS	NS
C20:2n-6	0.45 ± 0.07	0.67 ± 0.04	0.63 ± 0.08	0.67 ± 0.09	NS	NS	NS
C20:3n-6	0.63 ± 0.13	0.70 ± 0.07	0.36 ± 0.03	0.38 ± 0.09	<.01	NS	NS
C20:4n-6	8.83 ± 2.17	11.26 ± 1.14	10.66 ± 1.20	11.15 ± 0.99	NS	NS	NS
C22:0	1.01 ± 0.17	1.13 ± 0.16	1.10 ± 0.32	1.40 ± 0.20	NS	NS	NS
C22:4n-6	0.52 ± 0.11	0.85 ± 0.14	0.67 ± 0.09	0.68 ± 0.08	NS	NS	NS
C22:5n-3	0.54 ± 0.09	0.50 ± 0.04	0.57 ± 0.09	0.42 ± 0.05	NS	NS	NS
C22:6n-3	3.32 ± 0.46	3.81 ± 0.58	2.58 ± 0.41	2.91 ± 0.56	NS	NS	NS
C24:0	1.42 ± 0.21	1.62 ± 0.20	1.80 ± 0.23	1.85 ± 0.26	NS	NS	NS
C24:1n-9	0.26 ± 0.06	0.53 ± 0.05	0.29 ± 0.04	0.36 ± 0.05	NS	<.01	NS
Totals							
mg PL/g adipose	1.07 ± 0.17	1.07 ± 0.12	1.07 ± 0.10	1.46 ± 0.33	NS	NS	NS
SFA	44.66 ± 1.68	45.56 ± 1.94	39.34 ± 2.39	40.76 ± 2.74	<.05	NS	NS
MUFA	16.23 ± 2.77	17.26 ± 2.23	11.93 ± 1.87	12.81 ± 1.65	NS	NS	NS
PUFA	32.37 ± 1.50	31.97 ± 0.76	40.98 ± 1.27	38.42 ± 1.93	<.0001	NS	NS
SAT + MUFA + PUFA	93.27 ± 0.28	94.79 ± 0.25	92.25 ± 0.77	91.99 ± 0.92	<.01	NS	NS
n-9	12.53 ± 2.15	13.33 ± 1.82	9.94 ± 1.55	10.32 ± 1.45	NS	NS	NS
n-6	26.08 ± 1.84	27.10 ± 1.03[b]	35.13 ± 1.06	34.21 ± 2.07	<.0001	NS	NS
n-3	4.58 ± 0.40	4.86 ± 0.48	3.92 ± 0.32	4.21 ± 0.36	NS	NS	NS
Ratios							
MUFA/SFA	0.37 ± 0.07	0.39 ± 0.06	0.32 ± 0.08	0.33 ± 0.07	NS	NS	NS
PUFA/SFA	0.73 ± 0.03	0.71 ± 0.04	1.07 ± 0.10	0.97 ± 0.11	<.01	NS	NS
n-9/n-6	0.51 ± 0.10	0.49 ± 0.07	0.28 ± 0.04	0.30 ± 0.03	<.01	NS	NS
n-6/n-3	6.01 ± 0.98	5.89 ± 0.83	9.22 ± 0.82	8.45 ± 1.05	<.01	NS	NS
Δ9 DS index **	0.36 ± 0.08	0.36 ± 0.06	0.30 ± 0.08	0.30 ± 0.08	NS	NS	NS

Means ± SEM for n = 5 rats per group; main effects from ANOVA: genotype (*fa/fa* vs lean rats), lipid (0% vs 1.5% CLA), and lipid × genotype interaction. Geno indicates genotype; Pr, probability; NS, not significant.

* % Composition = grams per 100 g total fatty acids. Only fatty acids >0.25% are reported; see Fig. 5 for CLA isomer distribution.

** Δ9 DS index = [(16:1n-7 + 18:1-n9)/(16:0 + 18:0).

specific cellular membranes (eg, plasma, nuclear), but only total adipose tissue PL was analyzed. The *fa/fa* rats had approximately one half the amount of CLA present in adipose tissue TAG compared with the lean rats (Fig. 5) when expressed as a percentage of total fatty acids, suggesting that metabolism (eg, oxidation, storage) of CLA and other fatty acids is altered in the obese, insulin-resistant state; but the implications of this are not apparent in the current study. However, the changes in fatty acid composition due to CLA may be coupled to the increase in adiponectin because this adipokine has been shown to promote fatty acid oxidation [48].

In summary, the present study showed that CLA improved insulin sensitivity in *fa/fa* Zucker rats. This was in conjunction with the presence of smaller adipocytes, despite a slightly greater visceral adipose mass that was previously reported. The adipokine status of the CLA-fed *fa/fa* rats was also favorably modified—fasting serum leptin was reduced, and adiponectin was increased. The positive effect of CLA did not seem to be mediated by alterations in PPARγ expression or adipose tissue fatty acid composition. This study certainly adds to current evidence that smaller adipocytes improve insulin sensitization; however, it disputes popular claims that CLA reduces fat mass.

Acknowledgment

We would like to acknowledge Marilyn Latta and Dennis Labossiere for their technical assistance with gas chromatography; Dr John Kramer's laboratory (Agriculture and Agrifood Canada, Guelph, Ontario, Canada) for analyzing CLA oil used for diet preparation and expertise with fatty acid analysis; Dan Chateau for statistical expertise; and Melissa Fuerst, Jennifer Zahradka, and the staff of the Animal Holding Facility (University of Manitoba) for assistance with animal care. Financial

support was provided by the Dairy Farmers of Canada, the Natural Sciences and Engineering Research Council, and the Manitoba Institute of Child Health, a division of The Children's Foundation of Manitoba.

References

[1] Tremblay MS, Katzmarzyk PT, Willms JD. Temporal trends in overweight and obesity in Canada 1981-1996. Int J Obes Relat Metab Disord 2002;26:538-43.

[2] Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. Arch Pediatr Adolesc Med 2003;157:821-7.

[3] Lustig RH, Sen S, Soberman JE, Valasquez-Meiyer PA. Obesity, leptin resistance, and the effects of insulin reduction. Int J Obes Relat Metab Disord 2004;28:1344-8.

[4] Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature 2001;18:307-12.

[5] Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. J Clin Endocrinol Metab 2003;88:4848-56.

[6] Fehmann HC, Heyn J. Plasma resistin levels in patients with type 1 and type 2 diabetes mellitus and in healthy controls. Horm Metab Res 2002;34:671-3.

[7] Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochem Biophys Res Commun 2001;28:561-4.

[8] Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator–activated receptor–gamma action in humans. Diabetes 2001;50:2199-202.

[9] Putz DM, Goldner WS, Bar RS, Haynes WG, Sivitz WI. Adiponectin and C-reactive protein in obesity, type 2 diabetes, and monodrug therapy. Metabolism 2003;53:1454-61.

[10] Ukropec J, Reseland JE, Gasperikova D, Demcakova E, Madsen L, Berge RK, et al. The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression. Lipids 2003;38:1023-9.

[11] Haugen F, Zahid N, Dalen KT, Hollung K, Nebb HI, Drevon CA. Resistin expression in 3T3-L1 adipocytes in reduced by arachidonic acid. J Lipid Res 2004;46:143-53.

[12] Pariza MW. Conjugated linoleic acid, a newly recognized nutrient. Chemical Index 1997:464-6.

[13] Pariza MW, Park Y, Cook ME. The biologically active isomers of conjugated linoleic acid. Prog Lipid Res 2001;20:283-98.

[14] Pariza MW. Perspective on the safety and effectiveness of conjugated linoleic acid. Am J Clin Nutr 2004;79:1132S-6S.

[15] Gaullier JM, Halse J, Hoivik HO, Hoye K, Syvertsen C, Nurminiemi M, et al. Six months supplementation with conjugated linoleic acid induces regional-specific fat mass decreases in overweight and obese. Br J Nutr 2007;97:550-60.

[16] Wang YM, Rahman SM, Nagao K, Han SY, Buang Y, Cha JY, et al. Conjugated linoleic acid reduces hepatic microsomal triacylglycerol transfer protein activity and hepatic triacylglycerol mass in obese rats. J Oleo Sc 2003;52:129-34.

[17] Rahman SM, Wang YM, Youtsumoto H, Cha JY, Han SY, Inoue S, et al. Effects of conjugated linoleic acid on serum leptin concentration, body-fat accumulation, and β-oxidation of fatty acid in OLETF rats. Nutrition 2001;17:385-90.

[18] Ryder JW, Portocarrero CP, Song XM, Cui L, Yu M, Combatasisaris T, et al. Isomer-specific antidiabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes 2001;50:1149-57.

[19] Yamasaki M, Ikeda A, Oji M, Tanak Y, Hirao A, Kasai M, et al. Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets. Nutrition 2003;19:30-5.

[20] Clement L, Porier H, Niot I, Bocher V, Guerreo-Millo M, Krief S, et al. Dietary trans-10, cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J Lipid Res 2002;43: 1400-9.

[21] Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, et al. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 2002;49:1534-42.

[22] Medina EA, Horn WF, Keim NL, Havel PJ, Benito P, Kelley DS, et al. Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite. Lipids 2000;35:783-8.

[23] Riserus U, Arner P, Brismar K, Bessby B. Treatment with dietary trans10cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care 2002;25:1516-21.

[24] Belury MA, Mahon A, Banni S. The conjugated linoleic acid (CLA) isomer, t10c12-CLA, is inversely associated with changes in body weight and serum leptin in subjects with type 2 diabetes mellitus. J Nutr 2003;133:257S-60S.

[25] Nagao K, Inoue N, Wang YM, Yanagita T. Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (fa/fa) rats. Biochem Biophys Res Commun 2003;310:562-6.

[26] Purushotam A, Wendel A, Liu L-F, Belury M. Maintenance of adiponectin attenuates insulin resistance induced by dietary CLA in mice. J Lipid Res 2007;48:444-52.

[27] Smith SB, Hively TS, Cortese GM, Han JJ, Chung KY, Castenada P, et al. Conjugated linoleic acid depresses the Δ9 desaturase index and stearoyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. J Anim Sci 2002;80:2110-5.

[28] Choi Y, Kim YC, Han YB, Park Y, Pariza MW, Ntambi JM. The trans 10, cis 12 isomer of conjugated linoleic acid downregulates stearoyl-coA desaturase 1 gene expression in 3T3-L1 adipocytes. J Nutr 2000;130:1920-4.

[29] Lee KN, Pariza MW, Ntambi JM. Conjugated linoleic acid decreases stearoyl-coA desaturase mRNA expression. Biochem Biophys Res Commun 1998;248:817-21.

[30] Noto A, Zahradka P, Yurkova N, Xie X, Nitschman E, Ogborn M, et al. Conjugated linoleic acid reduces hepatic steatosis, improves liver function, and favorably modifies lipid metabolism in obese insulin-resistant rats. Lipids 2006;41:179-88.

[31] Noto A, Zahradka P, Ryz NR, Yurkova N, Xie X, Taylor CG. Dietary conjugated linoleic acid preserves pancreatic function and reduces inflammatory markers in obese, insulin resistant rats. Metabolism 2007;56:142-51.

[32] Canadian Council on Animal Care (CCAC). Guide to the care and use of experimental animals [cited Feb. 25, 2005]. Available from: http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GUIDES/ENGLISH/toc_v1.htm, 1993.

[33] Matthews DR, Hosker JP, Rudenki AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

[34] Zahradka P, Yurkova N, Litchie B, Moon MC, Del Rizzo DF, Taylor CG. Activation of peroxisome proliferator–activated receptor α and γ1 inhibits human smooth muscle cell proliferation. Mol Cell Biochem 2004;246:105-10.

[35] Yau L, Elliot T, Lalonde C, Zahradka P. Repression of phosphoenolpyruvate carboxykinase gene activity by insulin is blocked by 3-aminobenzamide but not by PD128763, a selective inhibitor of poly (ADP-ribose) polymerase. Eur J Biochem 1998;253:91-100.

[36] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-7.
[37] Tallman DL, Taylor CG. Effects of dietary fat and zinc on adiposity, serum leptin and adipose fatty acid composition in C57BL/6J mice. J Nutr Biochem 2002;14:17-23.
[38] Christie WW. Methylation of fatty acids. Preparation of methyl esters—part 2 [cited August 30, 2002]. Available from: http://www.lipid.co.uk/infores/topics/methests/index.htm, 1990.
[39] Kramer JKG, Felner V, Dugan MER, Sauer FD, Mossoba MM, Yurawecz MP. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids 1997;32:1219-28.
[40] Nagao K, Inoue N, Wang YM, Shirouchi B, Yanagita T. Dietary conjugated linoleic acid alleviates nonalcoholic fatty liver disease in Zucker (fa/fa) rats. J Nutr 2005;13:9-13.
[41] Bauche IB, El Mkadem SA, Pottier AM, Senou M, Many MC, et al. Overexpression of adiponectin targeted to adipose tissue in transgenic mice: impaired adipocyte differentiation. Endocrinology 2007;148:1539-49.
[42] Hargrave KM, Azin MJ, Kachman SD, Miner JL. Conjugated linoleic acid does not improve insulin tolerance in mice. Obes Res 2003;11:1104-15.
[43] Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, et al. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest 1998;101:1354-61.
[44] Wolins NE, Quaynor BK, Skinner JR, Schoenfish MF, Tzekov A, Bickel PE. S3-12, adipophilin, and TIP47 package lipid in adipocytes. J Biol Chem 2005:19146-55.
[45] Heid HW, Moll R, Schwetlick I, Rackwitz HR, Keenan TW. Adipophilin is a specific marker of lipid accumulation in diverse cell types and diseases. Cell Tissue Res 1998;294:309-21.
[46] Dalen KT, Schoonjans K, Ulven SM, Weedon-Fekjaer MS, Bentzen TG, Koutnikova H, et al. Adipose tissue expression of the lipid droplet-associating proteins S3-12 and perilipin is controlled by peroxisome proliferator–activated receptor–gamma. Diabetes 2004;53:1243-52.
[47] Whitehead JP, Richards AA, Hickman IJ, MacDonald GA, Prins JB. Adiponectin—a key adipokine in the metabolic syndrome. Diabetes Obes Metab 2005;8:264-80.
[48] Karbowska J, Kochan Z. Role of adiponectin in the regulation of carbohydrate and lipid metabolism. J Physiol Pharmacol 2006;57:103-13.
[49] Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipocyte expression and secretion. J Clin Endocrinol Metab 2007;92:1023-33.
[50] Teachey MK, Taylor ZC, Maier T, Saengsirisuwan V, Sloniger JA, Jacob S, et al. Interactions of conjugated linoleic acid and lipoic acid on insulin action in the obese Zucker rat. Metabolism 2003;52:1167-74.
[51] Henriksen EJ, Teachey MK, Taylor ZC, Jacob S, Ptock A, Kramer K, et al. Isomer-specific actions of conjugated linoleic acid on muscle glucose transport in the obese Zucker rat. Am J Physiol Endocrinol Metab 2003;285:E98-E105.
[52] Ealey KN, El-Sohemy A, Archer MC. Effects of dietary conjugated linoleic acid on the expression of uncoupling proteins in mice and rats. Lipids 2002;37:853-61.
[53] Choi JS, Jun MH, Park HS, Song J. Effect of conjugated linoleic acid isomers on insulin resistance and mRNA levels of genes regulating energy metabolism in high-fat–fed rats. Nutrition 2004;20:1008-17.
[54] Guo K-Y, Halo P, Leibel RL, Zhang Y. Effects of obesity on the relationship of leptin mRNA expression and adipocyte size in anatomically distinct fat depots in mice. Am J Physiol Regul Integr Comp Physiol 2004;287:112-9.
[55] Ohashi A, Matsushita Y, Kimura K, Miyashita K, Saito M. Conjugated linoleic acid deteriorates insulin resistance in obese/diabetic mice in association with decreased production of adiponectin and leptin. J Nutr Sci Vitaminol 2004;50:416-21.
[56] House RL, Cassady JP, Eisen EJ, Eling TE, Collins JB, Grissom SF, et al. Functional genomic characterization of delipidation elicited by trans-10, cis-12-conjugated linoleic acid (t10c12-CLA) in a polygenic obese line of mice. Physiol Genomics 2005;21:351-61.
[57] Field CJ, Ryan EA, Thomson ABR, Clandinin MT. Diet fat composition alters membrane phospholipid composition, insulin binding and glucose metabolism in adipocytes from control and diabetic animals. J Biol Chem 1990;265:11143-50.
[58] Azain MJ, Hausman DB, Sisk MB, Flatt WB, Jewell DE. Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. J Nutr 2000;130:1548-54.