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Dietary conjugated linoleic acid preserves pancreatic function and reduces inflammatory markers in obese, insulin-resistant rats

Amy Noto[a], Peter Zahradka[a,b], Natasha R. Ryz[a], Natalia Yurkova[b]
Xueping Xie[b], 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*

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Abstract

Pancreatic preservation is an important part of diabetes management that may occur with improved peripheral insulin sensitivity and attenuated low-grade adipose tissue inflammation. The objective of the current study was to determine the response of obese, insulin-resistant *fa/fa* Zucker rats vs lean controls to dietary conjugated linoleic acid (CLA) supplementation with respect to pancreatic islet size, insulin resistance, and markers of inflammation and adipose glucose uptake. Six-week-old *fa/fa* and lean Zucker rats (n = 20 per genotype) were fed either a 1.5% CLA mixture or control diet for 8 weeks. Oral glucose tolerance testing was conducted at 7.5 weeks. Fasting serum haptoglobin, insulin, and C-peptide were assayed, and select messenger RNA (mRNA) and protein markers of inflammation and glucose metabolism were measured in adipose and liver tissues. CLA-fed *fa/fa* Zucker rats had smaller islet cell size, improved oral glucose tolerance and insulinemia, and attenuated serum haptoglobin levels compared with control-fed *fa/fa* Zucker rats, despite no differences in body weight and a slightly higher visceral adipose mass. CLA did not alter insulin sensitivity or islet size in lean Zucker rats. The CLA-fed *fa/fa* rats also had greater adipose glucose transporter-4 mRNA and less adipose tumor necrosis factor α mRNA and protein compared with control-fed *fa/fa* rats. In contrast, other markers of inflammation and glucose metabolism including adipose macrophage inflammatory factor, macrophage inflammatory protein-2, and liver pyruvate carboxylase and pyruvate dehydrogenase kinase 4 were not significantly changed. These results suggest that CLA supplementation preserved pancreatic function in conjunction with improved peripheral glucose use and reduced inflammation in *fa/fa* Zucker rats.

1. Introduction

Type 2 diabetes mellitus is a major health concern that is occurring not only at higher rates [1] but also at younger ages [2]. Obesity, a major predisposing risk factor for type 2 diabetes mellitus, is associated with hyperinsulinemia leading to beta-cell hypertrophy [3,4]. Progression of type 2 diabetes mellitus results in net beta-cell death and pancreas exhaustion [4] requiring insulin therapy. Thus, intervention strategies that target both pancreatic preservation and insulin sensitization are important to maintain optimal blood glucose control in the prediabetes stage and during diabetes management to reduce progression to pancreatic exhaustion.

The relationship between the pancreas and adipose tissue is imperative. The thiazolidinediones (TZDs) are a group of peroxisome proliferator–activated receptor γ (PPARγ) agonists that are known to improve insulin sensitization in adipose tissue by promoting adipocyte differentiation and thus increasing the number of small adipocytes [5,6]. The TZDs also preserve beta-cell mass in Zucker diabetic fatty rats and db/db mice [7,8]. Dietary intervention with conjugated linoleic acids (CLAs), 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 linoleic acid (C18:2), similarly increases the number of small adipocytes present in adipose tissue in growing rats [9], although further studies are required in diabetic models. Therapeutic approaches that improve peripheral insulin sensitization may reduce the demand on the pancreas for insulin and reduce the likelihood of gluco- and lipotoxicity within the pancreas [10].

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

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Table 1
Diet formulations

Ingredients[a] (g/100 g)	CLA diet	CTL diet
Cornstarch[b]	348	348
Maltodextrin	132	132
Sucrose	100	100
Egg white	213	213
Cellulose	50	50
AIN-93M mineral mix (zinc free)	35	35
Zinc premix[c]	10	10
Potassium phosphate[d]	5.4	5.4
AIN-93-VX vitamin mix	10	10
Biotin premix[e]	10	10
Choline bitartate	2.5	2.5
tert-Butylhydroquinone[f]	0.014	0.014
Soybean oil[g]	70	85
CLA oil[h]	15	0

[a] Ingredients purchased from Harlan Teklad (Madison, WI) unless otherwise indicated.
[b] Best Foods (Etobicoke, Ontario, Canada).
[c] Zinc premix = 5.775 g/kg zinc carbonate in cornstarch.
[d] Provides mineral content equivalent to AIN-93G diet (Fisher Scientific, Fair Lawn, NJ).
[e] Biotin premix = 200 mg/kg biotin in cornstarch because egg white was the protein source.
[f] Fisher Scientific.
[g] Vita Health (Winnipeg, Manitoba, Canada).
[h] Nu-Chek Prep (Elyslan, MN).

A positive relationship between low-grade inflammation and insulin resistance has been established in epidemiologic studies [11,12]; however, mechanistically, the relationship is not as clear. It has been shown that TZDs, angiotensin-converting enzyme inhibitors, and angiotensin receptor antagonists reduce the relative risk of developing diabetes [13] and that markers of low-grade inflammation are blunted by treatment with PPARγ ligands such as the TZDs [14]. Tumor necrosis factor α (TNF-α) has a well-established negative impact on insulin sensitivity at least partially via its role as a proinflammatory adipokine [15]. Haptoglobin and angiotensinogen are also proinflammatory adipokines that may affect insulin metabolism [16,17]. Macrophage infiltration into the white adipose tissue occurs before insulin resistance in obese mice [18], and macrophages are a source of inflammatory mediators, such as macrophage inflammatory protein-2 (MIP-2) and macrophage migration inhibitory factor (MIF). It is possible that dietary factors, including CLA, may directly modulate proinflammatory factors associated with insulin resistance.

We hypothesized that CLA, a PPAR agonist [19], may have similar effects as the TZDs for preserving islet cell size, improving insulinemia and attenuating inflammation. Thus, the objective of the current 8-week study was to determine the response of obese, insulin-resistant *fa/fa* Zucker rats vs lean controls to dietary CLA supplementation with respect to pancreatic mass and islet cell size, insulin resistance, and markers of inflammation and adipose glucose uptake.

2. Methods

2.1. Animals and diets

After a 5- to 7-day acclimatization period, *fa/fa* (fa) and lean (ln) 6-week-old Zucker rats (n = 20 per genotype; Charles River, St Constant, Quebec, Canada) were randomly assigned to receive either the CLA diet (faCLA and lnCLA groups) or control diet (faCTL and lnCTL groups) for 8 weeks. The protocol for the animal care procedures was approved by the University of Manitoba Protocol Management and Review Committee. Hepatic indices for these rats have been reported elsewhere [20].

The diet formulation and fatty acid composition of the diets are shown in Tables 1 and 2. The predominant CLA isomers in the CLA diet were t10,c12 (4.6% of total fatty acids), c9,t11 (4.5%), c11,t13 (2.8%), and t8,c10 (2.4%). The dry ingredients for the diets were premixed and stored at 4°C. The CLA oil was aliquoted and stored at −20°C. Fresh, 6-kg batches of the diet, containing oil, were prepared weekly and stored at −20°C until used. Rats were given new feed cups with fresh feed 3 times per week. Rats were weighed weekly.

2.2. Oral glucose tolerance testing

After 7.5 weeks of feeding the test diets, oral glucose tolerance testing (OGTT) was completed after a 5-hour fast. Feed cups were removed at 8:00 AM and testing began at 1:00 PM. Initial blood samples (t = 0) were collected from the saphenous vein, followed by administration of an oral glucose dose (1 g glucose per kilogram body weight). Additional blood samples were collected at t = 15, t = 30 and t = 60 minutes after glucose administration. Blood samples were stored on ice until centrifuged at 1500 rpm for

Table 2
Fatty acid composition of experimental diets

	CLA	CTL
% Composition[a]		
C16:0	8.9	10.7
C18:0	3.7	4.5
C18:1n-9	18.6	22.6
C18:2n-6	42.1	51.0
C18:3n-3	5.6	6.8
CLA c9,t11	4.5	0.0
CLA t8,c10	2.4	0.0
CLA c11,t13	2.8	0.0
CLA t10,c12	4.6	0.0
Total SFA	13.3	16.1
Total MUFA	19.9	24.1
Total PUFA	63.7	58.4
Total n-9	18.6	22.7
Total n-6	43.0	51.6
Total n-3	5.6	6.8
Ratios		
PUFA/SFA	4.8	3.6
n-6/n-3	7.7	7.6

SFA indicates saturated; MUFA, monounsaturated; PUFA, polyunsaturated fatty acids.
[a] % Composition = g/100 g total fatty acids.

Table 3
PCR primers

Gene	GenBank accession no.	Sense	Antisense
PC[a]	U36585	(s)-tgcattaaggacatggcagg	(as)-ttcatggtagccgtgcaatc
PDK-4[a]	NM053551	(s)-caggaaacccaagccacatt	(as)-tttcttgatgctcgaccgtg
Haptoglobin[a]	AH002183	(s)-aatcgcacagccaaggacat	(as)-tctttggaaggcaggcagat
TNF-α[b]	X66539	(s)-cagcagatgggctgtacctt	(as)-ccggactccgtgatgtctaa
GLUT-4[b]	NM012751	(s)-aacgccccacagaaagtgat	(as)-aatgaggaaccgtccgagaa
MIP-2[a]	X65647	(s)-gggtgtcattttcctgaccaag	(as)-ttcttggacccaggtctctttg
MIF[a]	U62326	(s)-cggaccagctcatgacttttagt	(as)-ggctgcgttcatgtcgtaatag
L32	M13501	(s)-taagcgaaactggcggaaac	(as)-gctcgtctttctacgatggtt
GAPDH	NM017008	(s)-cgctgtgaacggatttggccgtat	(as)-agccttctccatggtggtgaagac

[a] L32 was the housekeeping gene.
[b] GAPDH was the housekeeping gene.

15 minutes at 4°C. Serum was aliquoted and stored at −80°C until glucose and insulin concentrations were determined. Rats were preexposed to the OGTT procedure, without blood collection, to reduce the stress response during the actual testing period.

Area under the curve (AUC) was calculated for both glucose (AUCg) and insulin (AUCi) from OGTT results by the trapezoidal method [21], a method that has been validated for glucose tolerance testing [22]. The glucose-insulin index was calculated as AUCg × AUCi [23,24] and used as a surrogate marker for insulin sensitivity.

2.3. Tissue collection

With cessation of the study period, rats were fasted overnight and euthanized by carbon dioxide asphyxiation and cervical dislocation according to the Canadian Council on Animal Care Guidelines [25]. Trunk blood was collected and immediately placed on ice until centrifuged to separate the serum fraction, which was stored at −80°C. Dissected organs including epididymal adipose and pancreas were weighed, immediately frozen in liquid nitrogen, and subsequently stored at −80°C. A portion of the pancreas tail was formalin fixed and paraffin blocks were prepared (Pathology, Health Science Centre, Winnipeg, Manitoba, Canada).

2.4. Serum biochemistry

Enzymatic colorimetric kits were used to determine OGTT serum glucose (Sigma Chemicals, St Louis, MO) and fasting serum haptoglobin (Tri-Delta Diagnostics, Wicklow, Ireland). Radioimmunoassays were completed for fasting and OGTT serum insulin, and fasting C-peptide (Linco Research, St Charles, MO).

2.5. Pancreas islet size

Islet size was determined by insulin localization in pancreas using standard procedures for indirect immunoperoxidase staining. Briefly, pancreas sections were incubated with CYTO Q Background Buster (Innovex Biosciences, Richmond, CA) for 20 minutes, monoclonal mouse anti-insulin antibody (1:50 dilution; clone E2/E3; Innovex Biosciences) for 20 minutes, secondary linking antibody for 10 minutes, peroxidase-streptavidin tertiary antibody for 10 minutes, 3,3′ -diaminobenzidine tetrahydrochloride substrate solution for 5 minutes (all from Stat Q kit, Innovex Biosciences), and 3% hematoxylin for 1 minute. Specificity of staining was confirmed by omission of the anti-insulin antibody (phosphate-buffered saline buffer only) and substitution of the anti-insulin antibody with mouse IgG1 κ (clone DAK-GO1; DAKO, Mississauga, Ontario, Canada). Computer images of immunostained sections were obtained and insulin-positive islet cell perimeter and area were quantified by using Northern Eclipse software (Empix Imaging, Toronto, Ontario, Canada).

2.6. Reverse transcription–polymerase chain reaction for analysis of messenger RNA

Markers of hepatic glucose oxidation and synthesis (pyruvate carboxylase [PC] and pyruvate dehydrogenase

Table 4
Feed intake, body weight, and adipose tissue-body weight ratios of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks

	faCLA	faCTL	lnCLA	lnCTL	Geno	Lipid	Geno × Lipid
Total feed intake (g)	1498 ± 40	1578 ± 35	1101 ± 34	1055 ± 28	<.0001	NS	NS
Final body weight (g)	552 ± 14	582 ± 12	355 ± 8	360 ± 9	<.0001	NS	NS
Body weight gain (g)	371 ± 10	394 ± 11	213 ± 5	221 ± 6	<.0001	NS	NS
Epididymal adipose tissue (g/100 g bwt)	3.4 ± 0.1[a]	2.8 ± 0.1[b]	1.7 ± 0.2[c]	1.7 ± 0.1[c]	<.0001	<.05	<.05
Perirenal adipose tissue (g/100 g bwt)	5.0 ± 0.2[a]	4.2 ± 0.1[b]	1.9 ± 0.2[c]	2.1 ± 0.2[c]	<.0001	<.05	<.01
Visceral adipose tissue* (g/100 g bwt)	8.5 ± 0.2[a]	7.0 ± 0.2[b]	3.6 ± 0.3[c]	3.7 ± 0.3[c]	<.0001	<.05	<.01

Values are expressed as means ± SEM for n = 10 per group. Main effects from ANOVA: Geno, genotype (*fa/fa* vs lean rats), Lipid (0% vs 1.5% CLA), and Geno × Lipid, 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; bwt, body weight; NS, not significant.

* Visceral adipose tissue = epididymal + perirenal adipose tissue.

kinase-4 [PDK-4] messenger RNA [mRNA] levels, respectively), hepatic acute phase inflammation (haptoglobin mRNA levels), adipose tissue inflammation (TNF-α, MIP-2, and MIF mRNA levels) and adipose tissue glucose transport (GLUT-4 mRNA levels), and a housekeeping gene (GAPDH or ribosomal protein L32 [rpL32]) were assessed by reverse transcription–polymerase chain reaction (RT-PCR) using previously published procedures [26]. Briefly, total RNA was isolated from epididymal adipose tissue by using TRIzol (Invitrogen, Burlington, ON, Canada). 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). The GenBank accession number and sequences for the PCR primers (Invitrogen Life Technologies, Burlington, Ontario, Canada) are shown in Table 3. Vistra green (Amersham, Baie d'Urfe, Quebec, Canada) was used to visualize RT-PCR products on 2% agarose gels by electrophoresis. Relative band intensity was quantified by densitometric scanning of the gel (Storm Fluorimager, Amersham) and results are expressed as arbitrary units relative to GAPDH or rpL32. The mRNA levels of GAPDH or rpL32 did not differ in the adipose tissue of lean vs *fa/fa* Zucker rats.

2.7. Western blot analysis

Frozen epididymal adipose tissue was ground with a mortar and pestle and 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 [27]. Horseradish peroxidase–conjugated secondary antibody was detected using the ECL chemiluminescent system (Amersham). Band intensity was quantified by scanning densitometry. Antibodies used included TNF-α, GLUT-4 (Santa Cruz Biotechnology, Santa Cruz, CA), angiotensinogen (Research Diagnostics, Concord, MA), and p42/44 mitogen-activated protein kinase (Cell Signaling Technology, Danvers, MA) as the loading control.

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 of preplanned comparisons (with the additional effect of gel treated as a block and 4 treatments per block). For mRNA data, the housekeeping gene was analyzed as a covariate. The significance level was $P < .05$.

3. Results

3.1. Feed intake, body weight, and adiposity

Dietary CLA supplementation did not alter feed intake, weight gain, or final body weight within each genotype (Table 4). However, the faCLA rats had greater relative

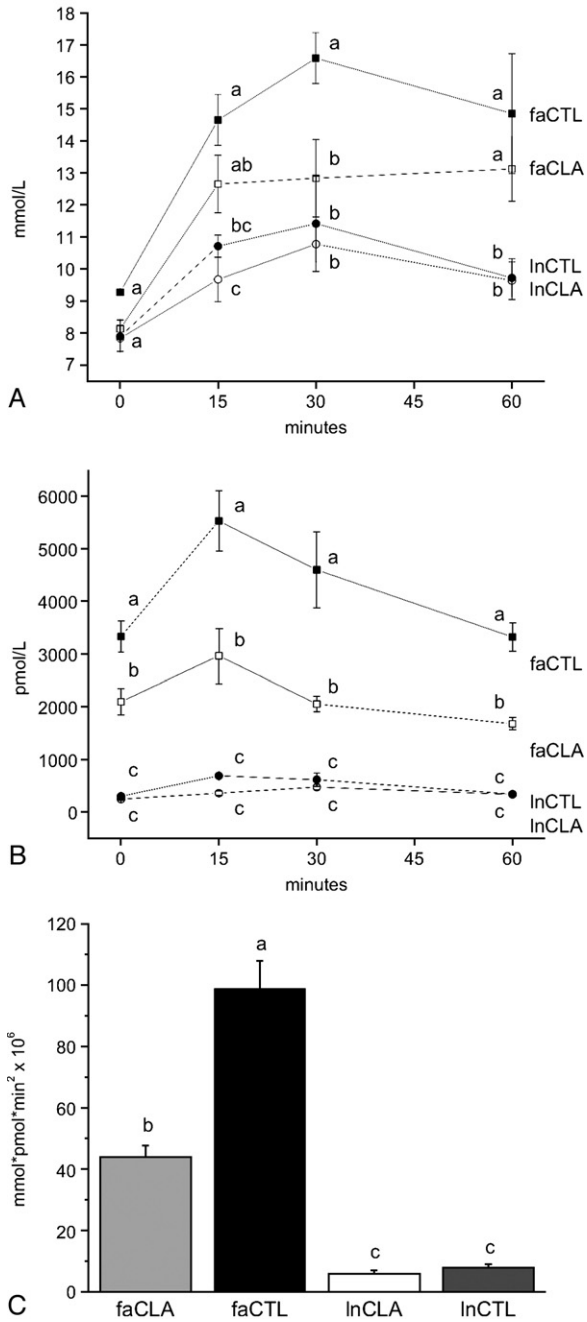


Fig. 1. Oral glucose tolerance testing: serum glucose (A), serum insulin (B), and glucose-insulin index (C) of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks. Values are means ± SEM for n = 8 to 10 rats per group; means with different superscript letters are significantly different ($P < .05$) by Duncan multiple range test. There was a significant ($P < .05$) genotype × lipid interaction for serum glucose (A), serum insulin (B), and glucose-insulin index (C). 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.

Table 5
Fasting serum insulin and C-peptide in *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks

	faCLA	faCTL	lnCLA	lnCTL	Geno	Lipid	Geno × Lipid
Insulin (pmol/L)	2159 ± 153[b]	6323 ± 973[a]	213 ± 25[c]	214 ± 25[c]	<.0001	.0002	.0002
C-Peptide (pmol/L)	5677 ± 652[b]	12388 ± 1394[a]	1277 ± 196[c]	1337 ± 151[c]	<.0001	.0001	.0001
Insulin–C-peptide	2.59 ± 0.22	2.13 ± 0.18	5.96 ± 0.73	7.30 ± 1.52	<.0001	NS	NS

Abbreviations are explained in the first footnote to Table 4.

epididymal, perirenal, and visceral adipose tissue weights than faCTL rats.

3.2. Glucose tolerance and insulin sensitivity

Conjugated linoleic acid improved insulin sensitivity in the *fa/fa* genotype based on results from fasting and OGTT serum, despite no differences in body weight between CLA- and CTL-fed *fa/fa* rats. During OGTT, the CLA-fed *fa/fa* rats had lower serum glucose concentrations at t = 30 minutes and lower serum insulin concentrations at all time points vs the CTL-fed *fa/fa* rats (Fig. 1A and B). AUCg and AUCi (data not shown) and the glucose-insulin index (AUCg × AUCi) were lower in *fa/fa* rats fed CLA compared with *fa/fa* rats fed the CTL diet (Fig. 1C). Fasting serum insulin and C-peptide concentrations were 65% and 54% lower, respectively, in CLA-fed *fa/fa* rats compared with CTL-fed *fa/fa* rats (Table 5). The insulin–C-peptide ratio was lower in the *fa/fa* rats compared with the lean rats. Fasting serum glucose and hepatic markers of glucose oxidation (PC mRNA) and synthesis (PDK-4 mRNA) were not different between the *fa/fa* groups (data not shown). GLUT-4 mRNA and protein were more abundant in the adipose tissue of CLA-fed *fa/fa* rats compared with CTL-fed *fa/fa* rats (Fig. 2). In addition, the CLA-fed lean rats had more GLUT-4 protein in adipose tissue than the CTL-fed lean rats.

3.3. Pancreas mass and islet size

The pancreas mass and pancreas-body weight ratio were not different among treatment groups or between genotypes (Fig. 3). The CLA-fed *fa/fa* rats had both smaller perimeter (46% reduction) and area (33% reduction) of islet cells compared with the CTL-fed *fa/fa* rats, suggesting pancreatic preservation by CLA. Islet cell perimeter and area did not differ between the CLA- and CTL-fed lean rats.

3.4. Inflammatory factors from serum, liver, and adipose

The CLA-fed *fa/fa* rats had lower fasting serum haptoglobin compared with CTL-fed *fa/fa* rats (Fig. 4). This result was not paralleled by changes in liver haptoglobin mRNA. Haptoglobin is also produced by adipose tissue, but adipose haptoglobin mRNA was not detectable by the methods used in the current study.

In adipose tissue, TNF-α mRNA and protein were lower in CLA-fed *fa/fa* rats compared with CTL-fed *fa/fa* rats (Fig. 5). Adipose TNF-α protein was also lower in CLA-fed lean rats compared with CTL-fed lean rats. There were no apparent effects of CLA on adipose MIP-2 and MIF. Interestingly, CTL-fed *fa/fa* rats had higher mRNA levels of TNF-α and MIP-2 than CTL-fed lean rats. Adipose angiotensinogen protein levels tended (P = .0872) to be

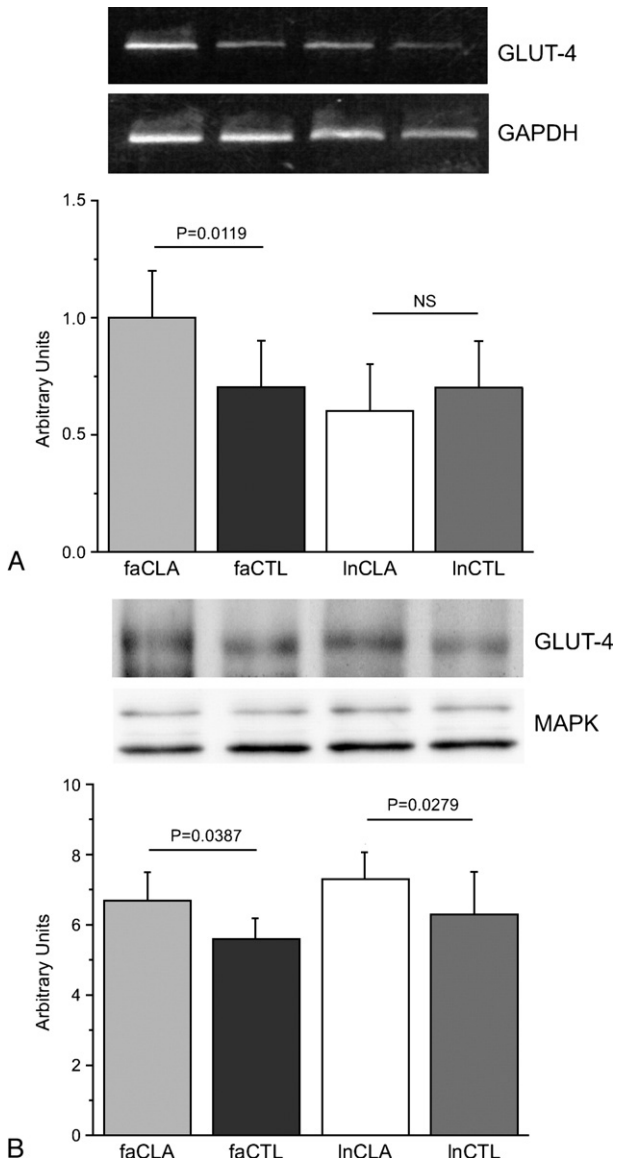
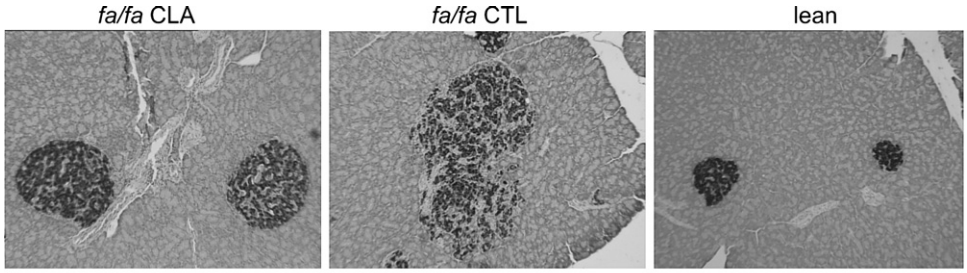


Fig. 2. Adipose GLUT-4 mRNA (A) and protein (B) of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks. Results for mRNA and protein levels are expressed as means for arbitrary units for n = 8 rats per group for GLUT-4 mRNA, n = 10 rats per group for GLUT-4 protein levels; mitogen-activated protein kinase was the loading control; P values from preplanned comparisons. See Fig. 1 for abbreviations.



	faCLA	faCTL	lnCLA	lnCTL
Islet cell perimeter ($\mu mx10^3$)	$3.7 \pm .0.7^{b}$	6.9 ± 0.8^{a}	2.1 ± 0.3^{bc}	1.6 ± 0.3^{c}
Islet cell area (μm^2x10^4)	3.6 ± 0.6^{b}	5.4 ± 0.8^{a}	2.1 ± 0.3^{c}	1.6 ± 0.2^{c}
Pancreas mass (g)	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Pancreas/body weight ($x10^{-3}$)	2.5 ± 0.1	2.3 ± 0.1	4.2 ± 0.2	4.0 ± 0.2

Fig. 3. Pancreas mass and islet size 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 superscript letters are significantly different ($P < .05$) by Duncan's multiple range test. See Fig. 1 for abbreviations.

lower in *fa/fa* rats fed CLA compared with *fa/fa* rats fed the CTL diet.

4. Discussion

Preserving pancreatic function and improving peripheral use of insulin to optimize glycemic control are major goals in the clinical management of type 2 diabetes mellitus. In the current study, dietary CLA both preserved pancreatic islet size (Fig. 3) and improved peripheral glucose use, assessed by oral glucose tolerance, in the *fa/fa* Zucker rat (Fig. 1), despite no differences in body weight and a slightly higher visceral adipose mass (Table 4). In CLA-fed *fa/fa* rats, the improved peripheral glucose utilization likely contributed to the reduction in pancreatic insulin production as indicated by the smaller islet size (ie, reduced beta-cell hypertrophy) and lower serum insulin (fasting and during OGTT) and C-peptide concentrations (Table 5 and Fig. 1) compared with the CTL-fed *fa/fa* rats. On the other hand, CLA feeding was associated with increased pancreatic beta-cell number in a mouse model that displays fat ablation, hepatic steatosis, and insulin resistance [28].

The improved peripheral glucose disposal in CLA-fed *fa/fa* rats was also associated with the presence of more adipose GLUT-4 mRNA and protein (Fig. 2) compared with CTL-fed *fa/fa* rats. This suggests that CLA enhances insulin sensitization given that GLUT-4 translocation is a major outcome of the insulin-signaling pathway [29], although membrane-bound and intracellular GLUT-4 levels were not differentiated in the current study. In lean rats, the higher levels of GLUT-4 protein due to CLA feeding may not affect glucose disposal (and AUC) until the glucose load exceeds the capacity of GLUT-4.

Furthermore, there was no difference between the *fa/fa* groups in liver glucose synthesis and oxidation as marked by hepatic PC and PDK4 mRNA, respectively, emphasizing the important role of peripheral glucose use. Likewise,

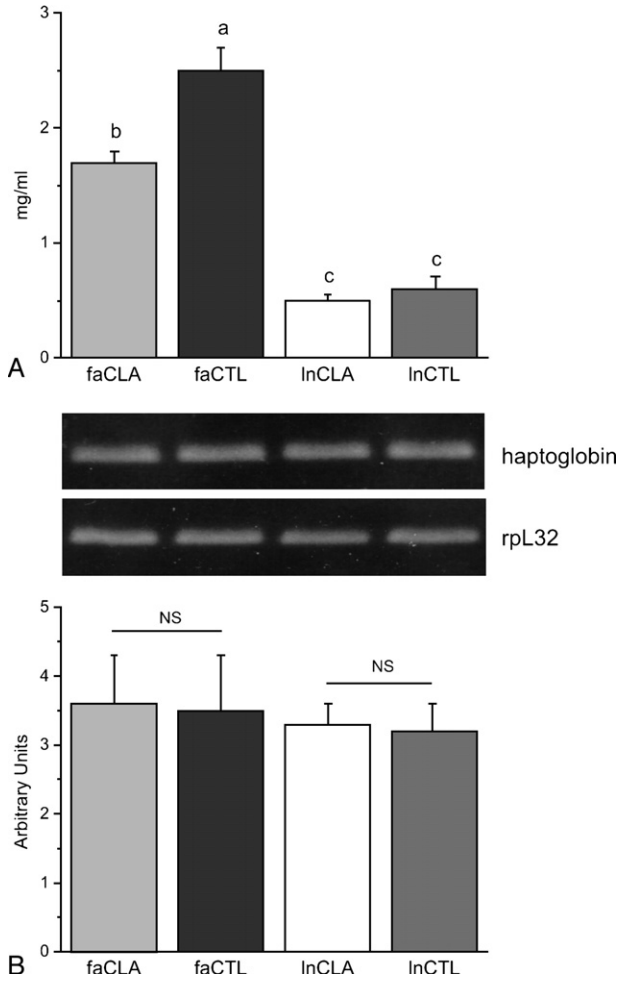


Fig. 4. Serum haptoglobin (A) and liver haptoglobin mRNA (B) of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks. Results for serum haptoglobin are means ± SE for n = 10 rats per group; means with different letters are significantly different ($P < .05$) by Duncan multiple range test. Arbitrary units for haptoglobin mRNA levels are means ± SEM for n = 6 rats per group; *P* values from preplanned comparisons. See Fig. 1 for abbreviations.

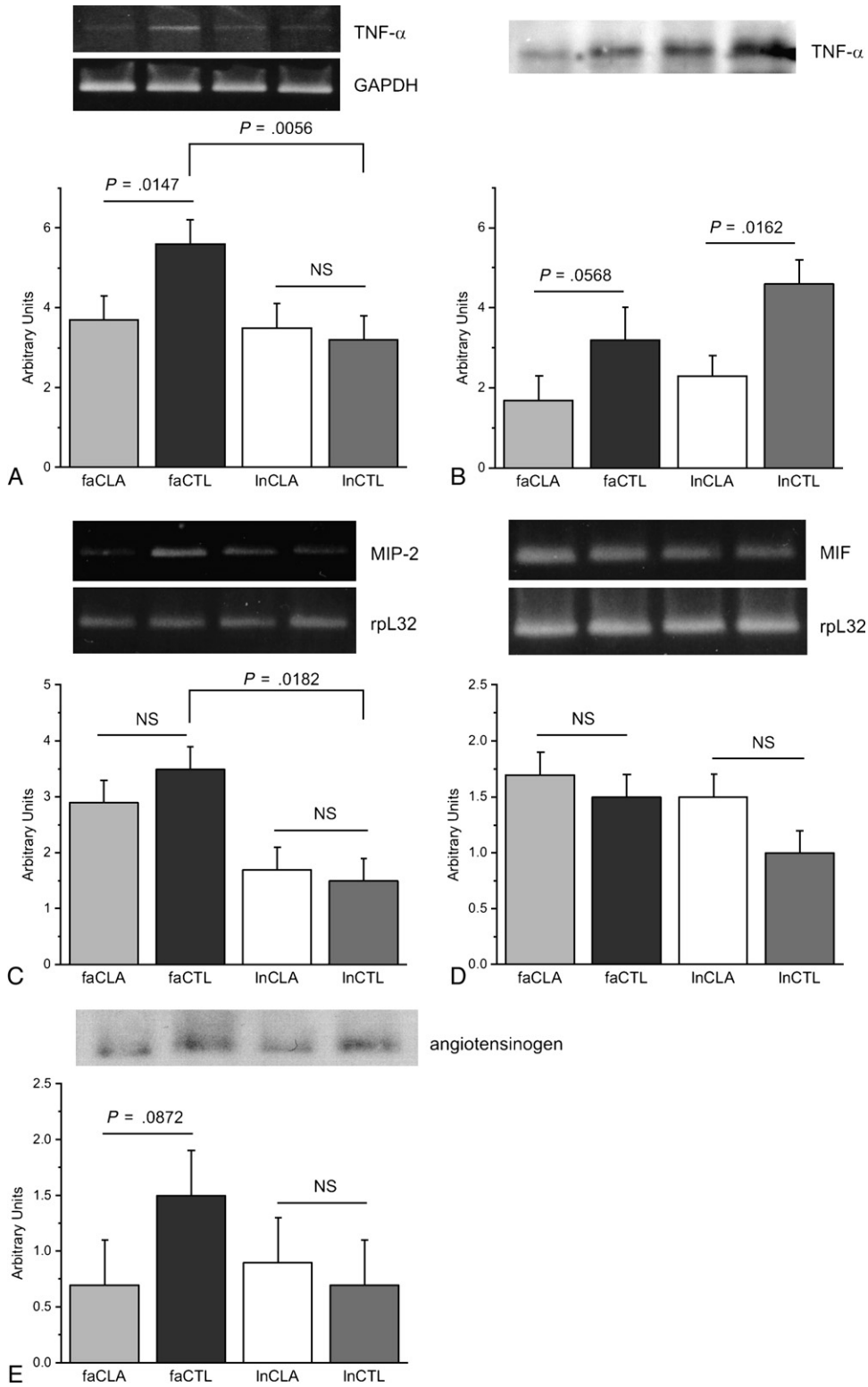


Fig. 5. Adipose TNF-α mRNA (A), TNF-α protein (B), MIP-2 mRNA (C), MIF mRNA (D), and angiotensinogen protein (E) of *fa/fa* and lean Zucker rats fed 0% or 1.5% CLA for 8 weeks. Results for mRNA and protein levels are expressed as means for arbitrary units for n = 8 rats per group for TNF-α mRNA, n = 10 rats per group for TNF-α and angiotensinogen protein levels, and n = 4 rats per group for MIP-2 and MIF mRNA; *P* values from preplanned comparisons. Appropriate loading control can be seen in Fig. 2B. See Fig. 1 for abbreviations.

others have shown that CLA-fed ZDF and *fa/fa* Zucker rats have improved muscle glucose transport [30,31]. In ob/ob mice fed t10,c12-CLA, reduced levels of adipose GLUT-4 mRNA were associated with elevated fasting glucose and insulin concentrations [32], again displaying the divergent effects of CLA in mice compared with obese Zucker rats.

The improved oral glucose tolerance and insulin sensitivity (Fig. 1) in CLA-fed *fa/fa* Zucker rats was not explained by differences in total feed intake, final body weight, or weight gain, and they occurred despite a slightly higher visceral adipose mass (Table 4). Insulin sensitizers known as TZDs are associated with weight gain and increased fat mass in obese *fa/fa* Zucker rats [33]. Both TZDs and CLA are PPAR agonists [19]. Improvements in insulin sensitivity associated with PPARγ agonists include a redistribution of lipid deposition from nonlipid storage tissues to adipose tissue [34]. Similarly, CLA-fed *fa/fa* rats had reduced hepatic steatosis [20] and elevated visceral fat mass in conjunction with improved oral glucose tolerance.

Others have reported that CLA supplementation worsens insulin tolerance testing in lean mice [35-37]; however, there was no detectable difference in oral glucose tolerance (Fig. 1) or fasting serum insulin levels (Table 5) in lean Zucker rats fed CLA or CTL diet. In fact, CLA-fed lean rats had elevated adipose GLUT4 protein compared with CTL-fed lean rats, but this did not translate into an effect on fasting glucose concentrations. It is interesting, however, that no genotype effect was observed for adipose GLUT-4, although such an effect might be assumed given the reduced insulin potency in the *fa/fa* rats. Adipocyte GLUT-4 mRNA and protein levels (on a per cell basis) are elevated in 5- to 10-week-old *fa/fa* Zucker rats and reduced in 20-week-old *fa/fa* rats compared with age-matched lean rats [38]; thus, the 14-week-old rats in the present study would be in the midst of this age-related transition of GLUT-4 levels in adipose tissue.

Another explanation for improved glucose tolerance and improved insulin sensitivity in CLA-fed *fa/fa* rats may be related to the state of inflammation in these animals. The concept that low-grade inflammation promotes insulin resistance is relatively new and undefined. Trayhurn and Wood [39] have put forth the argument that inflammation is a response to hypoxia that occurs because of expanded adipose tissue mass. Xu et al [18] have provided evidence for macrophage infiltration into the white adipose tissue of ob/ob mice, db/db mice, and diet-induced obese C57BL/6J mice before insulin resistance, and they have shown that inflammatory genes that are known to function in macrophages are consistently up-regulated in the white adipose tissue of these models. In the current study, the CLA-fed *fa/fa* rats showed a favorable reduction in proinflammatory markers including serum haptoglobin (Fig. 4A) and adipose TNF-α mRNA and protein levels (Fig. 5A and B) in conjunction with improved insulin utilization and GLUT4 mRNA levels (discussed above). The effect of CLA on adipose TNF-α levels is similar to the action of the TZD family of PPARγ agonists [40] and consistent with CLA acting as a PPAR ligand [19]. There was no difference in liver haptoglobin mRNA levels so it may be speculated that adipose haptoglobin levels were lower in CLA-fed compared with CTL-fed *fa/fa* rats, although we do not have data on the same. Contrary to the current study and specific to adipose tissue, Chung et al [41] showed that t10,c12-CLA treatment induced nuclear factor κB and subsequently inflammatory cytokine (eg, TNF-α) secretion while suppressing GLUT-4 mRNA levels in differentiated adipocytes and stromal vascular cells, nondifferentiated stromal vascular cells, and adipose tissue explants from human subcutaneous adipose tissue. There is limited supporting information about the effect of CLA on inflammation in vivo, although it has been shown that CLA, specifically t10,c12-CLA, increased inflammation, marked by C-reactive protein, in men with the metabolic syndrome [42-44].

Although macrophage infiltration may contribute to inflammatory cytokine production and insulin resistance in various obese models, the improved glucose tolerance observed with dietary CLA in *fa/fa* rats was not associated with alterations in adipose MIP-2 and MIF (Fig. 5C and D). The present study used semiquantitative PCR and subtle changes in gene expression (MIP-2, MIF, and other genes) may have not been detected. However, *fa/fa* rats had elevated adipose MIP-2 mRNA levels compared with lean rats. MIP-2 is known to attract macrophages, but current evidence also suggests that it may be up-regulated in preadipocytes, thus playing a role in adipogenesis [45]. There was no obvious difference between obese and lean rats with respect to adipose MIF mRNA levels, a marker that is proportional to body mass index in people [46] and up-regulated in vitro by TNF-α [47]. Future studies need to investigate additional mechanisms for CLA modulation of adipose proinflammatory cytokine production, including the role of other adipokines such as leptin and adiponectin [39], and the role of activation of adipose AMP-activated protein kinase by PPARγ agonists such as CLA [48].

Obesity, insulin resistance, and hypertension are a cluster of risk factors that are associated with the metabolic syndrome but the relationship is not fully elucidated. Recently, it has been shown that suppressing renin-angiotensin system activity (via angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists) aids in insulin sensitivity [49-51]. CLA has been reported to lower systolic blood pressure in Otsuka Long-Evans Tokushima Fatty (OLETF) rats [52]; however, the relationship with glucose tolerance was not examined. In the current study, there was a trend ($P = .09$) for lower adipose angiotensinogen mRNA levels (Fig. 5E) in CLA-fed *fa/fa* rats concurrent with improved insulin sensitivity (Fig. 1). Further studies are required to determine if the effects of CLA are mediated, in part, by the renin-angiotensin system, and to examine potential relationships among CLA isomers, insulin sensitivity, and vascular function.

In summary, the current study has shown that CLA supplementation leads to preservation of pancreas islets, improvements in peripheral glucose utilization, and sup-

pression of inflammation in *fa/fa* Zucker rats. However, adipose MIP-2 and MIF mRNA, markers of macrophage infiltration, were not affected by dietary CLA under these experimental conditions.

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