Dietary trans-10, cis-12 Conjugated Linoleic Acid Reduces Early Glomerular Enlargement and Elevated Renal Cyclooxygenase-2 Levels in Young Obese fa/fa Zucker Rats1–3

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Abstract
Conjugated linoleic acid (CLA) slows the progression of disease in models of chronic kidney disease. Because obesity is associated with nephropathy and increased renal cyclooxygenase (COX) levels, the effects of dietary CLA on kidney function, morphology, and COX protein levels in the kidneys of young obese (fa/fa) Zucker rats, a model of metabolic syndrome, were examined. In study 1, 6-wk-old fa/fa and lean Zucker rats were given a mixture of CLA isomers (1.5% CLA, wt:wt) or the control diet (CTL) with no CLA for 8 wk. To examine specific isomer effects, study 2 used the same model with the following diets: 0.4% (g/g) cis-9, trans-11 (c9, t11) CLA; 0.4% trans-10, cis-12 (t10, c12) CLA; a combination of these 2 isomers (0.4% each); or CTL diets with no CLA. In study 1, fa/fa rats given the CLA mixture had 11% smaller kidney weights and 28% smaller glomeruli, and feed intake and body weight did not differ from the CTL rats. In study 2, diet also did not affect body weights, but fa/fa rats given a diet containing t10, c12 CLA had 7% lower kidney weights, 20% smaller glomeruli, and 39% lower COX-2 protein levels than CTL rats. In conclusion, dietary t10, c12 CLA reduces the enlargement of glomeruli in young obesity-associated nephropathy and is associated with lower protein levels of renal COX-2. Long-term studies with CLA supplementation are required to determine whether these changes would lead to reduction in development of renal disease associated with obesity. J. Nutr. 139: 285–290, 2009.

Introduction
Conjugated linoleic acid (CLA) describes a group of positional (carbon 7–12) and geometric (cis-cis, cis-trans, trans-cis, and trans-trans) isomers of octadecadienoic acid in which the double bonds are conjugated (1). CLA in the food supply is found in dairy products and ruminant meats, in which the predominant isomer is cis-9, trans-11 (c9, t11) isomer. The next most abundant isomer naturally occurring in food is trans-7, cis-9 (t7, c9), followed by c11, t13, t8, c10, and t10, c12. The formation of synthetic CLA also can occur under strong alkaline conditions during commercial hydrogenation of linoleic acid-rich vegetable oils at high temperatures, resulting in the formation of predominantly c9, t11 and t10, c12 isomers (2,3).

CLA has been associated with several health benefits, including antidiopogenic, antidiabetogenic, anticarcinogenic, and antiatherosclerotic effects. The mechanisms of these effects are hypothesized to involve modulation of prostanooid production, enhanced oxidation of lipids, cell apoptosis, and/or differentiation, decreased cell proliferation, and alterations in gene expression (2,3). The majority of research investigating the effects of CLA has been with dietary supplementation of a CLA isomer mixture; however, recent studies have moved toward uncovering which CLA isomer(s) are responsible for the effects of CLA.

Although the concept of CLA supplementation in individuals with obesity and the metabolic syndrome currently is garnering much interest (4), its effects on the kidney in obesity are not known. Obesity and the metabolic syndrome, often described as a type 2 diabetes “prediabetic” state, are independently associated with increased risk of microalbuminuria and kidney disease (5,6). In chronic kidney disease models, CLA appears to reduce disease...
progression (7–9). Therefore, the hypothesis of the current study was that early CLA supplementation in young obese rats would alter one of the earliest signs of nephropathy associated with obesity, namely glomerular enlargement (10–13).

Glomerular hypertrophy in obesity appears to result from chronically increased afferent arteriole flow caused by an increase in vasodilatory mediators such as prostanooids (12,14). Renal prostanooid production and rate-limiting enzymes involved in prostanooid production, such as cyclooxygenase (COX)-1 and COX-2, are altered in models of established kidney disease (7,15–20). CLA alters COX enzymes and their products in several other tissues (21–24), possibly by reducing the arachidonic acid content of membrane phospholipids, by inhibiting prostanoid production, such as cyclooxygenase (COX)-1 and COX-2, enzymes or by acting as an antagonist for COX-1 and/or COX-2. Because reducing the production of COX-derived prostanooids may be among the potential effects of CLA, we further hypothesized that renal COX-1 and COX-2 levels would be affected by CLA supplementation in young obese rats.

Thus, the objective of study 1 was to determine the effect of a CLA isomer mixture on glomeruli size and steady-state renal levels of COX-1 and COX-2 in young fa/fa rats. In study 2, the objective was to determine specific isomer effects by supplementing the diet with either c9,t11, r10,c12, or a combination of the 2 CLA isomers.

### Materials and Methods

#### Animals and diets.
Male fa/fa and lean Zucker rats (n = 10 per group) were used in both studies; however, rats were obtained from different sources (Charles River for study 1 and Harlan for study 2). Rats were received at 5 wk of age and acclimated for 5–7 d before the start of each study. The protocols for the animal care procedures were approved by the University of Manitoba Protocol Management and Review Committee.

Diet formulations were based on the AIN-93G diet (25) and contained 8.5% total lipid (wt/wt) in both studies (Table 1). Dry ingredients were premixed and fresh batches of feed containing oil were prepared weekly and stored at −20°C. Both studies originally included a weight-matched group that was to be fed restricted amounts of control (0% CLA) diet such that their body weight would not differ from the treatment group with the lowest body weight, within each genotype. This approach was used because pair-feeding would not result in similar body weights if feed efficiency differed among the groups. During both studies, however, feed intake of the rats designated to be the weight-matched group did not have to be restricted. Statistical analyses also determined that final body weights did not differ within genotypes in both studies. Thus, the weight-matched group was removed from further analyses. Both studies lasted for 8 wk, during which all rats consumed the diet ad libitum with fresh feed 3 times/wk.

In study 1, fa/fa and lean rats were each randomly assigned to 1 of 2 treatment groups, where they were offered diet containing 1.5% (wt/wt) CLA (faCLA and lnCLA) or control (CTL) containing no CLA (faCTL and lnCTL) for 8 wk (n = 10 in each group). The CLA mixture (86% purity, FFA form) contained 4 major isomers: r10,c12, c9,t11, c11,t13, and c8,c10, found at final amounts in the diet at 3.91, 3.83, 2.38, and 2.04 g/kg, respectively. The dose of CLA was chosen to parallel research in Zucker Diabetic Fatty rats demonstrating improved glucose tolerance and attenuated hyperinsulinemia (26). Details of the CLA composition, diet formulations, diet fatty acid composition, and other indices for these rats related to bone mass and immune function have been reported elsewhere (27–29).

In study 2, fa/fa and lean rats were each randomly assigned to 1 of 4 treatment groups for 8 wk: 0.4% (g/g) c9,t11 CLA (fa9,11 and ln9,11); 0.4% (g/g) r10,c12 CLA (fa10,12 and ln10,12); 0.4% (g/g) c9,t11 plus 0.4% (g/g) r10,c12 CLA (faT9G and lnT9G); or the CTL group with no CLA (faCTL and lnCTL) (n = 10 for each group). The percent CLA in each diet group was based on the amount of c9,t11 and r10,c12 isomers present in study 1. Final amounts of CLA isomers in each of the diets are given in Table 1. Details of the CLA composition, diet formulations, fatty acid compositions, and other indices for these rats related to bone mass and immune function have been reported elsewhere (27,30,31).

Rats were housed individually in hanging stainless steel wire-bottom cages, and kept in a controlled environment with temperature maintained at 21–23°C, 55% relative humidity, and a 14-h-light:10-h-dark cycle. During this time, feed consumption (corrected for spillage) and weekly body weights were recorded. After 7 wk, rats were food deprived overnight (12 h) in metabolic cages for urine collection.

### Table 1

**Lipid composition of study diets**

<table>
<thead>
<tr>
<th>Diet</th>
<th>c9,t11 CLA</th>
<th>r10,c12 CLA</th>
<th>Soybean oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>g/kg diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLA</td>
<td>3.83&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.91&lt;sup&gt;2&lt;/sup&gt;</td>
<td>70</td>
</tr>
<tr>
<td>CTL</td>
<td>—</td>
<td>—</td>
<td>85</td>
</tr>
<tr>
<td>Study 2</td>
<td>9,11</td>
<td>4.00&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>10,12</td>
<td>0.19&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;2&lt;/sup&gt;</td>
<td>81</td>
</tr>
<tr>
<td>TTO</td>
<td>4.19&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.03&lt;sup&gt;3&lt;/sup&gt;</td>
<td>77</td>
</tr>
<tr>
<td>CTL</td>
<td>—</td>
<td>—</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>1</sup> The dry ingredients included: cornstarch (963 g/kg), maltodextrin (122 g/kg), sucrose (100 g/kg), egg white (213 g/kg), cellulose (50 g/kg), AIN-93M mineral mix for study 1 (35 g/kg), AIN-93G mineral mix for study 2 (35 g/kg), AIN-93-VX vitamin mix (10 g/kg), biotin premix (10 g/kg) prepared with 200 g biotin/kg cornstarch, choline (2.5 g/kg), and tert-butylhydroquinone (0.0144 g/kg). All dry ingredients, were purchased from Harlan Teklad, except cornstarch, which was supplied Best Foods (study 1), and tert-butylhydroquinone, supplied by Fisher Scientific (study 1) or Aldrich Chemical (for study 2).

<sup>2</sup> NuChek Prep.

<sup>3</sup> Natural ASA.

<sup>4</sup> Vita Health for study 1 and Harlan Teklad (study 2).

### Table 2

**Final feed intake, body weight, kidney weight, and kidney/body weight ratios of lean and fa/fa rats fed 0 or 1.5% CLA for 8 wk (study 1)**

<table>
<thead>
<tr>
<th></th>
<th>Lean Zucker</th>
<th>Obese fa/fa Zucker</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lnCTL</td>
<td>lnCLA</td>
</tr>
<tr>
<td>Total feed intake, g/rat</td>
<td>1055 ± 28</td>
<td>1101 ± 34</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>360 ± 9</td>
<td>355 ± 8</td>
</tr>
<tr>
<td>Absolute kidney weight, g</td>
<td>2.9 ± 0.1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.9 ± 0.1&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative kidney weight, g/100 g body weight</td>
<td>0.80 ± 0.01</td>
<td>0.81 ± 0.02</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SEM; n = 10. Means in a row with superscripts without a common letter differ, P < 0.05.

<sup>2</sup> Significant in the 2-way ANOVA: genotype (G, P < 0.0001), interaction (I, P = 0.0225), body weight, kidney weight, and kidney/body weight ratios of lean and fa/fa rats fed CLA isomers alone or together for 8 wk.
TABLE 3  Final feed intake, body weight, kidney weight, and kidney:body weight ratios of lean and fa/fa rats fed CLA isomers alone or together for 8 wk (study 2)*

<table>
<thead>
<tr>
<th></th>
<th>Lean Zucker</th>
<th>Obese fa/fa Zucker</th>
<th>Significant effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lnCTRL</td>
<td>ln8,11</td>
<td>ln10,12</td>
</tr>
<tr>
<td>Total feed intake, g/rat</td>
<td>1022 ± 24a</td>
<td>959 ± 21a</td>
<td>998 ± 17a</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>328 ± 5</td>
<td>338 ± 7</td>
<td>334 ± 9</td>
</tr>
<tr>
<td>Absolute kidney weight, g</td>
<td>2.3 ± 0.1a</td>
<td>2.4 ± 0.1a</td>
<td>2.4 ± 0.1a</td>
</tr>
<tr>
<td>Relative kidney weight, g/100 g body weight</td>
<td>0.71 ± 0.02a</td>
<td>0.72 ± 0.02a</td>
<td>0.71 ± 0.01b</td>
</tr>
</tbody>
</table>

* Values are expressed as means ± SEM, n = 10. Means in a row with superscripts without a common letter differ, P < 0.05.

** Signiﬁcant in the 3-way ANOVA: genotype [G], P < 0.0001; 1preplanned contrast for lean vs. fa for 10,12 vs. CTL (P = 0.0670); 1preplanned contrast for lean vs. fa for TOG vs. CTL (P = 0.0338); 1interaction [I, between genotype and 10,12 level (P = 0.0025)]; II between genotype and 10,12 level (P = 0.0025) and between 10,12 and 9,11 levels (P = 0.0013); 1preplanned contrast for lean vs. fa for 10,12 vs. CTL (P = 0.0049).

Tissue collection. At the end of 8 wk, rats were food deprived overnight (12 h) and asphyxiated with CO₂. Trunk blood was collected for serum analysis and kidneys were cleaned and weighed. Half of 1 kidney was taken and stored in 10% phosphate-buffered formalin (Fisher Scientiﬁc) for histological assessment of glomerular size. The remainder of the kidneys were frozen immediately in liquid nitrogen and stored at −80°C.

Histology and image analysis. Formalin-fixed kidney cross sections were embedded in paraffin, sectioned at 5 microns, and processed using previously described methods for histologic analysis (32). By random stage movement through the sections, the diameter of at least 30 randomly chosen glomeruli per kidney was measured by light microscopy with an Olympus BX60 microscope (Olympus Optical) using 20× magnification. After being captured using a Spot Junior CCD high-resolution camera (Diagnostic Instruments), images were analyzed using Image Pro Plus software (32). We converted the images to grayscale across gels. Dose-response curves were used to determine the linear range of response (14–20 μg of protein was used).

Statistical analysis. Two-way (genotype; ± CLA) and 3-way (genotype; c9,r11 CLA level; r10,c12 CLA level) ANOVA were used to determine significant main and all interaction effects (SAS Institute) for studies 1 and 2, respectively. Data were analyzed for normality using the Kolmogorov–Smirnov D statistic and for homogeneity of variance using Levene’s test. In study 2, because of the larger number of groups and interactions observed in study 1, preplanned contrasts also were used to detect interactions. Preplanned contrasts compared lean vs. fa/fa for each of the following: ± 9,11; ± 10,12; ± TOG; 9,11 vs. 10,12; 10,12 vs. TOG; 9,11 vs. TOG.

Western immunoblotting. Western immunoblotting of steady-state protein levels of COX-1 and COX-2 was performed as described (18,19). Briefly, 30 mg of liophilized kidney was homogenized in 100 volumes of ice-cold homogenization buffer [pH 7.4, 50 mmol/L Tris-HCl, 250 mmol/L sucrose, 2 mmol/L EDTA, 1 mmol/L EGTA, 10 mmol/L β-mercaptoethanol, 100 μmol/L sodium orthovanadate, 1 mg/L soybean trypsin inhibitor, 144 μmol/L 4-(2-aminoethyl) benzene-sulfonyl fluoride, and 2.5 mg/L of aprotinin, pepstatin, and leupeptin (all reagents from Sigma-Aldrich)]. Homogenates were centrifuged at 100,000 g for 35 min at 4°C and the supernatant containing the cytosolic fraction was removed. The remaining pellet was resuspended in 15–20 volumes of homogenization buffer containing 1% Triton X-100, incubated on ice for 10 min, and centrifuged at 100,000 g for 35 min at 4°C. The supernatant was stored at −80°C for later analysis of COX proteins.

After SDS-PAGE, detection of COX-1 (1:250, Santa Cruz Biotechnology) and COX-2 (1:250, Cayman Chemical) was carried out by incubating blots overnight at 4°C with primary antibodies, followed by incubation for 1 h at room temperature with a peroxidase-conjugated secondary antibody (1:20,000, Sigma). Immunoblots were incubated with ChemiGlow (Fisher Scientific) and image analysis and quantification of immunoreactive bands were performed using the Fluorchem FC digital imaging system (Alpha Innotech). A reference kidney homogenate was loaded on each gel in duplicate so that results could be compared across gels. Dose-response curves were used to determine the linear range of response (14–20 μg of protein was used).

FIGURE 1  Glomerular size in lean and fa/fa rats fed 0 or 1.5% CLA for 8 wk (study 1). Data are expressed as means ± SEM, n = 9–10. Labeled bars without a common letter differ, P < 0.05.

FIGURE 2  Representative hematoxylin- and eosin-stained kidney sections of lean (A, C) and fa/fa (B, D) rats at 14 wk of age in study 1 fed control (A, B) or CLA diets (C, D) (20× magnification). Arrows point to glomeruli in sections.

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Results

Feed intake, body, and kidney weight. In study 1, \(fa/fa\) compared with lean rats had 43\% higher feed intakes, 58\% higher body weights, 21\% greater kidney weights, and 23\% lower kidney weights relative to body weight (Table 2). Dietary CLA supplementation did not alter feed intake or body weight within each genotype. Whereas CLA did not alter kidney size in lean rats, kidneys were 11\% smaller by weight in \(fa/CLA\) rats than in \(fa/CTL\) rats.

In study 2, \(fa/\) compared with lean rats had 54\% higher feed intakes, 67\% higher body weights, 22\% larger kidney weights, and 27\% lower kidney weights relative to body weight (Table 3). With respect to CLA effects, dietary \(10,12\) CLA supplementation in \(fa/\) resulted in 9–16\% lower feed intakes than \(fa/\) on the other diets. Also, \(fa/\) ate 8\% less feed than \(fa/CTL\) rats. Analysis of weekly feed intake data revealed that all of the differences in feed intake occurred within the first 5 wk of the study and that weekly feed intakes within each genotype did not differ during the last part of the study. This accounts for the similar body weights at the end of the study. As in study 1, CLA supplementation did not alter kidney size in lean rats, but in \(fa/\) rats, the \(10,12\) group had lower kidney weights (by 7\%) than \(fa/CTL\) rats.

Glomerular volume. In study 1, kidney glomeruli were 46\% larger in \(fa/CTL\) vs. \(In/CTL\) (Fig. 1 and 2). Whereas CLA supplementation did not alter glomerular size in lean rats, it did result in 28\% smaller glomeruli volumes in the \(fa/CTL\) vs. \(fa/CTL\) rats. However, the glomeruli of \(fa/CLA\) rats remained larger than the lean rats.

In study 2, the glomeruli were 37\% higher in \(fa/CTL\) vs. \(In/CTL\) rats (Figs. 3 and 4). Again, CLA isomer supplementation did not alter the glomerular size in lean rats. In \(fa/\) rats, only the \(10,12\) had significantly smaller glomerular volumes than the \(fa/CTL\) rats, whereas the glomeruli in the other 2 groups did not significantly differ from either the \(10,12\) or \(fa/CTL\) groups.

Urinary variables. In study 1, \(fa/\) had 32\% higher urine volume, 31\% lower urine creatinine, 55\% higher serum creatinine, 66\% lower creatinine clearance, and 109\% higher urine protein excretion than lean rats. However, dietary CLA supplementation did not alter any of these variables (Supplemental Table 1).

In study 2, there also was a genotype effect observed in all urinary variables, where \(fa/\) had 47\% higher urine volume, 31\% lower urine creatinine, 25\% higher serum creatinine, 49\% lower creatinine clearance, and 76\% higher urine protein excretion than lean rats. Again, dietary CLA supplementation did not alter any of these variables (Supplemental Table 2).

Protein levels of COX enzymes. In study 1, COX-1 levels did not differ (Supplemental Fig. 1A) between \(fa/\) and lean rat kidneys, but \(fa/\) had 187\% higher COX-2 protein levels (Supplemental Fig. 1B) than lean rats. CLA supplementation did not alter COX-1 or COX-2 protein levels. In study 2, COX-1 levels also did not differ (Supplemental Fig. 2) between \(fa/\) and lean rats, but \(fa/CTL\) rats had 67\% higher COX-2 protein levels (Fig. 5) than \(In/\) rats. As with the glomerular size values, when CLA was given to \(fa/\) rats, COX-2 protein levels were lower but only significantly different from CTL when \(10,12\) alone was given (reduced by 39\%).

Discussion

The current study demonstrates that CLA supplementation, particularly the \(10,12\) isomer, significantly reduces the early glomerular enlargement and the higher levels of COX-2 in the
young obese fa/fa Zucker rat model of metabolic syndrome. In both studies, larger glomeruli were observed in fa/fa compared with lean rats, consistent with previous reports in this rat model (10,11,37). Glomerular hypertrophy in obesity and its associated metabolic abnormalities appear to be due to afferent but not efferent arteriole dilation, which leads to glomerular hyperfiltration, glomerular enlargement, thickening of the basement membrane, mesangial expansion, proteinuria, and renal disease (12,14). This also has been observed in obesity-related glomerulopathy, a common occurrence in obese patients that is characterized by glomerulomegaly often accompanied by focal segmental glomerulosclerosis (38).

Interestingly, dietary CLA affected fa/fa but not lean rats, suggesting that CLA may not affect the kidney unless there is a perturbation in glomerular filtration. It may do this by altering the production of COX metabolites. In this regard, fa/fa rats have significantly higher renal COX-2 protein levels than lean rats, as demonstrated in the current studies. Other studies have demonstrated similarly elevated renal COX-2 levels in fa/fa and Zucker Diabetic Fatty rats (15,16,20,37). Prostanoids produced by COX enzymes are important in the regulation of renal hemodynamics, the renin-angiotensin system, and renal pathogenesis (39). Elevated prostanoids have been implicated in renal injury in both animal models of obesity-associated nephropathy (15–17,20,38) and in patients with type 2 diabetes (41). With COX-2 inhibition, prostanooid levels and renal injury are reduced in the fa/fa rat (15,20) and in patients with Type 2 diabetes (40). Supplementation of this isomer may ameliorate renal disease through the alteration of COX metabolites as in the Han:SPRD-cy rat model of kidney disease, which displays elevated COX-2 activity and in which dietary CLA competes with linoleic acid and inhibits the production of prostaglandin E₂ (7,19). The ability of CLA to alter prostanooid production has been demonstrated in other tissues (21–24). In animal models of obesity and diabetic nephropathy, increased COX-2 may be due to the effects of high glucose on protein kinase C activation of prostanooid production (21–24) and/or be due to activation of the renin-angiotensin system (20). Alternatively, it may be associated with the increased glomerular size observed in these kidneys, because COX-2 in the kidney is primarily associated with the macula densa. In the same way, the possibility that COX-2 protein levels in fa/fa rats given t₁₀,c₁₂ CLA are reduced because of changes in glomerular size also cannot be ruled out in the current study.

We have reported other effects of CLA mixture supplementation in the fa/fa rat, including preservation of pancreatic islets, improvement in peripheral glucose utilization, and reduction in inflammatory markers (28). As well, CLA reduces hepatic steatosis, improves liver function, and favorably modifies lipid metabolism (27). It also has recently been shown to decrease adipocyte size and favorably modify adipokine status and insulin resistance (29). In the JCR:LA-cp rat model of metabolic syndrome, insulin resistance and vascular disease, cosupplementation of chromium picolinate and CLA improves lipid profile, hyperinsulinemia, and circulating adipokines. As well, supplementation of CLA alone improves renal function by reducing urinary albumin levels and glomerulosclerosis (9). However, long-term studies on the efficacy, safety, and mechanism of action of the t₁₀,c₁₂ isomer are warranted to determine whether the renal effects are beneficial in the long term, especially because dietary supplementation with the t₁₀,c₁₂ isomer exhibits pro-oxidative, pro-inflammatory, and proinflammatory properties in humans (41,42).

Acknowledgments

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