Dietary trans-10,cis-12 CLA Reduces Murine Collagen-Induced Arthritis in a Dose-Dependent Manner1–3

Shane M. Huebner,4 Jake M. Olson,7 James P. Campbell,5 Jeffrey W. Bishop,5 Peter M. Crump,6 and Mark E. Cook4,5,7*

Departments of 4Nutritional Sciences, 4Animal Sciences, and 6Computing and Biometry, and 7Molecular and Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI

Abstract

Dietary trans-10,cis-12 (t10c12) conjugated linoleic acid (CLA) has been shown to reduce inflammation in a murine collagen-induced arthritis (CA) model. To understand the anti-inflammatory potential of t10c12-CLA in the diet, the minimum dose of pure dietary t10c12-CLA capable of reducing CA was investigated. Because plasma inflammatory cytokines often do not reflect the progression of late-stage arthritis, inflamed tissue cytokine concentrations were also investigated in relation to increasing dietary t10c12-CLA amounts. Mice were randomly assigned to the following dietary treatments upon the establishment of arthritis: corn oil (CO) or 0.125%, 0.25%, 0.375%, or 0.5% t10c12-CLA (wt:wt) for 84 d. Sham mice (no arthritis) were fed CO and served as controls. Arthritic paw score, based on subjective assessment of arthritic severity, and paw thickness decreased linearly overall [16–65% (P < 0.001) and 0.5–12% (P < 0.001), respectively] as dietary t10c12-CLA increased (P < 0.001, R² < 0.81). Increasing dietary t10c12-CLA was associated with a decrease in plasma interleukin (IL)-1β at days 21 and 42 compared with CO-fed arthritic mice, such that mice fed ≥0.25% t10c12-CLA had IL-1β concentrations that were similar to sham mice. Plasma cytokines returned to sham mice concentrations by day 63 regardless of treatment; however, an arthritis-induced elevation in paw IL-1β decreased linearly as dietary t10c12-CLA concentrations increased at day 84 (P = 0.007, R² = 0.92). Similarly, increasing dietary t10c12-CLA linearly decreased paw tumor necrosis factor (TNF)-α (P = 0.05, R² = 0.70). In conclusion, ≥0.125% t10c12-CLA dose-dependently reduced inflammation in a murine CA model. J. Nutr. 144: 177–184, 2014.

Introduction

CLA is a naturally occurring class of conjugated dienoic FA isomers of linoleic acid (LA; 18:2n–6) [e.g., c9-t11, trans-11 (c9t11-CLA) and trans-10, cis-12 (t10c12-CLA)] (1). CLA is synthesized in ruminants through the biohydrogenation of specific PUFAs (e.g., 18:3n–3) (2). Dairy fat concentrations of total CLA naturally vary from 75–90% and 0–6% of total CLA, respectively, depending on the ruminant diet (3–6). Whereas humans have been estimated to consume 150–400 mg CLA/d (resulting in plasma t10c12-CLA concentrations of ~0.4–1.2 μg/mL), the anti-inflammatory significance at these amounts is largely unknown (7–9).

Synthetically derived CLA for experimental research is typically achieved by alkali isomerization of oils rich in LA (e.g., corn, sunflower, and safflower oil) and often consists of equal parts c9t11- and t10c12-CLA (10). Synthetic dietary CLA has been shown to have anti-inflammatory properties in a variety of animal disease models, including the following: type I hypersensitivity (11–16), immune-induced cachexia (17–22), lupus (19, 23–25), inflammatory bowel disease (26–28), and arthritis (29, 30). By using pure isomers, Huebner et al. (30) showed that both dietary c9t11- and t10c12-CLA at 0.5% wt:wt of diet separately reduced inflammation in a murine collagen-induced arthritis (CA) model over 84 d of treatment. However, t10c12-CLA was 12% more effective than c9t11-CLA in reducing clinical arthritis score. Inhibition of the NF-κB signaling pathway, which is a key regulator of inflammatory cytokine production, has been proposed as an anti-inflammatory mechanism for t10c12-CLA (31,32).

Amounts of pure, dietary t10c12-CLA (0.25% or 0.5%) shown to have anti-inflammatory effects in murine models (21,30,31) far exceed those typically found in natural sources. Thus, the minimum anti-inflammatory concentration of pure t10c12-CLA was determined to estimate the anti-inflammatory value of...
t10c12-CLA naturally found in foods. Since dietary t10c12-CLA exerts antiarthritic effects long after proinflammatory plasma cytokines have returned to baseline concentrations (30), the second objective was to determine if t10c12-CLA continued to influence proinflammatory cytokine concentrations directly in arthritic tissues after plasma cytokines returned to baseline.

Materials and Methods

This experiment was conducted in accordance with protocols approved by the College of Agricultural and Life Sciences Animal Care and Use Committee.

Collagen-induced arthritis model. Four-wk-old male DBA/1 mice (n = 84) were purchased (Harlan) and housed in shoebox units (3 mice per box) with a 12:12-h light-dark cycle. Mice were fed a standard rodent diet (8604; 24.5% protein, 4.4% fat, 46.6% nitrogen-free extract; Harlan) during a 7-d environmental acclimation period, which upon completion, was replaced with a 99% complete, casein-based, semipurified diet based on the American Institute of Nutrition (AIN)–76A diet (TD94060; Harlan) supplemented with 1% corn oil (CO) (33). CO has been used as a reference dietary lipid source in multiple CA studies (29,30,34,35).

Mice were immunized at the completion of 3-wk maintenance on the CO diet as previously described to induce arthritis (29,30,36). Briefly, 8-wk-old mice were randomly assigned to 2 groups for immunization against chick type II collagen (IICl; Chondrex; n = 75) or 0.1 mL of acetic acid (sham; n = 9) emulsified with 4 µL Mycobacterium tuberculosis complete Freund’s adjuvant (Difco Laboratories).

When the first mouse per shoebox developed definite clinical signs of arthritis, defined as a clinical arthritic score of 2 on at least 1 paw (designated day 0), it was randomly assigned to 1 of 5 dietary treatments as follows: 1.0% CO diet, 0.125% t10c12-CLA (Natural Lipids), 0.25% t10c12-CLA, 0.375% t10c12-CLA, or 0.5% t10c12-CLA. The t10c12-CLA was 90% pure, determined by gas chromatography (37–40), and the remaining 10% FA composition was as follows: 18:1c9 (1%), 18:2c9t11 (5%), 18:2c9c11 (0.5%), 18:2c10c12 (1%), and other CLA isomers (1%). All diets, based on the AIN-76A diet (TD94060; Harlan), were a 99% complete, casein-based, semipurified diet until supplemented up to 1% t10c12-CLA plus CO. Because the onset of arthritis is unpredictable, procedures were used to ensure that dietary treatments were initiated after arthritis onset while preventing extended periods of singly housed mice. Sham-injected mice were maintained on the CO diet.

Mice remained on their respective dietary treatments for 84 d. Clinical arthritic score (described below) was monitored 3 times/wk for each mouse throughout the dietary treatment period. Additionally, paw thickness measurements (described below) were recorded once per week on and after day 0.

Clinical arthritic score. Mice were examined 3 times/wk by 2 trained observers, blinded to treatment, as detailed previously (29,30,36). Briefly, individual paws were assigned an inflammation severity score on the basis of the following criteria: 0 = normal (no inflammation); 1 = mild (definite redness and swelling of the ankle or wrist or apparent redness and swelling limited to individual digits, regardless of the number of affected digits); 2 = moderate (redness and swelling of ankle and wrist); 3 = severe (redness and swelling of the entire paw including digits); 4 = maximally inflamed limb involving multiple joints. The sum of all 4 paws was calculated, averaged between the 2 observers, and reported as the “clinical arthritic score.”

Paw thickness measurements. Immediately upon the diagnosis of clinical arthritis, paw thickness was recorded for each paw by using a pressure-sensitive caliper (SPI) once per week. Paw thickness measurements were used as an objective measure of arthritic swelling and to confirm the subjective clinical arthritic score.

Plasma collection and preparation. On days 0, 21, 42, 63, and 84 of arthritis and dietary treatment, blood was collected in heparinized microtainer tubes (BD) by venous puncture of the maxillary vein. Plasma was separated by centrifugation at 3000 × g for 10 min and stored at −80°C until analyses. Sampling times were selected on the basis of the length of time required for maximal CLA incorporation into tissues (between days 7 and 21) while allowing for the chronic inflammation of CA to progress (~70 d) (29,30,36).

Paw tissue preparation. On day 84 immediately after mice were killed by exsanguination, front and hind paws were harvested, snap-frozen in liquid nitrogen, and separately weighed then crushed under liquid nitrogen. Total protein was extracted by tissue homogenization using a custom protein extraction/lysis buffer (20 mmol/L MOPS, 2 mmol/L EGTA, 5 mmol/L EDTA, 30 mmol/L sodium fluoride, 40 mmol/L β-glycerophosphate, 20 mmol/L sodium pyrophosphate, 1 mmol/L sodium orthovanadate, 1 mmol/L PMSE, 3 mmol/L benzamidine, 10 mg/mL pepstatin A, 10 mg/mL leupeptin, 0.5% Triton X; adjusted to a final pH of 7.2) at 5:1 v:v buffer to paw tissue. Samples were incubated on ice for 1 h and centrifuged at 3000 × g for 30 min at 4°C. Supernatant was collected and stored at −80°C. Samples were adjusted to 3 mg protein/mL by using a bicinchoninic acid protein assay (Thermo) before cytokine quantization (41).

Cytokine analysis. Plasma and concentrations of IL-1β, IL-6, IL-10, and TNF-α were quantified using Bio-Plex Pro mouse cytokine assays according to the manufacturer’s specifications (Bio-Rad Laboratories). Fluorescein was measured using the Luminex 100 system (Bio-Rad Laboratories), and results were analyzed using Bio-Plex Manager software (Bio-Rad Laboratories).

Plasma anti-Ⅱc antibody ELISA. Plasma samples were analyzed as previously described for the total concentration of anti-Ⅱc immunoglobulin (Ig) G (29,30), with the exception that anti-Ⅱc IgG2a antibody concentrations were measured as a concentration of anti-Ⅱc IgG2a monoclonal antibody standard (Chondrex).

Liver FA determination. Total lipids of 0.5-g liver samples were extracted using chloroform/methanol (2:1 v:v) according to the methods of Folch et al. (37). FA methyl esters were prepared by acid-catalyzed methylation (38,39). Relative percentages of liver FA methyl esters were determined by an Agilent 6890N GC (Agilent Technologies) equipped with a 100m CP-Sil 88 column (Varian) (40).

Statistical analysis. Data for arthritic score, paw thickness, plasma cytokines, and antibodies were analyzed by using the SAS procedure MIXED (SAS Institute) to determine differences due to dietary treatment and time. The fixed effects were dietary treatment, time, and interaction of dietary treatment and time in addition to the random effect of mouse within each treatment. Data analyzed included non-normalized and normalized measurements of both arthritic score and paw thickness. Normalization was achieved by subtracting the arthritic score or paw thickness of each mouse on day 0 from the subsequent measurements recorded after day 0. A post hoc t test was conducted to determine differences between sham and arthritic mice for each treatment and day. Data at day 0 were also analyzed by 1-factor ANOVA to determine if significant differences existed between sham and arthritic mice before initiation of dietary treatment. Subsequent analyses excluded day 0 data because dietary treatments had not begun. Paw cytokines were analyzed by 1-factor ANOVA. When Levene’s test for homogeneity of variances was significant, a nonparametric ANOVA was used. Liver FA data were analyzed by 1-factor ANOVA.

When the effect of dietary treatment was significant, a post hoc analysis, using a least-squared difference mean separation test was conducted to identify individual treatment differences. In the case of repeated measurements taken on the same experimental unit over time (i.e., clinical arthritic score), the SAS procedure MIXED was used with a REPEATED statement with a first-order autoregressive covariance structure to account for auto-correlated errors. A different error variance for each treatment was achieved by using the GROUP option for the REPEATED statement. Values are means ± SEMs except where noted. Because this experiment investigated markers of inflammation in response to varied concentrations of t10c12-CLA, linear regressions were conducted for each response.
variable on all concentrations of t10c12-CLA. All statistical tests were considered significant at the $P < 0.05$ level.

**Results**

**Clinical arthritic score.** The day 0 mean clinical arthritic score was 2.9 and did not differ between dietary treatment groups. The mean scores of all arthritic mice were greater than sham mice (Table 1). The mean score of CO-fed arthritic mice peaked at 6.1 on day 9 and remained between 4.5 and 6 until day 84 (Fig. 1A). An interaction between dietary treatment and time was observed (Table 1), such that as t10c12-CLA increased in the diet, arthritic score decreased earlier (Fig. 1A). Arthritic mice fed 0.5%, 0.375%, 0.25%, and 0.125% dietary t10c12-CLA had reduced scores beginning on days 7, 21, 21, and 49, respectively. By day 84, arthritic mice fed 0.5%, 0.375%, 0.25%, and 0.125% t10c12-CLA had significantly reduced scores by 68%, 37%, 37%, and 32%, respectively, compared with CO-fed arthritic mice. The decrease in arthritis with increasing t10c12-CLA was linear for arthritic score.

**Paw thickness measurements.** Paw thickness was recorded to provide an objective measurement of the anti-inflammatory effect of t10c12-CLA (Fig. 1B). Preceding dietary intervention, paw thickness of arthritic mice increased 11% (0.18 mm) above that in sham mice (1.58 mm; Fig. 1B, day 0). The addition of t10c12-CLA decreased arthritic paw thickness, linearly, over the entire study period (days 0–84, main effect of treatment; Table 1). Overall, a t10c12-CLA concentration $>0.25\%$ wt:wt diet was needed to significantly reduce paw thickness relative to the CO arthritic group. Unlike arthritic score, there was no interaction between treatment and time for paw thickness (Table 1). When non-normalized paw thickness was analyzed at day 84, mice fed 0.25%, 0.375%, or 0.5% t10c12-CLA had significantly reduced paw thickness by 27.5%, 25.5%, and 24.7%, respectively, compared with CO.

**Plasma cytokine concentrations.** On day 0 before randomization to diet, arthritic mice had significantly increased plasma IL-1$\beta$ (69 $\pm$ 7 vs. 32 $\pm$ 6 ng/L), TNF-$\alpha$ (154 $\pm$ 15 vs. 26 $\pm$ 6 ng/L), and IL-6 (37 $\pm$ 3 vs. 13 $\pm$ 4 ng/L), whereas IL-10 (20 $\pm$ 4 vs.

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**TABLE 1** Clinical arthritic score and paw thickness of arthritic mice fed CO or t10c12-CLA and sham mice fed CO-containing diets for an 84-d treatment period postinitiation of arthritis$^1$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Arthritic score</th>
<th>Paw thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-normalized</td>
<td>Normalized</td>
</tr>
<tr>
<td>Sham$^2$ ($n = 9$)</td>
<td>0.0 $\pm$ 0.0</td>
<td>—</td>
</tr>
<tr>
<td>CO ($n = 12$)</td>
<td>5.1 $\pm$ 0.3$^a$</td>
<td>2.7 $\pm$ 0.3$^a$</td>
</tr>
<tr>
<td>0.125% t10c12-CLA ($n = 9$)</td>
<td>4.3 $\pm$ 0.4$^{b, a}$</td>
<td>1.3 $\pm$ 0.4$^{b, a}$</td>
</tr>
<tr>
<td>0.25% t10c12-CLA ($n = 9$)</td>
<td>3.9 $\pm$ 0.4$^b$</td>
<td>0.8 $\pm$ 0.4$^b$</td>
</tr>
<tr>
<td>0.375% t10c12-CLA ($n = 11$)</td>
<td>3.8 $\pm$ 0.4$^b$</td>
<td>1.1 $\pm$ 0.3$^b$</td>
</tr>
<tr>
<td>0.5% t10c12-CLA ($n = 10$)</td>
<td>1.8 $\pm$ 0.4$^a$</td>
<td>$-$1.2 $\pm$ 0.5$^a$</td>
</tr>
</tbody>
</table>

$P$ values

- Treatment $<0.001$ $<0.001$ $<0.001$ $<0.001$ $<0.001$ $<0.001$ $<0.001$
- Time$^3$ $<0.001$ $<0.001$ 0.12 0.15
- Treatment $\times$ time$^3$ 0.004 0.012 0.98 0.98
- Linear regression $<0.001$ $<0.001$ $<0.001$ $<0.001$

$R^2$

- 0.85 0.82 0.91 0.88

$^1$ Values are means $\pm$ SEMs. There were no differences detected on day 0 after randomization to dietary treatments. Baseline data were excluded from subsequent statistical analysis. Shams were not included in normalized arthritic data. Arthritic means within a column without a common letter differ, $P < 0.05$. CO, corn oil.

$^2$ CO and all CLA treatments were significantly different from shams based on separate 1-factor ANOVAs.

$^3$ See Fig. 1 for time-related effects.
When plasma IL-1β was analyzed at each time point, mice fed ≥0.25% t10c12-CLA at day 21 and ≥0.375% at day 42 had reduced plasma IL-1β compared with CO-fed arthritic mice, with values similar to shams. However, when plasma cytokines in arthritic mice were analyzed by linear regression across all time points (pooled data not shown), plasma concentrations of IL-1β, TNF-α, and IL-6 significantly decreased with increasing dietary amounts of t10c12-CLA ($R^2 = 0.91$, 0.7, and 0.98, respectively), and plasma IL-10 significantly increased ($R^2 = 0.96$).

**FIGURE 2** Plasma concentrations of IL-1β (A), TNF-α (B), IL-6 (C), and IL-10 (D) in sham mice fed CO and arthritic mice fed CO or t10c12-CLA-containing diets for 84 d postestablishment of arthritis. Values are means ± SEMs, n = 6. Labeled means at a time without a common letter differ, $P < 0.05$. CO, corn oil.

**Paw cytokine concentrations.** Paws were collected at day 84 and analyzed for IL-1β, TNF-α, IL-6, and IL-10 cytokine concentrations (Fig. 3). Only paw IL-1β was significantly increased (Fig. 3A) in CO-fed arthritic mice compared with sham mice (77 ± 16 vs. 19 ± 3 ng/g protein, respectively). Paw IL-10 was decreased in CO arthritic mice compared with sham mice (3.6 ± 0.2 vs. 4.2 ± 0.2 ng/g protein; Fig. 3D). Dietary t10c12-CLA significantly reduced paw IL-1β concentrations in arthritic mice fed at 0.375% and 0.5% CLA compared with CO (40 ± 10 and 30 ± 12 vs. 77 ± 16 ng/g protein, Fig. 3A). Additionally, IL-1β in the 0.5% t10c12-CLA group was reduced to similar concentrations found in sham mice (30 ± 12 vs. 19 ± 3 ng/g protein; Fig. 3A). A significant linear decrease in both IL-1β and TNF-α was associated with increasing dietary t10c12-CLA ($R^2$ = 0.92 and 0.70, respectively).

**Plasma anti-tIIc antibody concentrations.** Anti-tIIc total IgG, IgG1, and IgG2a antibodies were not detectable in sham-injected mice; however, they were detectable at the onset of arthritis in tIIc immunized mice (day 0; [Supplemental Table 1](#)). Dietary treatments had no effect on plasma total IgG, IgG1, and IgG2a anti-tIIc antibodies concentrations. As a main effect of time, both anti-tIIc total IgG and IgG1 declined from day 0 to day 21, whereas IgG2a increased during this period. Total IgG and IgG2a were stable from days 21 to 84, whereas IgG1 significantly increased from day 21 to day 84 (main effect of time).

**Relative liver FA composition.** At day 84, livers were analyzed for relative FA content. CO-fed arthritic mice had reduced relative amounts of 18:3$\omega$3 (−35%) and increased 20:5n–3 (+260%) compared with sham mice ([Table 2](#)). The t10c12-CLA-isomer was not detectable in sham or CO livers; however, as t10c12-CLA increased in the diet of arthritic mice, hepatic t10c12-CLA significantly increased linearly ($R^2 = 0.98$). Relative amounts of hepatic LA and 18:3$\omega$3 were significantly reduced by as much as −22% and −60%, respectively, in t10c12-CLA compared with CO-fed arthritic mice. Hepatic c9t11-CLA was elevated in arthritic mice fed 0.5% t10c12-CLA compared with CO, which was likely due to low amounts of c9t11-CLA (<5% v:v, determined by GC analyses) in the t10c12-CLA preparation.

**Discussion**

One objective of the current study was to determine the minimum effective anti-inflammatory concentration of dietary t10c12-CLA for estimating the anti-inflammatory potential of food products naturally containing CLA. Similar to previously reported data (30), we found 0.5% wt:wt dietary t10c12-CLA decreased clinical signs of arthritis in CA mice over an 84-d period. However, in the present study, arthritis was reduced within 10 d of treatment compared with 20 d, as previously observed (30). The
earlier effectiveness of t10c12-CLA in this study may be due to increased clinical arthritis severity, possibly due to the use of 4 mg/mL *M. tuberculosis* during immunization (42) compared with 1 mg/mL in the previous study (30).

Arthritic mice fed a diet containing 0.125% t10c12-CLA showed reductions in clinical arthritis severity. These mice consumed ~125 mg t10c12-CLA/kg body weight · d (assuming 3 g food consumption by a 30-g mouse). Using metabolic efficiency scaling for a 70-kg person according to Kleiber’s law (43), this amount is equal to a human daily dose of 3.025 g t10c12-CLA. Dairy fat has been reported to have concentrations of t10c12-CLA as high as 1.1 g/kg fat (6). On the basis of this information, a person is unlikely to realize an antiarthritic benefit from t10c12-CLA in enriched dairy fat, because ~2.75 kg fat/d need to be consumed to obtain a minimally effective anti-inflammatory dose of t10c12-CLA. Dietary CLA supplements, however, typically contain 400 mg/g t10c12-CLA. A person consuming 7.5 g/d of such a supplement would receive a minimally effective anti-inflammatory dose of t10c12-CLA.

The dietary percentage of t10c12-CLA and treatment duration both affected arthritic outcome. At the 0.125% level, t10c12-CLA needed ~60 d of treatment to reduce clinical arthritis severity relative to CO-fed arthritic mice. Interestingly, the reduction in severity observed with the 0.125% level was similar to the reduction observed with feeding 0.25% or 0.375% t10c12-CLA for 30 d. Cellular turnover of t10c12-CLA is rapid relative to c9t11-CLA (44); hence, it may have taken longer for tissues (i.e., inflamed joints) to accumulate enough t10c12-CLA to become as effective as higher concentrations, an observation needing further examination. Arthritic mice fed 0.25% and 0.375% t10c12-CLA had similar mean arthritic scores, paw thickness, and both plasma and paw cytokine concentrations, even though there was considerably more terminal hepatic t10c12-CLA content in mice fed 0.375% than in those fed 0.25%. These similarities among inflammatory variables suggest a threshold was reached at the 0.25% level, such that higher concentrations failed to further reduce inflammation, at least in conditions in which maximum (similar to CO-fed) arthritic severity had been reached. For example, arthritic mice fed CO or 0.125%, 0.25%, or 0.375% t10c12-CLA all had mean peak arthritic scores >5.5, which were reduced to a mean score no less than 3 throughout the study period, whereas the 0.5% t10c12-CLA group had a mean peak arthritic score of 4 (27% lower than all other peak scores), which was reduced to 1.1 (65% lower than all other minimum scores). A possible explanation for reduced severity in the 0.5% t10c12-CLA group, relative to all other doses, may be due to a reduction in IL-1β, a potent proinflammatory cytokine, at the onset of arthritis. We have previously shown that 0.5% t10c12-CLA significantly reduces plasma IL-1β as early as 7 d after onset of arthritis (30). Furthermore, anti-IL-1β antibodies injected 7 d after the onset of arthritis reduced clinical arthritic scores as early as 2 d after injection (45). In this study, a linear decrease in both paw and plasma IL-1β with increasing dietary t10c12-CLA concentration showed that 0.5% most effectively reduces IL-1β, which may explain the uniquely low arthritic score peak observed before day 10.

An overexpression of IL-1β is associated with increased autoimmunity to tIIc (46), whereas a reduction in IL-1β is consistent with known therapeutic mechanisms that ameliorate CA (45,47–51). Dietary CLA (mixed isomers) has previously been shown to decrease IL-1β mRNA and protein expression in vivo and in vitro (52,53), which was further supported in this study by a decrease in paw and plasma IL-1β (day 21) at all t10c12-CLA concentrations. Additionally, a dose-dependent effect of

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**FIGURE 3** Paw concentrations of IL-1β (A), TNF-α (B), IL-6 (C), and IL-10 (D) in sham mice fed CO and arthritic mice fed CO or t10c12-CLA–containing diets at 84 d postestablishment of arthritis. Values are means ± SEMs, n = 6. Labeled means at a time without a common letter differ, *P* < 0.05. CO, corn oil.
t10c12-CLA at reducing IL-1β in both paws and plasma was further supported by significant inverse linear relations ($R^2 = 0.92$ and 0.91, respectively). Concentrations of IL-1β were considerably higher in paws than in plasma by day 84 in CO-fed arthritic mice (230 ng/L tissue lysate vs. 38 ng/L plasma), suggesting that it is likely higher in paws than in plasma by day 84 in CO-fed arthritic mice had increased plasma TNF-α, whereas plasma TNF-α in all t10c12-CLA-treated mice was similar to baseline concentrations ($P = 0.06$). Additionally, both plasma and paw TNF-α decreased linearly with increasing dietary t10c12-CLA ($P = 0.01$, $R^2 = 0.7$, and $P = 0.05$, $R^2 = 0.7$, respectively). These results further support that dietary t10c12-CLA, but not c9t11-CLA, increased proinflammatory cytokines in arthritic tissue and plasma, others have reported that anti-TNF-α and anti–IL-1β treatments are significantly more effective at reducing CA when administered in unison rather than separately (60). Hence, the ability of t10c12-CLA to reduce both of these cytokines may be advantageous.

Whereas dietary t10c12-CLA reduced proinflammatory cytokines in arthritic tissue and plasma, others have reported that dietary t10c12-CLA, but not c9t11-CLA, increased proinflammatory cytokines in adipose tissue of healthy animals (61–63). The mechanism(s) by which t10c12-CLA decreases TNF-α in circulation and in arthritic tissue. It should be noted that anti-TNF-α and anti–IL-1β antibodies or deletion of genomic TNF-α dramatically decreased histologic and clinical signs of joint inflammation in several murine CA trials (50,54–56) and has led to the development of human anti–TNF-α antibodies (i.e., etanercept, infliximab, adalimumab) for treating rheumatoid arthritis (57,58). Whereas multiple studies have shown that CLA (mixed and individual isomers) has the ability to reduce TNF-α (19,21,22,28,29,47,59), the responsible isomer is still debatable (19,22). In the study presented here, CO-fed arthritic mice had increased plasma TNF-α, whereas plasma TNF-α in all t10c12-CLA–treated mice was similar to baseline concentrations ($P = 0.06$). Additionally, both plasma

### TABLE 2 Relative hepatic FA compositions of sham mice fed CO and arthritic mice fed CO or t10c12-CLA-containing diets for an 84-d treatment period postinitiation of arthritis

<table>
<thead>
<tr>
<th>FA</th>
<th>Sham</th>
<th>Arthritic</th>
<th>0.125% CO</th>
<th>0.25% CO</th>
<th>0.375% CO</th>
<th>0.5% CO</th>
<th>SEM²</th>
<th>P</th>
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<td>12:0</td>
<td>0.28</td>
<td>0.40</td>
<td>0.36</td>
<td>0.37</td>
<td>0.30</td>
<td>0.32</td>
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<td>0.00</td>
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<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
<td>0.72</td>
</tr>
<tr>
<td>16:0</td>
<td>25.9</td>
<td>26.9</td>
<td>28.3</td>
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<td>25.8</td>
<td>26.9</td>
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<td>0.20</td>
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<td>0.31</td>
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¹ Values are means, $n = 3$. Means in a row without a common superscript differ, $P < 0.05$. CO, corn oil; ND, not detectable.

² Pooled SEM.

³ Increasing dietary t10c12-CLA in arthritic mice was linearly associated with an increase in hepatic t10c12-CLA ($P < 0.001$, $R^2 = 0.98$).
mice. Therefore, there is no evidence that t10c12-CLA reduced inflammation through modification of antibody responses to collagen.

In summary, our data suggest that the t10c12-CLA concentration in natural sources is unlikely to provide an antiarthritic effect in humans equivalent to our observations in the murine CA model. Evidence that t10c12-CLA had no effect on articular cartilage, synovium, or menisci, as well as the absence of adverse effects, suggests that the antiarthritic effects of t10c12-CLA are mediated through reduced proinflammatory cytokines. In this study, a maximum anti-inflammatory concentration of t10c12-CLA was not determined. Consequently, dietary concentrations >0.5% may be more effective in reducing inflammation, especially at the onset of arthritis.

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Literature Cited

184 Huebner et al.
