# Conjugated Linoleic Acid Isomers Reduce Blood Cholesterol Levels but Not Aortic Cholesterol Accumulation in Hypercholesterolemic Hamsters

**Thomas A. Wilson[a,*], Robert J. Nicolosi[a], Andrew Saati[a], Timothy Kotyla[a], and David Kritchevsky[b]**

[a]Department of Clinical Laboratory and Nutritional Sciences, Center for Health and Disease Research, University of Massachusetts Lowell, Lowell, Massachusetts 01854-5125, and [b]Wistar Institute, Philadelphia, Pennsylvania 19104-4268

**ABSTRACT:** The aim of the present study was to characterize plasma lipids and lipoprotein cholesterol and glucose concentrations in hamsters fed either *cis*-9,*trans*-11 CLA (9*c*,11*t* CLA); *trans*-10,*cis*-12 CLA (10*t*,12*c* CLA); or linoleic acid (LA) on the accumulation of aortic cholesterol in hypercholesterolemic hamsters. One hundred male $F_1B$ strain Syrian Golden Hamsters (*Mesocricetus auratus*) (BioBreeders Inc., Watertown, MA) approximately 9 wk of age were housed in individual stainless steel hanging cages at room temperature with a 12-h light/dark cycle. Hamsters were given food and water *ad libitum*. Following a 1-wk period of acclimation, the hamsters were fed a chow-based (nonpurified) hypercholesterolemic diet (HCD) containing 10% coconut oil (92% saturated fat) and 0.1% cholesterol for 2 wk. After an overnight fast, the hamsters were bled and plasma cholesterol concentrations were measured. The hamsters were then divided into 4 groups of 25 based on similar mean plasma VLDL and LDL cholesterol (nonHDL-C) concentrations. Group 1 remained on the HCD (control). Group 2 was fed the HCD plus 0.5% 9*c*,11*t* CLA isomer. Group 3 was fed the HCD plus 0.5% 10*t*,12*c* CLA isomer. Group 4 was fed the HCD plus 0.5% LA. Compared with the control, both CLA isomers and LA had significantly lower plasma total cholesterol and HDL cholesterol concentrations ($P <$ 0.001) after 12 but not 8 wk of treatment and were not significantly different from each other. Also, both CLA isomers had significantly lower plasma nonHDL-C concentrations ($P < 0.01$) compared with the control after 12 but not 8 wk of treatment and were not significantly different from each other or the LA-fed hamsters. Plasma TG concentrations were significantly higher ($P$ < 0.004) with the 10*t*,12*c* CLA isomer compared with the other treatments at 8 but not at 12 wk of treatment. Plasma TG concentrations were also significantly lower ($P < 0.03$) with the 9*c*,11*t* CLA isomer compared with the control at 12 wk of treatment. Also, the 10*t*,12*c* CLA isomer and LA had significantly higher plasma glucose concentrations compared with the control and 9*c*,11*t* CLA isomer ($P < 0.008$) at 12 wk of treatment, whereas at 8 wk, only the LA treatment had significantly higher plasma glucose concentrations ($P < 0.001$) compared with the 9*c*,11*t* CLA isomer. Although liver weights were significantly higher in 10*t*,12*c* CLA isomer-fed hamsters, liver total cholesterol, free cholesterol, cholesterol ester, and TG concentrations were significantly lower in these hamsters compared with hamsters fed the control, 9*c*,11*t* CLA isomer, and LA diets ($P < 0.05$). The 9*c*,11*t* CLA isomer and LA diets tended to reduce cholesterol accumulation in the aortic arch, whereas the 10*t*,12*c* CLA isomer diet tended to raise cholesterol accumulation compared with the control diet; however, neither was significant. In summary, no differences were observed between the CLA isomers for changes in plasma lipids or lipoprotein cholesterol concentrations. However, the 9*c*,11*t* CLA isomer did appear to lower plasma TG and glucose concentrations compared with the 10*t*,12*c* CLA isomer. Such differences may increase the risk of insulin resistance and type 2 diabetes in humans when the 10*t*,12*c* CLA isomer is fed separately.

Paper no. L9699 in *Lipids 41*, 41–48 (January 2006).

Animal studies in our laboratory as well as in others have shown that feeding a predominantly linoleic acid (LA)-containing diet such as corn oil or safflower oil results in dramatic decreases in blood LDL cholesterol (LDL-C) concentrations, which are associated with reductions in atherosclerosis (1,2). However, in these experiments blood LDL-C is reduced so dramatically that the nature of the LDL is unimportant. In contrast, in human studies, the magnitude of the blood LDL-C reduction with LA-rich diets is usually on the order of 5–10%; thus, these LDL particles enriched in LA are more susceptible to oxidation (3) and are presumably more atherogenic (4). Support for the atherogenicity of LA-enriched LDL comes from studies recently completed in our laboratory in which hypercholesterolemic animals fed a diet enriched in sunflower oil (LA-containing) and cholesterol had dramatically more aortic atherosclerosis than animals fed TriSun® oil (oleic-containing; SVO Enterprises, Eastlake, OH) plus cholesterol despite similar, albeit elevated, LDL-C levels (5). Moreover, the increase in aortic atherosclerosis with sunflower oil feeding was highly associated with greater LDL oxidative susceptibility and accumulation of aortic oxidized LDL (5).

These observations beg the question of whether CLA would be more effective than LA in terms of reducing the progression of the aortic lesion. Although several studies have confirmed the anticancer properties of CLA, only a few published reports support its antiatherosclerotic effects, and only one has shown the influence of the purified 9-*cis*,11-*trans* (9*c*,11*t*) CLA iso-

*To whom correspondence should be addressed at 3 Solomont Way, Suite 4, Department of Clinical Laboratory and Nutritional Sciences, Center for Health and Disease Research, University of Massachusetts Lowell, Lowell, MA 01854-5125. E-mail: Thomas_Wilson@uml.edu

Abbreviations: 9*c*,11*t* CLA, *cis*-9,*trans*-11 CLA; 10*t*,12*c* CLA, *trans*-10,*cis*-12 CLA; HDL-C, HDL cholesterol; LA, linoleic acid; LDL-C, LDL cholesterol; nonHDL-C, VLDL and LDL cholesterol.

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mer. Preliminary evidence from a non-dose–response study from our laboratory would suggest that animals fed mixed CLA isomers had less atherosclerosis than those fed LA (6). However, for reasons mentioned hereafter, these preliminary studies must be interpreted with caution. Also, a recent study (7) showed that CLA feeding promoted fatty streak formation in the C57BL/6 mouse atherosclerosis model. However, this finding may be specific to this animal model and not to others. A study by Lee *et al.* (8) in rabbits showed that at the only concentration studied, 0.5% (w/w), CLA as the FFA reduced aortic atherosclerosis. Recently, a study published by Gavino *et al.* (9) showed that feeding a diet containing an isomeric mixture of CLA significantly reduced plasma TG and total cholesterol levels in hamsters compared with LA and the 9*c*,11*t* CLA isomer. However, their study did not investigate the development of atherosclerosis.

Our recent study in hamsters fed a chow-based diet containing 0.05, 0.1, and 1.0 en% mixed CLA isomers as the FFA also showed an antiatherogenic effect that was associated with increased plasma vitamin E levels (10). Our preliminary study (6) suggested that the FFA was more effective than TG as an antiatherogenic compound. In our earlier mixed-CLA isomer study (10), hamsters were extremely hypercholesterolemic (plasma cholesterol levels >700 mg/dL), raising the question of the efficacy of CLA during more moderate hypercholesterolemia. Also, in that study (10), the hamsters were housed grouped, which may be the reason for the very high levels of blood cholesterol in those animals; in another study in our laboratory, group housing of hamsters was shown to be associated with adverse effects on plasma lipids (11). Data have also indicated that mixed and individual isomers of CLA are effective inhibitors of atherogenesis and also cause regression of established atherosclerosis in rabbits (12–14).

Much of the previous work that has been performed in animals fed CLA has been done using the mixed-isomer formulation of commercially available CLA. The current study examines the purified form of either 9*c*,11*t* or *trans*-10,*cis*-12 (10*t*,12*c*) CLA in the hypercholesterolemic hamster model to determine whether they have similar hypocholesterolemic, glucose hemostatic, and antiatherogenic properties.

## EXPERIMENTAL PROCEDURES

*Experimental design and diets.* One hundred male $F_1B$ strain Syrian Golden Hamsters (*Mesocricetus auratus*) (BioBreeders Inc., Watertown, MA) approximately 9 wk of age were housed in individual stainless steel hanging cages at room temperature with a 12-h light/dark cycle. Hamsters were given food and water *ad libitum*. Animals were fed Purina chow 5001 (Ralston Purina, St. Louis, MO) for a period of 1 wk prior to the start of the study to become acclimated to the facility. All 100 hamsters were fed a chow-based (nonpurified) hypercholesterolemic diet (HCD) containing 10% coconut oil (92% saturated fat) and 0.1% cholesterol for 2 wk. A nonpurified diet, rather than a semipurified diet, was used because published data from our laboratory (15) and those from another laboratory (16) indicated that hamsters on a nonpurified diet were more responsive to various cholesterolemic interventions and a resultant lipoprotein profile [VLDL and LDL cholesterol (nonHDL-C) > HDL cholesterol (HDL-C)] which was similar to that of humans. After an overnight fast, the hamsters were bled and plasma cholesterol concentrations were measured. The hamsters were then divided into 4 groups of 25 based on similar mean plasma nonHDL-C concentrations. Group 1 remained on the HCD (control). Group 2 was fed the HCD plus 0.5% 9*c*,11*t* CLA. Group 3 was fed the HCD plus 0.5% 10*t*,12*c* CLA. Group 4 was fed the HCD plus 0.5% LA. Dietary CLA isomers in pure form and LA were added to the diets as the FFA form and were obtained from Matreya, Inc. (Pleasant Gap, PA). The two CLA isomers and LA were >98% pure by GC. The FA composition of the treatment diets is provided in Table 1. Dietary treatments were fed for 12 wk. Plasma lipids were measured at 0, 8, and 12 wk of dietary treatment. At the end of dietary treatment, the hamsters were sacrificed and aortas were collected. Food disappearance and body weights were measured on a daily basis throughout the study. The animals were maintained in accordance with the guidelines of the Committee on Animal Care of the University of Massachusetts Lowell Research Foundation, as well as the guidelines prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication no. 85–23, revised 1985), following approval by the Institutional Animal Care and Use Committee.

**TABLE 1**
**FA Composition of Dietary Treatments (mg/g of diet)**

| FA | Control | 9*c*,11*t* CLA | 10*t*,12*c* CLA | LA[a] |
|---|---|---|---|---|
| 8:0 | 6.7 | 6.7 | 6.7 | 6.7 |
| 10:0 | 5.8 | 5.8 | 5.8 | 5.8 |
| 12:0 | 49.0 | 49.0 | 49.0 | 49.0 |
| 14:0 | 18.3 | 18.3 | 18.3 | 18.3 |
| 16:0 | 8.8 | 8.8 | 8.8 | 8.8 |
| 18:0 | 2.7 | 2.7 | 2.7 | 2.7 |
| 18:1 | 6.7 | 6.7 | 6.7 | 6.7 |
| 18:2 (LA[a]) | 1.5 | 1.6 | 1.6 | 6.5 |
| 9*c*,11*t* CLA | — | 4.9 | — | — |
| 10*t*,12*c* CLA | — | — | 4.9 | — |

[a]LA, linoleic acid.

*Plasma lipoprotein cholesterol, TG, and glucose measurements.* Blood samples were collected after an overnight fast (16 h). Blood from fasted hamsters, anesthetized with ultrapure 50:50 $CO_2/O_2$ (Northeast Airgas, Salem, NH), was collected *via* the retro-orbital sinus into heparinized tubes, and plasma was harvested after low-speed centrifugation at 2500 × *g* for 15 min at room temperature. Plasma was frozen at –80°C until analyzed for plasma total cholesterol, HDL-C, nonHDL-C, TG, and glucose concentrations. Plasma cholesterol (17) and TG (18) were measured enzymatically, and after the apoB-containing lipoproteins VLDL and LDL were precipitated with phosphotungstate reagent (19), the supernatant was assayed for HDL-C (Sigma, St. Louis, MO). Plasma nonHDL-C was calculated from the difference between total cholesterol and HDL-C. Plasma lipid determinations are standardized by participa-

tion in the Center for Disease Control–National Heart, Lung, and Blood Institute Standardization Program. Plasma glucose concentrations were also measured enzymatically using Kit #17–100 (Sigma).

*Liver lipid measurements.* Liver lipid concentrations were measured by a previously described method (20). A 100-mg (wet wt) portion of liver was homogenized with 50 mg of sodium sulfate. Four milliliters of methanol was then added and the tissue was homogenized a second time, followed by addition of 8 mL of chloroform. After mixing, 3 mL of a solution containing 1.25% KCl and 0.05% $H_2SO_4$ was added and centrifuged at 400 × *g* at room temperature for 10 min. The bottom layer was transferred, and the supernatant was re-extracted with 3 mL of chloroform/methanol (2:1) and centrifuged at 400 × *g* at room temperature for 10 min. The bottom layer was transferred and pooled with the previous supernatant. The solution was placed in a 37°C water bath under $N_2$. When approximately half of the solution was evaporated, 1 mL of chloroform containing 1% Triton-100 was added, mixed, and evaporated to dryness at 37°C under $N_2$. Five hundred microliters of distilled water was added to the samples, which were mixed and placed in a shaking water bath at 37°C for 20 min to solubilize the lipid. After incubation, liver total and free cholesterol and phospholipid concentrations were determined enzymatically using Wako total and free cholesterol C and phospholipid kits (Wako Chemicals, Richmond, VA). The hepatic cholesterol ester concentration was determined as the difference between the total and free cholesterol concentrations. Liver TG concentrations were as stated above for plasma TG measurements.

*Collection of aortas.* At the time of sacrifice (week 12), hamsters were anesthetized with an ip injection of sodium pentobarbital (62.5 mg/mL at a dosage of 0.2–0.25 mL/200 g body weight) (Henry Schein, Port Washington, NY), and aortic tissue was obtained for aortic cholesterol analysis (21). The heart and thoracic aorta were removed and stored in vials containing PBS at 4°C for subsequent analysis. To measure the extent of the aortic cholesterol accumulation in the aortic arch, a piece of thoracic aortic tissue extending from as close to the heart as possible to the branch of the left subclavian artery was used.

*Aortic cholesterol measurement.* The tissue was weighed and placed in a 25-mL screw-capped test tube (21). Four milliliters of methanol and 8 mL of chloroform were added. After mixing, the solution was allowed to stand at room temperature for 48 h for extraction of cholesterol from the blood vessel wall. After extraction, the aortic tissue was removed and the solution was placed in a 37°C water bath under $N_2$. When approximately half of the solution was evaporated, 1 mL of chloroform containing 1% Triton-100 was added, mixed, and evaporated to dryness at 37°C under $N_2$. Two hundred microliters of distilled water was added to the samples, which were mixed and placed in a shaking water bath at 37°C for 20 min to solubilize the lipids. After incubation, aortic total and free cholesterol concentrations were determined enzymatically using Wako total and free cholesterol C kits (Wako Chemicals). The aortic cholesteryl ester concentration was determined as the difference between the total and free cholesterol concentrations. A pilot study was conducted to evaluate the extent to which this procedure removed tissue cholesterol. Aortic cholesterol concentrations were determined after tissue was placed in solvent (4 mL of methanol and 10 mL of chloroform) overnight with frequent vigorous mixing, and were compared with the concentrations obtained following tissue mincing or homogenization as reported previously (22). No significant differences in aortic cholesterol content were observed between the different cholesterol extraction procedures.

*Statistical methods.* SigmaStat software was used for all statistical evaluations (Jandel Scientific, San Rafael, CA) (23). Differences between time points were determined by using repeated-measures one-way ANOVA followed by Student–Neuman–Keuls *post hoc* test. Differences between the dietary treatments were determined using one-way ANOVA followed by Student–Neuman–Keuls *post hoc* test. All values are expressed as mean ± SD, and significance was set at $P < 0.05$.

## RESULTS

All the animals survived the treatment period. As seen in Table 2, there were no differences between any of the animals for initial or final body weights and between food consumption over the course of the study. Also, all the animals in each group gained body weight over the course of the study. Liver weights were significantly greater in the animals fed the 10*t*,12*c* CLA isomer compared with the control (18%), 9*c*,11*t* CLA isomer (14%), and LA (16%) animals ($P < 0.03$) (Table 2).

No significant differences were observed for plasma lipids within dietary treatments between the week 8 and week 12 data. However, statistical differences between groups for plasma lipid measurements were different at 8 and 12 wk; thus, the data have been shown separately. No differences between any of the dietary treatments were observed for plasma total cholesterol, nonHDL-C, and HDL-C concentrations after 8 wk

**TABLE 2**
**Initial and Final Body Weights (g), Food Consumption (g/d), and Organ Weights (g) After 12 wk of CLA or LA Treatment[a]**

| Diet | Initial body weight | Final body weight | Food consumption | Liver weight | Adipose (perirenal) weight |
|---|---|---|---|---|---|
| Control | 93.5 ± 8.69 | 125.5 ± 23.9 | 14.7 ± 2.20 | 4.11 ± 0.50[a] | 0.91 ± 0.23 |
| 0.5% 9*c*,11*t* CLA | 91.5 ± 11.7 | 130.2 ± 24.8 | 14.9 ± 1.41 | 4.26 ± 0.66[a] | 0.87 ± 0.17 |
| 0.5% 10*t*,12*c* CLA | 94.7 ± 11.2 | 129.4 ± 24.5 | 14.7 ± 1.28 | 4.86 ± 0.88[b] | 0.80 ± 0.20 |
| 0.5% LA | 95.2 ± 8.20 | 128.0 ± 27.3 | 14.9 ± 2.13 | 4.19 ± 0.63[a] | 0.85 ± 0.15 |

[a]Values are mean ± SD; $n = 25$. Values in a column not sharing a roman superscript are significantly different at $P < 0.05$. For abbreviation see Table 1.

**TABLE 3**
**Plasma Cholesterol, Lipoprotein Cholesterol, TG, and Glucose Concentrations in Hamsters (week 8) (mg/dL)[a]**

| Diet | TC | nonHDL-C | HDL-C | TG | TC/HDL-C | Glucose |
|---|---|---|---|---|---|---|
| Control | 310.0 ± 94.1 | 241.6 ± 95.4 | 68.3 ± 3.38 | 479.5 ± 248.6[a] | 4.57 ± 1.45 | 110.5 ± 18.3[a,b] |
| 0.5% 9*c*,11*t* CLA | 268.3 ± 85.5 | 200.3 ± 86.0 | 68.1 ± 5.32 | 562.6 ± 310.9[a] | 3.97 ± 1.32 | 109.0 ± 21.8[a] |
| 0.5% 10*t*,12*c* CLA | 288.0 ± 36.3 | 223.3 ± 38.7 | 64.8 ± 6.41 | 794.7 ± 341.4[b] | 4.50 ± 0.81 | 126.1 ± 16.4[a,b] |
| 0.5% LA | 260.4 ± 73.4 | 194.2 ± 73.3 | 66.2 ± 3.97 | 483.5 ± 137.0[a] | 3.95 ± 1.13 | 128.5 ± 16.9[b] |

[a]Values are mean ± SD; $n = 25$. Values in a column not sharing a roman superscript are significantly different at $P < 0.05$. TC, total cholesterol; nonHDL-C, VLDL and LDL cholesterol; HDL-C, HDL cholesterol; for other abbreviation see Table 1.

**TABLE 4**
**Plasma Cholesterol, Lipoprotein Cholesterol, TG, and Glucose Concentrations in Hamsters (week 12) (mg/dL)[a]**

| Diet | TC | nonHDL-C | HDL-C | TG | TC/HDL-C | Glucose |
|---|---|---|---|---|---|---|
| Control | 316.7 ± 131.6[a] | 222.4 ± 152.7[a] | 94.4 ± 17.1[a] | 413.0 ± 235.7[a] | 3.58 ± 2.14 | 115.3 ± 15.3[a] |
| 0.5% 9*c*,11*t* CLA | 203.1 ± 29.0[b] | 124.3 ± 31.6[b] | 78.8 ± 9.57[b] | 199.0 ± 39.9[b] | 2.62 ± 0.51 | 99.4 ± 13.5[a] |
| 0.5% 10*t*,12*c* CLA | 205.5 ± 31.9[b] | 133.8 ± 34.1[b] | 71.8 ± 7.23[b] | 304.4 ± 80.6[a,b] | 2.90 ± 0.59 | 153.3 ± 34.6[b] |
| 0.5% LA | 227.5 ± 46.8[b] | 157.9 ± 45.1[a,b] | 69.5 ± 7.78[b] | 291.5 ± 106.0[a,b] | 3.29 ± 0.67 | 129.9 ± 16.9[b] |

[a]Values are mean ± SD; $n = 25$. Values in a column not sharing a roman superscript are significantly different at $P < 0.05$. For abbreviations see Tables 1 and 3.

of dietary treatment (Table 3). Plasma TG, however, were significantly higher in the hamsters fed the 10*t*,12*c* CLA isomer compared with hamsters fed the control (40%), 9*c*,11*t* CLA isomer (29%), and LA (39%) diets after 8 wk of treatment ($P < 0.004$). Also, hamsters fed the LA diet had significantly higher plasma glucose concentrations compared with hamsters fed the 9*c*,11*t* CLA isomer (15%) after 8 wk of treatment ($P < 0.001$).

After 12 wk of dietary treatment, plasma total cholesterol and HDL-C concentrations were significantly lower in the hamsters fed the 9*c*,11*t* CLA isomer (−35 and −17%, respectively), 10*t*,12*c* CLA isomer (−35 and −24%, respectively), and LA (−28 and −26%, respectively) diets compared hamsters fed the control diet ($P < 0.001$) (Table 4). Plasma nonHDL-C concentrations were significantly lower in hamsters fed the 9*c*,11*t* CLA isomer (−44%) and the 10*t*,12*c* CLA isomer (−40%) diets but not in those fed the LA diet, compared with hamsters fed the control diet after 12 wk of treatment ($P < 0.001$). Plasma TG concentrations were significantly lower in the hamsters fed the 9*c*,11*t* CLA isomer compared with those fed the control (−52%) diet after 12 wk of treatment ($P < 0.03$). Also, hamsters fed the 10*t*,12*c* CLA isomer and the LA diets had significantly higher plasma glucose concentrations compared with those fed the control (25 and 11%, respectively) and 9*c*,11*t* CLA isomer (35 and 23%, respectively) diets after 12 wk of treatment ($P < 0.008$).

Although liver weights were significantly higher in hamsters fed the 10*t*,12*c* CLA isomer diet, liver total cholesterol, free cholesterol, cholesterol ester, and TG concentrations were significantly lower in these hamsters than in those fed the control (−51, −28, −59, and −45%, respectively), 9*c*,11*t* CLA isomer (−53, −33, −60, and −46%, respectively), and LA (−47, −30, −53, and −40%, respectively) diets ($P < 0.05$) (Table 5). None of the other treatment groups were significantly different from each other for these same variables. Also, no treatment groups were significantly different from each other for liver phospholipid concentrations (Table 5).

Table 6 shows the data for aortic cholesterol accumulation measurements after 12 wk of dietary treatment. Although there were no statistically significant differences between any of the dietary treatments for aortic cholesterol accumulation, a small trend was observed. The 9*c*,11*t* CLA isomer tended to reduce total cholesterol and cholesteryl ester accumulation in the aortic arch compared with the 10*t*,12*c* CLA isomer (−18 and −13%, respectively, $P = 0.11$), but not significantly.

## DISCUSSION

Our results indicate that supplementing the diets of hypercholesterolemic hamsters with individual CLA isomers reduces plasma cholesterol concentrations when compared with controls, without any changes in body weight and aortic cholesterol accumulation. The effects of CLA on body weight and composition have been controversial in both animal and human studies. In the current study, no differences in final body

**TABLE 5**
**Liver TC, Free Cholesterol (FC), Cholesterol Ester (CE), TG, and Phospholipid (PL) Concentrations in Hamsters (mg/g liver tissue)[a]**

| Diet | TC | FC | CE | TG | PL |
|---|---|---|---|---|---|
| Control | 11.15 ± 3.93[a] | 2.99 ± 0.76[a] | 8.16 ± 3.47[a] | 6.29 ± 2.98[a] | 14.89 ± 5.44 |
| 0.5% 9*c*,11*t* CLA | 11.50 ± 5.33[a] | 3.20 ± 0.86[a] | 8.30 ± 4.56[a] | 6.35 ± 2.63[a] | 15.60 ± 3.53 |
| 0.5% 10*t*,12*c* CLA | 5.46 ± 0.88[b] | 2.14 ± 0.59[b] | 3.33 ± 2.31[b] | 3.43 ± 1.28[b] | 12.72 ± 3.23 |
| 0.5% LA | 10.22 ± 3.68[a] | 3.07 ± 0.82[a] | 7.15 ± 3.11[a] | 5.76 ± 2.16[b] | 15.48 ± 3.68 |

[a]Values are mean ± SD; $n = 25$. Values in a column not sharing a roman superscript are significantly different at $P < 0.05$. For other abbreviations see Tables 1 and 3.

**TABLE 6**
**Aortic Cholesterol Concentrations (μg/mg of tissue)[a]**

| Diet | FC | CE | TC |
|---|---|---|---|
| Control | 1.96 ± 1.48 | 0.80 ± 0.45 | 2.76 ± 1.36 |
| 0.5% 9*c*,11*t* CLA | 1.93 ± 1.54 | 0.74 ± 0.54 | 2.63 ± 1.37 |
| 0.5% 10*t*,12*c* CLA | 2.13 ± 1.65 | 0.90 ± 0.53 | 3.03 ± 1.75 |
| 0.5% LA | 1.91 ± 1.33 | 0.79 ± 0.60 | 2.71 ± 1.53 |

[a]Values are mean ± SD, *n* = 25. For abbreviations see Tables 1, 3, and 5.

weights were observed between hamsters fed the control diet or diets supplemented with CLA isomers or LA. Previous work in young rats fed diets supplemented with 0.5% CLA showed, in animals fed the CLA, an increase in body weight without changes in food consumption compared with controls (24). In contrast to this study, Park *et al.* (25) reported in mice that the effects of CLA on body composition changes appeared to be due in part to reduced fat deposition and increased lipolysis in adipocytes. Animal studies in general have suggested that the 10*t*,12*c* isomer, and not the 9*c*,11*t* isomer, has the most potent body fat-reducing properties (9,25). In the current study, no differences were observed for perirenal adipose tissue weight. However, the 10*t*,12*c* CLA isomer did reduce it slightly compared with the control. One possible explanation for the nonsignificant effect of either CLA isomer on the reduction of fat tissue is that the effects of CLA on the perirenal depot are more sensitive when diets are low in fat (4%), but not when diets are high in fat (10%) (26), as in the current study. In humans, the majority of studies have shown no effect on body weight or composition when individual or mixed isomers of CLA were fed (27–31). However, one recent long-term trial (1 yr) showed that CLA feeding decreased body fat mass without any adverse effects (32). Other differences between earlier studies that showed positive effects and this study on body weight changes may be due to feeding the mixed CLA isomer in the earlier studies compared with feeding the individual isomers in the current study, or to species differences. Since previous work in hamsters fed a mixed-isomer diet showed significantly less weight gain than did either the 9*c*,11*t* isomer- or LA-supplemented groups (9) and showed that the mixed-isomer diet decreased fat depot weights compared with the 9*c*,11*t* isomer diet in other studies (25,33), there may be synergistic effects on body weight or composition when both the 9*c*,11*t* and 10*t*,12*c* CLA isomers are supplemented simultaneously. Overall, however, there is no conclusive evidence to suggest that consumption either of mixtures of CLA isomers or of highly enriched preparations of individual isomers results in a significant reduction in body composition in animals, including humans.

Some studies have shown that feeding only the 10*t*,12*c* CLA isomer is associated with greater liver weights in hamsters and with a significant decrease or no change in the amount of TG accumulation in the liver (33–35). However, other studies in mice have shown the same hepatomegoly with mixed CLA isomer feeding, and this was associated with greater TG accumulation (36–39). In the current study, larger livers were also found in hamsters fed the 10*t*,12*c* CLA isomer but not in hamsters fed the 9*c*,11*t* CLA isomer or LA. Similar to previous work in hamsters (35), this increase in liver weights in hamsters fed the 10*t*,12*c* CLA isomer was not due to increased lipid accumulation. In fact, levels of liver TG, cholesterol esters, and free cholesterol were significantly decreased in these hamsters compared with the effects of other dietary treatments. Differences between the current study and those by others in which an increase in lipid accumulation was observed (36–39) may be due to the feeding of individual isomers vs. mixed CLA or due to different species (mice vs. hamsters) being studied. However, it is still unknown what may have led to the increase in liver weights in hamsters fed the 10*t*,12*c* CLA isomer in the current study, but it could be due to an increase in the total number of hepatocytes with the 10*t*,12*c* CLA isomer, as previously observed (35).

Previous studies from our laboratory (6,10) and from others (8,9) have shown reductions in plasma cholesterol concentrations in mixed CLA-fed animals compared with controls. The results of the current study with the individual CLA isomer formulations compared with controls are consistent with these previous studies (40) for plasma cholesterol lowering. However, in the current study we observed no significant differences between feeding the individual CLA isomers and LA for plasma cholesterol lowering, whereas an earlier study from our laboratory did show that mixed CLA feeding produced significantly lower plasma cholesterol levels compared with LA (10), but another did not (6). Presently, there is no explanation for this discrepancy, although the difference may be due to the different diets that were used in these studies. In the present study, we fed the different isomers individually in the diets, compared with feeding a mixed CLA isomer formulation in the earlier study (10). Previous work (9) showed that the 9*c*,11*t* CLA isomer had no effect on plasma cholesterol concentrations compared with control hamsters. However, in that study (9) only 0.2% of the 9*c*,11*t* CLA isomer was fed to hamsters, whereas in the current study 0.5% was fed to hamsters. Another study (40) also showed a reduction in plasma cholesterol concentrations with both the 9*c*,11*t* and 10*t*,12*c* CLA isomers and with LA when compared with control hamsters. Similar contradictory results have been observed in human studies. Tricon *et al.* (27) showed a significant effect of isomer supplementation on blood cholesterol concentrations in humans. Their data suggest a trend toward a decrease in plasma cholesterol and LDL-C concentrations after supplementation with the 9*c*,11*t* CLA isomer, but not the 10*t*,12*c* CLA isomer (27). Other studies have also shown a cholesterol-lowering effect of CLA in humans

(30,41). However, more studies appear to have shown no effect on blood cholesterol levels in humans when fed CLA (31,42,43). The observation in the present study that individual or mixed CLA isomer feeding reduced plasma HDL-C is similar to some previous studies in animals (31,36) and humans (44), but not to others (6,9,10,27,42,43).

We also observed an increase in plasma TG when feeding the 10*t*,12*c* CLA isomer compared with the other treatments after 8 wk of supplementation, but not after 12 wk. By 12 wk of treatment, the 9*c*,11*t* CLA isomer had significantly lowered plasma TG compared with hamsters fed the control. These results are similar to our previous study (6), which showed that feeding mixed-isomer CLA increased plasma TG more than did LA. In contrast, previous studies in hamsters (9,10) and humans (29) have shown that feeding the mixed CLA either reduced or had no effect on plasma TG concentrations. The differences between those studies and the current study may be due to the different concentrations of purified isomers used in these studies or to the use of mixed CLA isomers vs. individual isomers. However, one study (27) in healthy humans showed that plasma TG concentrations were higher during supplementation with the 10*t*,12*c* CLA isomer than during supplementation with the 9*c*,11*t* CLA isomer. The TG-raising potential of mixed CLA may be predominantly due to the 10*t*,12*c* isomer, as shown in the current study and in others (27). Along with the slight changes in plasma TG concentrations with the different CLA isomers, we also observed changes in plasma glucose concentrations. In the current study, the 9*c*,11*t* CLA isomer resulted in significantly lower plasma glucose concentrations compared with the 10*t*,12*c* CLA isomer and LA diets, similar to previous studies in humans (27,44). Risérus *et al.* (44) also showed that feeding the 9*c*,11*t* CLA isomer decreased insulin resistance whereas feeding the 10*t*,12*c* CLA isomer increased insulin resistance in obese men. Also, a study by Moloney *et al.* (45) showed that supplementation with mixed CLA significantly increased fasting glucose concentrations. In contrast, CLA feeding was shown to improve glucose concentrations in type 2 diabetics (46) and in rats (47). The difference between these studies (46,47) and the current one may again be due to the use of mixed CLA vs. individual isomers, or to species differences.

The present study also showed that feeding CLA as individual isomers did not produce a significant change in early aortic atherosclerosis, as measured by aortic cholesterol accumulation, compared with both LA and the control diet, a result that is inconsistent with previous work (9,10). However, this observation in the former study (10) was not significant until the data for the three different doses of CLA feeding were combined and then compared with the HCD-fed animals; also, the CLA used for the earlier studies was mixed CLA. Additionally, in our earlier study (10), the hamsters that were fed CLA had a significantly greater plasma tocopherol/total cholesterol ratio than did hamsters fed a control diet or LA-containing diet. These results suggest that CLA may be tocopherol sparing and thus act directly or indirectly as an antioxidant *in vivo*, which may contribute to its antiatherogenic properties. Another possible explanation for the discrepancy is that earlier studies have either used fatty streak area (10) or visual staining procedures (15), measures of early atherosclerosis that may not be as appropriate as the biochemical measurement of aortic cholesterol accumulation used in the current study. Recently, Mitchell *et al.* (40) showed a similar result when feeding the individual isomers compared with LA, in that there were no significant differences in aortic fatty streak lesions but that a slight decrease was observed with the CLA isomers.

Although, in the current study, feeding LA to hamsters produced a significant decrease in plasma total cholesterol and HDL-C concentrations, similar to CLA feeding, it did not produce a significant reduction in nonHDL-C concentrations, as did the CLA isomers after 12 wk of dietary treatment or in early atherosclerosis. This nonsignificant reduction in aortic atherosclerosis with LA feeding in the present study may be due to an increase in LDL oxidative susceptibility that is associated with LA feeding, which was not measured. Previous studies have shown that LDL particles enriched with LA are more susceptible to oxidation and presumably more atherogenic than are oleate-rich diets (3,4).

In summary, both CLA isomers produced significant lowering in plasma total cholesterol and nonHDL-C concentrations; however, the majority of human studies have shown no effect. Also, no significant differences were observed between the CLA isomers (9*c*,11*t* vs. 10*t*,12*c*) for changes in plasma lipids or lipoprotein cholesterol concentrations; however, the 10*t*,12*c* CLA isomer did raise plasma glucose concentrations compared with the 9*c*,11*t* CLA isomer, and only the 9*c*,11*t* CLA isomer reduced plasma TG compared with the control. The lower plasma glucose concentrations, along with the lower plasma TG concentrations, may decrease the risk of insulin resistance and type 2 diabetes in humans when the 9*c*,11*t* CLA isomer is fed alone.

## ACKNOWLEDGMENTS

This project was funded by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen's Beef Board and state councils by the National Cattlemen's Beef Association and Kraft General Foods (T.A.W.). The authors would like to thank Benjamin Woolfrey, Catherine Jones, Wendy DeSimone, and Subbiah Yoganathan for their technical support and Maureen Faul for her administrative support.

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[Received January 25, 2005; accepted December 15, 2005]