Effect of Abomasal Infusions of Geometric Isomers of 10,12 Conjugated Linoleic Acid on Milk Fat Synthesis in Dairy Cows

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ABSTRACT: The *trans*-10,*cis*-12 isomer of conjugated linoleic acid (CLA) decreases TAG accumulation in 3T3-L1 adipocytes, reduces lipid accretion in growing animals, and inhibits milk fat synthesis in lactating mammals. However, there is evidence to suggest that other FA may also exert antilipogenic effects. In the current experiment, the effects of geometric isomers of 10,12 CLA on milk fat synthesis were examined using four Holstein-British Friesian cows in a 4 × 4 Latin Square experiment with 14-d periods. Treatments consisted of abomasal infusions of skim milk, or skim milk containing *trans*-10,*cis*-12 CLA (T1), *trans*-10,*trans*-12 CLA (T2), or a mixture of predominantly 10,12 isomers containing (g/100 g) *trans*-10,*cis*-12 (35.0), *cis*-10,*trans*-12 (23.2), *trans*-10,*trans*-12 (14.9), and *cis*-10,*cis*-12 (5.1). CLA supplements were prepared from purified ethyl linoleate and infused as nonesterified FA. Infusions were conducted over a 4-d period with a 10-d interval between treatments and targeted to deliver 4.5 g/d of 10,12 CLA isomers. Compared with the control, *trans*-10, *trans*-12 CLA had no effect ($P > 0.05$) on milk fat yield, whereas treatments T1 and T3 depressed ($P < 0.05$) milk fat content (19.8 and 22.9%, respectively) and decreased milk fat output (20.8 and 21.3%, respectively). Comparable reductions in milk fat synthesis to 4.14 and 1.80 g *trans*-10,*cis*-12/d supplied by treatments T1 and T3 indicate that other 10,12 geometric isomers of CLA have the potential to exert antilipogenic effects. The relative abundance of *cis*-10,*trans*-12 CLA in treatment T3 and the low transfer efficiency of this isomer into milk suggest that *cis*-10,*trans*-12 CLA was the active component..

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It is well established that isomers of CLA are involved in the regulation of lipid metabolism. Supplements of a mixture of CLA isomers containing *trans*-8,*cis*-10; *cis*-9,*trans*-11; *trans*-10,*cis*-12; and *cis*-11,*trans*-13 have been shown to reduce body fat in growing animals and decrease milk fat content in several species including the pig, cow, and human (1). Investigations with pure isomers in the 3T3-L1 adipocyte cell culture model (2), mice (3), and lactating dairy cows (4) have revealed that the antilipogenic effects are attributable to the *trans*-10,*cis*-12 isomer. In addition, studies in dairy cows have also shown that *cis*-9,*trans*-11 (4) or a mixture of CLA isomers containing *trans*-8,*cis*-10 or *cis*-11,*trans*-13 (5) has no effect on milk fat content. Thus far, *trans*-10,*cis*-12 is the only isomer of CLA shown unequivocally to reduce milk fat synthesis in lactating dairy cows.

Formation of *trans*-10,*cis*-12 CLA in the rumen is enhanced when high concentrate–low fiber diets are fed, with an increase in ruminal formation of this isomer being related to decreases in milk fat secretion (6,7). However, during diet-induced milk fat depression, concentrations of *trans*-10,*cis*-12 CLA in milk fat are less than half those when comparable reductions in milk fat secretion are induced with postruminal *trans*-10,*cis*-12 CLA infusions (8). Furthermore, small or negligible increases in *trans*-10,*cis*-12 CLA have been reported during milk fat depression when diets containing marine oils are fed (9,10). Overall, these findings tend to suggest that other biohydrogenation intermediates formed in the rumen also may have a role in the regulation of milk fat synthesis, but the identity of these FA remains unclear.

Park and Pariza (11) reported that a mixture of conjugated nonadecadienoic acid (CNA) isomers, containing *cis*-10,*trans*-12 CNA and *trans*-11,*cis*-13 CNA as major components, decreased fat deposition in mice to a greater extent than *trans*-10,*cis*-12 CLA. Since the CNA supplement contained comparable amounts of *cis*-10,*trans*-12 CNA and *trans*-11,*cis*-13 CNA, isomer-specific effects could not be determined. Owing to the structural analogy with *trans*-10,*cis*-12 CLA, the antilipogenic effects were attributed to the *trans*-11,*cis*-13 isomer of CNA (11). However, it is possible that the antilipogenic effects of *trans*-10,*cis*-12 CLA relate to the position of the 10,12 conjugated double bond structure relative to the carboxyl rather than the methyl group of the FA moiety, with the implication that the reduction in tissue lipid accretion of CNA isomers in mice could be a direct response to *cis*-10,*trans*-12 CNA.

To test this hypothesis, the effects of a mixture of 10,12 CLA isomers containing a structural 18-carbon analog (*cis*-10,*trans*-12 CLA) as a major component on milk fat synthesis in the lactating dairy cow were examined in the current study, relative to *trans*-10,*trans*-12 CLA and the known inhibitor *trans*-10,*cis*-12 CLA.

MATERIALS AND METHODS

Experimental animals and diet. Four multiparous lactating Holstein-British Friesian cows (679 ± 10.3 kg live weight and 303 ± 25.9 d in lactation; mean ± SE) fitted with rumen fistula (100 mm i.d.; Bar Diamond, Parma, ID) were used in the present experiment lasting 56 d. All procedures used were licensed, regulated, and inspected by the United Kingdom Home Office under the Animals (Scientific Procedures) Act of 1986.

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Abbreviations: CNA, conjugated nonadecadienoic acid; T1, treatment with *trans*-10,*cis*-12 CLA; T2, treatment with *trans*-10,*trans*-12 CLA; T3, treatment with a mixture of 10,12 geometric isomers of CLA.

The animals were housed in individual stalls within the Metabolism Unit of the Centre for Dairy Research, University of Reading, United Kingdom. Cows were offered a total mixed ration formulated (Table 1) to meet or exceed nutrient requirements according to the Agricultural and Food Research Council (12). Diets were offered *ad libitum* and fed as equal meals at 0800 and 1600. Cows had continuous access to water and trace-mineralized salt blocks (Baby Red Rockies, Winsford, Cheshire, United Kingdom) and were milked at approximately 0600 and 1630.

Experimental design and treatments. Cows were randomly assigned treatments in a 4 × 4 Latin Square design. Treatments consisted of abomasal infusions targeted to provide 0 (control) or 4.5 g/d of *trans*-10,*cis*-12 CLA (T1), *trans*-10,*trans*-12 CLA (T2), or a mixture of 10,12 geometric isomers of CLA (T3). Infusions started at 1630 and lasted for 4 d with a 10-d interval between infusion periods to minimize treatment carry-over effects. Postruminal infusion was used as a convenient means of delivering FA to the small intestine, avoiding ruminal biohydrogenation and possible effects on the microbial population in the rumen and enabling the amount of FA supplied by each treatment to be accurately determined. Infusion lines were established using Nalgene tubing that passed through the rumen fistula and reticulo-omasal orifice and a peristaltic pump (Model 202; Watson-Marlow, High Wycombe, United Kingdom) calibrated to deliver 3 kg of infusate/d. Infusions were conducted over a period of 23 h/d at a rate of 130 g/h to allow time for daily line flushing to prevent deterioration of infusion rates. Four-day infusions were used, since earlier studies showed that reductions in milk fat synthesis due to *trans*-10,*cis*-12 CLA reach a nadir after this period of infusion (4,13).

TABLE 1
Diet Formulation and Chemical Composition

Composition	
Ingredient (g/100 g dry matter)	
Grass silage	10.0
Corn silage	30.0
Cracked wheat	16.7
Ground barley	9.2
Rapeseed meal[a]	4.1
Soybean meal	6.1
Molassed sugarbeet pulp[b]	9.2
Wheat feed (middlings)	8.2
Blended cane molasses and urea[c]	4.0
High protein corn gluten meal (Prairie meal)	1.0
Minerals and vitamins[d]	1.5
Chemical composition (g/100 g dry matter)	
Dry matter[e]	50.7
Organic matter	93.0
Crude protein	17.8
Neutral detergent fiber	26.0
Starch	31.5
Water-soluble carbohydrate	2.9
Metabolizable energy (MJ/kg dry matter)	11.8

[a]Solvent-extracted rapeseed meal of low glucosinolate content.
[b]By-product of the manufacture of sugar comprised of dried shredded sugarbeet pulp, to which molasses has been added.
[c]Regumaize 44 (SvG Intermol Limited, Bootle, Merseyside, United Kingdom). Declared composition (g/100 g dry matter) crude protein (44.0), water-soluble carbohydrate (55.0), and metabolizable energy content (11.8 MJ/kg dry matter).
[d]Proprietary mineral and vitamin supplement (Dairy Direct, Bury St. Edmonds, United Kingdom) contained (g/kg) calcium (270), magnesium (60), sodium (40), phosphorus (40), zinc (5.0), manganese (4.0), copper (1.5); (mg/kg), iodine (500), cobalt (50), selenium (15); (IU/g) retinyl acetate (500), cholecalciferol (100), dl-α-tocopheryl acetate (0.5).
[e]Oven dry matter content corrected for volatile losses (16).

The CLA treatments in the form of nonesterified FA preparations were infused as an emulsion with skim milk. Three separate skim milk and CLA emulsions (13.5 L/treatment) were prepared in the morning before each infusion period by mixing the appropriate amount of CLA material and skim milk (85°C) with a high-shear mixer (Model L4RT; Silverson Machines, Chesham, United Kingdom) and passing each mixture through a single-stage pressure homogenizer (APV; Rannie, Copenhagen, Denmark). Two passes were performed at pressures of 200 and 50 bar, respectively, to ensure uniform dispersion of the CLA supplements in skim milk. Once prepared, emulsions were stored at 4°C before infusion.

Measurements and sampling. Individual cow intakes were measured daily during each infusion period. Representative samples of fresh diets and feed refusals were composited daily and stored at −20°C. Dry matter contents were determined after drying at 100°C for 24 h. Frozen feed samples were analyzed for volatile components or dried at 60°C, ground, and submitted for the determination of chemical composition using accredited and Parliament-approved procedures for feedstuff analysis (14,15) by a commercial laboratory (Natural Resources Management, Bracknell, United Kingdom). Dry matter content of silage was corrected for volatile losses according to Porter *et al.* (16).

Milk yields were recorded daily. Samples of milk for the determination of fat, protein, and lactose were collected from each cow at each milking 24 h before and during the 96-h infusion period. Milk samples were treated with potassium dichromate preservative (1 mg/mL; Lactabs; Thompson & Capper, Runcorn, United Kingdom) and stored at 4°C prior to analysis. Milk fat, crude protein, and lactose were determined using a Milko-Scan 133B analyzer (Foss Electric Ltd., York, United Kingdom). FA composition was determined in untreated samples of milk collected during the last 24 h of each infusion period and bulked according to yield. Milk samples for FA determinations were stored at −20°C until submitted for analysis.

Synthesis of CLA supplements. The CLA supplements were synthesized by Natural ASA (Hovdebygda, Norway) using a FA ethyl ester distillate prepared from safflower oil (Cognis GmbH, Düsseldorf, Germany) as the starting material. After dissolving in acetone and cooling to −60°C, precipitated oleic acid was removed by filtration, and the ethyl ester contained approximately 95% ethyl linoleate after solvent removal. The purified ethyl linoleate concentrate was heated at 120°C for 3 h with catalytic amounts of potassium ethoxide and ethanol (17) to yield a mixture of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 ethyl esters, which contained less than 3 g/100 g of other CLA isomers. Thereafter, the mixture of conjugated ethyl esters was distilled in a molecular distillation plant, and a concentrate of *trans*-10,*cis*-12 was obtained by low-temperature crystallization at −60°C.

Precipitated material was removed and dried under vacuum. Analysis by GC confirmed the composition of the precipitate as containing (g/100 g total CLA) primarily *trans*-10,*cis*-12 (93.8) and small amounts of *cis*-9,*trans*-11 (4.0).

Part of the *trans*-10,*cis*-12 ethyl ester-enriched fraction was retained, and the remainder was isomerized by heating at 85°C under a nitrogen atmosphere and strong agitation for 9 h with 2% (by weight) concentrated nitric acid (85%) to yield a mixture of 10,12 conjugated ethyl esters. After numerous washes with hot water, oil was dried under vacuum and subjected to repeated low-temperature crystallizations, yielding two fractions containing *trans*-10,*trans*-12 or a mixture of isomers enriched with *cis*-10,*trans*-12. All conjugated ethyl ester fractions synthesized were converted to nonesterified CLA by reaction with aqueous potassium hydroxide and methanol. Reaction mixtures were neutralized with citric acid (18), and the oil obtained was washed with distilled water, dried under vacuum, and stored under nitrogen until infused.

Lipid extraction and preparation of FAME. Before extraction, milk samples were thawed, heated to 40°C, cooled to 20°C, and mixed (IKA-Ultraturrax T50; Janke & Kunkel, Staufen, Germany) for few seconds. Lipid in 1 mL of milk was extracted in duplicate using diethylether/hexane (5:4, vol/vol) according to reference procedures (IDF 1C:1987; IDF 16C:1987; International Dairy Federation, Brussels, Belgium). Extracts were combined and evaporated to dryness at 60°C under nitrogen for 1 h. Samples were dissolved in hexane and methyl acetate and transesterified to FAME using freshly prepared methanolic sodium methoxide (19). The mixture was neutralized with oxalic acid (1 g oxalic acid in 30 mL diethyl ether), centrifuged, and dried using calcium chloride (20). Lipid in samples of infused emulsified CLA treatments was extracted using the same procedures used for milk. FA in CLA supplements and infusion samples were converted to FAME using 1% (vol/vol) sulfuric acid in methanol at 50°C for 30 min (21), and the amount of CLA isomers provided by each infusion treatment was determined using methyl pentacosanoate (Sigma, St. Louis, MO) as an internal standard.

GC analysis of FAME. The FAME prepared from CLA supplements, infused treatments, and milk samples were separated and quantified using a gas chromatograph (Model 6890; Hewlett-Packard, Wilmington, DE) equipped with an FID, quadrupole mass selective detector (Model 5973N; Agilent Technologies Inc.), automatic injector, split injection port, and a 100-m fused-silica capillary column (i.d., 0.25 mm) coated with a 0.2-μm film of cyanopropyl polysiloxane (CP-SIL 88; Chrompack 7489, Middelburg, The Netherlands) using hydrogen as the carrier gas operated at constant pressure (20 psi) and flow rate of 0.5 mL/min. Injector and detector temperatures were maintained at 255°C. The total FAME profile in a 2-μL sample at a split ratio of 1:50 was determined using a temperature gradient program (22). Peaks were identified using authentic FAME standards (GLC #463 and GLC #606; Nu-Chek-Prep, Elysian, MN) and comparison of EI ionization spectra of each peak with that of known standards and an online reference library (http://www.lipids.co.uk/infores/masspec.html; Christie, W.W., personal communication). Individual isomers of 18:1, 18:2, and CLA methyl esters were further resolved in a separate analysis under isothermal conditions at 170°C (22). Under these conditions, it was possible to resolve the *cis*-10,*trans*-12, *cis*-10,*cis*-12, and *trans*-10,*cis*-12 isomers, but *trans*-10,*trans*-12 CLA could not be separated from *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA, and *trans*-9,*trans*-11 CLA, which co-elute as a single GC peak (Fig. 1).

Ag^+-HPLC analysis of FAME. The distribution of isomers in CLA supplements was determined using an HPLC system (Model 1090; Hewlett-Packard), equipped with autosampler, photodiode array detector, 20-μL injection loop, heated column compartment, and four silver-impregnated silica columns (ChromSpher 5 Lipids, 250 × 4.6 mm, 5 μm particle size; Varian Ltd., Walton-on-Thames, United Kingdom) coupled in series. Methyl esters of CLA were separated under isocratic conditions at 22°C using 0.1% (by vol) of acetonitrile (Rathburn

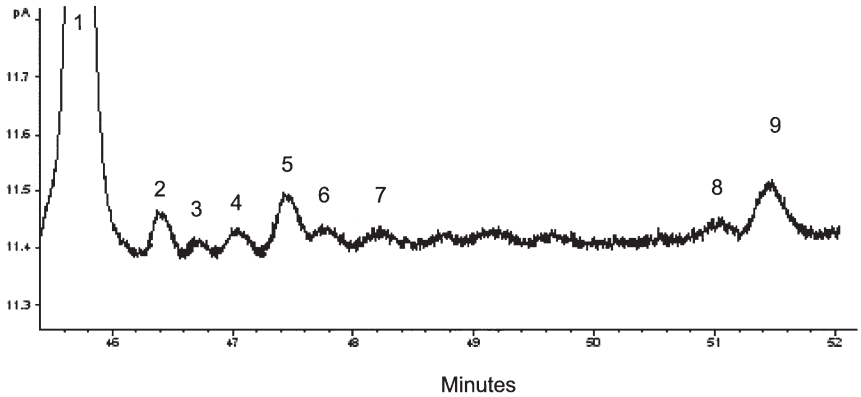

FIG.1. Partial gas chromatogram indicating typical separation of methyl esters of CLA prepared from milk from cows receiving postruminal infusions of a mixture of geometric 10,12 CLA isomers (treatment T3) using a 100-m fused-silica capillary column (CP-SIL 88) under isothermal conditions at 170°C. Peak identification: (1) unresolved *cis*-9,*trans*-11 CLA, *trans*-7,*cis*-9 CLA, and *trans*-8,*cis*-10 CLA, (2) *cis*-10,*trans*-12 CLA, (3) *trans*-9,*cis*-11 CLA, (4) 21:0, (5) *trans*-10,*cis*-12 CLA, (6) unresolved *cis*-9,*cis*-11 CLA and *trans*-11,*cis*-13 CLA, (7) *cis*-10,*cis*-12 CLA, (8) *trans*-11,*trans*-13 CLA, and (9) unresolved *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA, *trans*-9,*trans*-11 CLA, and *trans*-10,*trans*-12 CLA

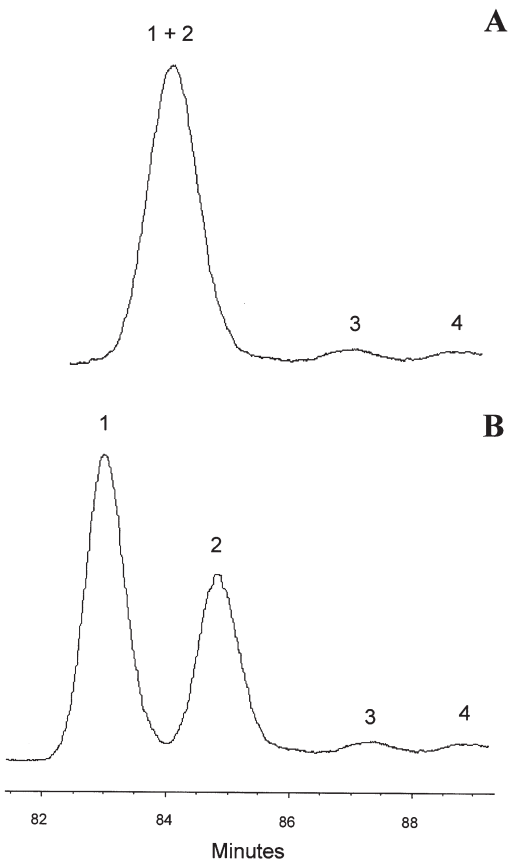

FIG.2. Partial silver ion-high performance liquid chromatograms indicating the separation of *cis-trans* and *trans-cis* 9,11 and 10,12 isomers of CLA methyl esters prepared from treatment T3 attained using four silver impregnated silica columns coupled in series and a mobile phase containing (A) 0.1% (vol/vol) acetonitrile in heptane (22), or (B) 2.0% (vol/vol) acetic acid (23) under isocratic conditions at 22°C. Peak identification: (1) *trans*-10,*cis*-12 CLA, (2) *cis*-10,*trans*-12 CLA, (3) *cis*-9,*trans*-11 CLA, and (4) *trans*-9,*cis*-11 CLA

Chemicals Limited, Walkerburn, United Kingdom) in heptane at a flow rate of 1 mL/min (total run time 100 min) and monitoring column effluent at 233 and 210 (reference wavelength) nm (22). Even though most isomers were well-resolved, *cis*-10,*trans*-12 and *trans*-10,*cis*-12 CLA co-elute under these conditions (Fig. 2). To achieve baseline separation of these isomers, Ag^+-HPLC analysis was repeated under isocratic conditions at 22°C using 2.0% (vol/vol) of acetic acid in heptane as the mobile phase (23) at a flow rate of 1 mL/min (total run time 100 min) and monitoring column effluent at 233 and 210 (reference wavelength) nm. Typical injection volumes were 10–20 μL, representing <250 μg lipid. Identification of CLA isomers was performed using commercially available CLA methyl ester standards (Matreya Incorporated, Pleasant Gap, PA; Sigma), comparison of the elution order reported in the literature (23), and cross-referencing the GC analysis of CLA supplements with reference milk samples for which the distribution of CLA isomers had previously been determined by Ag^+-HPLC (22,24). Only the isomer profile in CLA supplements was determined by Ag^+-HPLC, since most of the isomers of interest in milk samples, other than *cis*-9,*cis*-11 CLA and *trans*-10,*trans*-12 CLA, could be determined by GC analysis.

Calculations and statistical analysis. Milk FA composition was expressed as a weight percentage of total FA using response factors determined by analysis of butter oil of known FA composition (CRM 164 milk fat reference standard, Community Bureau of Reference, Brussels, Belgium). Yields of individual FA were determined using tritridecanoin (Nu-Chek-Prep) as an internal standard. Concentrations of specific conjugated isomers in CLA supplements were calculated based on proportionate peak area responses determined by both Ag^+-HPLC methods and total CLA weight percentage determined by GC. Apparent transfer efficiencies of infused 10,12 CLA isomers from the abomasum into milk were calculated as: [(amount of isomer secreted in milk during CLA infusion − amount of isomer secreted during the control infusion)/amount of isomer infused]. The apparent transfer of *cis*-9,*trans*-11 CLA and total CLA were not determined, since postruminal infusions of *trans*-10,*cis*-12 CLA inhibit the conversion of *trans*-11 18:1 and reduce endogenous synthesis of *cis*-9,*trans*-11 (4,13).

Nutrient intake, milk production, and milk FA composition data measured on the last day of infusion were evaluated by ANOVA for a 4×4 Latin Square design using the mixed procedure of SAS (25). The statistical model included the random effects of cow and the fixed effects of period and experimental treatment. Treatment effects were considered significant at $P < 0.05$.

RESULTS

Supplements of nonesterified FA preparations used to supply *trans*-10,*cis*-12 CLA or *trans*-10,*trans*-12 CLA were relatively pure, whereas the mixture of geometric 10,12 isomers also contained several other positional isomers of CLA (Table 2). Preparation of CLA supplements as emulsions in skim milk supplied the targeted amounts of total CLA for treatment T2, but the amounts of *trans*-10,*cis*-12 CLA and total 10,12 CLA isomers infused for treatments T1 and T3, respectively, were marginally lower than originally intended (Table 3).

Relative to the control treatment, infusions of *trans*-10,*trans*-12 CLA had no effect on dry matter intake or milk production, whereas treatments T1 and T3 reduced ($P < 0.05$) milk fat content and output (Table 4). Infusion of treatments T1 and T3 resulted in a progressive reduction in milk fat content and secretion (Fig. 3). With the exception of reduced concentrations of lactose ($P < 0.05$) for T3 compared with T2, treatments had no effect.

Treatments caused significant increases in the concentration of specific 10,12 isomers corresponding to those contained in the infused CLA supplement (Table 5). In addition, CLA supplements also resulted in significant ($P < 0.05$) reductions in the *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-9 18:1/18:0, and *cis*-9,*trans*-11 CLA/*trans*-11 18:1 desaturase ratios, with relatively minor, but often significant, effects on the concentration of other FA in milk (Table 5). Compared with the control, treatment T2 had no effect on milk FA yield other than increasing ($P < 0.05$) the output of *trans*-10,*trans*-12 CLA in milk, but treatments T1 and T3 decreased the secretion of FA in milk

TABLE 2
FA Composition of CLA Supplements

FA[a] (g/100 g)	Treatment T1	T2	T3
cis-9 18:1	0.89	0.00	2.76
trans-10 18:1	0.17	0.00	0.00
trans-11 18:1	0.19	0.00	0.00
cis-9,*cis*-11 CLA	0.05	0.02	3.08
cis-9,*trans*-11 CLA	5.89	0.29	2.80
trans-9,*cis*-11 CLA	0.00	0.06	2.12
trans-9,*trans*-11 CLA	1.08	1.30	9.40
cis-10,*cis*-12 CLA	0.09	0.31	5.13
cis-10,*trans*-12 CLA	0.00	0.00	23.18
trans-10, *cis*-12 CLA	90.22	2.09	35.00
trans-10,*trans*-12 CLA	1.14	95.67	14.93
Other CLA	0.00	0.25	1.23
Total CLA	98.46	100.00	96.87

[a]FA composition determined in duplicate based on a combination of GC and Ag^+-HPLC analysis of methyl esters. Separation of CLA methyl esters by Ag^+-HPLC analysis was conducted under isocratic conditions at 22°C using both 0.1% (vol/vol) acetonitrile in heptane (22) and 2.0% acetic acid (vol/vol) in heptane (23) as the mobile phase.

(Table 6). Reductions in the secretion of $C_{4–14}$, C_{16}, and $C_{18–24}$ FA accounted for proportionately 0.33, 0.42, and 0.17, and 0.34, 0.40, and 0.22 of the total decrease in milk FA output during abomasal infusions of *trans*-10,*cis*-12 CLA and the mixture of 10,12 geometric CLA isomers, respectively.

Apparent transfer of infused *trans*-10,*cis*-12 and *cis*-10,*trans*-12 at the abomasum was similar, but *trans*-10,*trans*-12 and *cis*-10,*cis*-12 were transferred at a higher efficiency (Table 7).

DISCUSSION

Although *trans*-10,*cis*-12 is the only CLA isomer shown unequivocally to reduce milk fat synthesis in the dairy cow, there is indirect evidence that other FA produced during ruminal biohydrogenation of dietary PUFA also may elicit antilipogenic effects that are similar to or even more potent than those of *trans*-10,*cis*-12 CLA. This comes in part from examination of milk FA composition, which indicates little or no change in *trans*-10,*cis*-12 CLA during diet- induced milk fat depression

TABLE 3
Mean Amounts of CLA Isomers Delivered to the Abomasum During 4-d Infusion Periods

Amount infused[a] (g/d)	Treatment T1	T2	T3
cis-9,*cis*-11 CLA	0.002 ± <0.001	0.001 ± <0.001	0.159 ± 0.006
cis-9,*trans*-11 CLA	0.270 ± 0.037	0.014 ± 0.001	0.144 ± 0.005
trans-9,*cis*-11 CLA	ND	0.003 ± <0.001	0.109 ± 0.004
trans-9,*trans*-11 CLA	0.049 ± 0.007	0.063 ± 0.003	0.484 ± 0.018
cis-10,*cis*-12 CLA	0.004 ± 0.0003	0.015 ± 0.001	0.264 ± 0.010
cis-10,*trans*-12 CLA	ND	ND	1.194 ± 0.044
trans-10,*cis*-12 CLA	4.137 ± 0.564	0.101 ± 0.005	1.802 ± 0.067
trans-10,*trans*-12 CLA	0.052 ± 0.007	4.615 ± 0.216	0.769 ± 0.029
ΣCLA	4.52 ± 0.62	4.82 ± 0.23	4.99 ± 0.19

[a]Values represent the amount (mean ± SD) of CLA isomers supplied during 4-d infusions to four cows for each treatment. ND, isomers not detected.

TABLE 4
Intake and Milk Production of Dairy Cows During Abomasal Infusions of CLA Supplements

Variable[c]	Treatment[a] Control	T1	T2	T3	SEM[b]
Intake (kg dry matter/d)	19.7	20.4	19.7	20.4	0.38
Yield					
Milk (kg/d)	19.7	21.0	19.9	20.2	1.92
Fat (g/d)	677^{x}	536^{y}	694^{x}	533^{y}	41.5
Protein (g/d)	734	754	712	753	52.5
Lactose (g/d)	866	931	895	870	81.1
Concentration					
Fat (g/kg)	36.3^{x}	29.1^{y}	37.2^{x}	28.0^{y}	1.66
Protein (g/kg)	37.9	36.8	36.6	37.9	1.34
Lactose (g/kg)	$43.1^{x,y}$	$43.6^{x,y}$	44.6^{x}	42.1^{y}	0.59

[a]Cows received 4-d abomasal infusions (3 kg/d) of skim milk (control), or skim milk containing emulsified supplements of *trans*-10,*cis*-12 CLA (T1), *trans*-10,*trans*-12 CLA (T2), or a mixture of geometric isomers of 10,12 CLA (T3). See Table 2 for the FA composition of CLA supplements.
[b]Error DF, 6.
[c]Values represent the mean for day 4 of infusion for four cows. Means within row not sharing common roman superscripts differ significantly ($P < 0.05$).

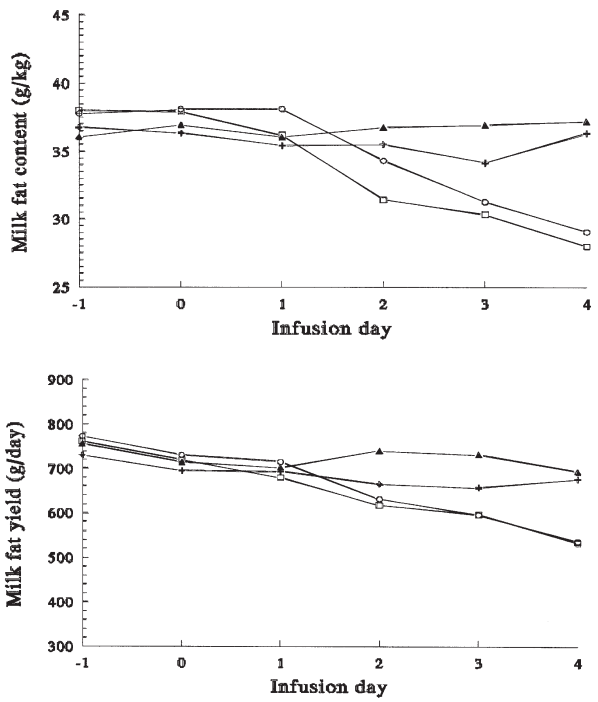

FIG.3. Temporal changes in milk fat content and milk fat yield during abomasal infusion of mixtures of CLA supplements. Infusions were continuously infused during a 4-d period. Values represent means from four animals. SEM for milk fat content and milk fat yield was 1.66 g/kg and 53.8 g/d, respectively. The mean amounts of CLA isomers infused for each treatment are listed in Table 3. (+) Control, (○) T1, (▲) T2, (□) T3. For treatment descriptions see Figure 1.

when marine oils are fed and from the observation that supplements of rumen-protected CLA isomers cause larger decreases in milk fat secretion than would be expected based on milk fat *trans*-10,*cis*-12 CLA content (8,26). Reductions in milk fat yield have been reported in response to postruminal infusions of a mixture of CLA isomers devoid of *trans*-10,*cis*-12 CLA (27), and more recent studies have established that *cis*-9,*trans*-11 (4), *trans*-8,*cis*-10, and *cis*-11,*trans*-13 CLA (5) are not involved in the regulation of milk fat synthesis in the dairy cow.

The unique antilipogenic effect of *trans*-10,*cis*-12 CLA also has been demonstrated in growing mice (3) and cultured 3T3-L1 adipocytes (2), indicating a consistency in the response to *trans*-10,*cis*-12 CLA between these experimental models and the inhibitory effects on milk fat synthesis in the lactating bovine (4,13). The lactating dairy cow represents a convenient and robust means for examining potential antilipogenic effects, since changes in milk fat output can be readily quantified over a short period of time (4,13,27), occur in a predictable manner, and are sufficiently sensitive to respond to small amounts of infused FA with antilipogenic activity (28,29). Furthermore, administering CLA treatments by postruminal infusions enables the amount of FA supplied by each treatment to be accurately determined, while avoiding possible effects on the microbial population in the rumen.

It was not possible to produce *cis*-10,*trans*-12 CLA in sufficient quantities for the present experiment, and therefore a mixture of CLA isomers had to be used. The *cis*-10,*trans*-12-enriched CLA supplement was composed of three main positional 10,12 isomers that accounted for proportionately 0.755 of total CLA content, but it also contained positional 9,11 isomers. The occurrence of 9,11 isomers can be attributed to the small amounts of *cis*-9,*trans*-11 ethyl esters in the *trans*-10,*cis*-12 fraction used to synthesize other positional 10,12 CLA isomers, which during isomerization were converted to ethyl esters of *trans*-9,*trans*-11 CLA, *trans*-9,*cis*-11 CLA, and *cis*-9,*cis*-11 CLA.

To account for the contribution of the major 10,12 isomers other than *cis*-10,*trans*-12 to the overall response to the mixture of CLA isomers in treatment T3, the effect of relatively pure preparations of *trans*-10,*cis*-12 CLA and *trans*-10,*trans*-12 CLA on milk fat synthesis also were evaluated. Treatments T1, T2, and T3 supplied 4.19, 4.73, and 4.03 g of geometric 10,12 CLA isomers/d, respectively. Postruminal infusions of 4.62 g *trans*-10,*trans*-12 CLA/d (treatment T2) had no effect on milk fat synthesis but decreased the ratios of *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-9 18:1/18:0, and *cis*-9,*trans*-11 CLA/*trans*-11 18:1, which serve as a proxy for Δ-9 stearoyl CoA desaturase in the mammary gland (1). Reductions in desaturase ratios in the absence of changes in milk fat yield are consistent with earlier observations (30) and underline the lack of a direct involvement of Δ-9 stearoyl CoA desaturase in the inhibition of mammary lipid metabolism.

Since postruminal *trans*-10,*trans*-12 CLA infusion had no effect on milk fat yield, the contribution of this isomer to the overall reduction in milk fat yield to treatment T3 can be discounted. Treatments T1 and T3 supplied 4.14 and 3.00 g/d of the other major 10,12 isomers, *trans*-10,*cis*-12 CLA and *cis*-10,*trans*-12 CLA, but resulted in similar decreases in milk fat secretion compared with the control (20.8 and 21.3%, respectively). The reduction in milk fat yield in response to postruminal infusions of *trans*-10,*cis*-12 CLA from treatment T1 is in line with a predicted decrease of 24.8% based on the relationship developed using data from several experiments (28). However, comparable decreases in milk fat output in response to infusions of 4.14 and 1.80 g *trans*-10,*cis*-12 CLA/d supplied by treatments T1 and T3 indicate that other conjugated isomers in the T3 supplement also inhibit milk fat synthesis.

Treatment T3 supplied small amounts of *cis*-10,*cis*-12 CLA (0.26 g/d). The possible involvement of this isomer in the decrease in milk fat synthesis can be examined by comparing the apparent transfer efficiencies of the major CLA isomers from the abomasum into milk fat. Apparent transfer of the known inhibitor *trans*-10,cis-12 CLA is typically lower than that of other CLA isomers that are not involved in the regulation of milk fat synthesis (5,27). These observations point toward a relatively low apparent transfer efficiency being a characteristic of conjugated FA that exert antilipogenic effects. It is interesting to note that the transfer of *trans*-10,*cis*-12 CLA and *cis*-10,*trans*-12 CLA from treatment T3 into milk were virtually identical, whereas the transfer efficiency for *cis*-10,*cis*-12 CLA was substantially higher, leading to the overall conclusion that *cis*-10,*cis*-12 CLA is unlikely to have been a causal factor underlying the reduction in milk fat yield to treatment T3 in the

TABLE 5
Milk FA Composition During Abomasal Infusions of CLA Supplements (g/100 g FA)

	Treatment[a]				
FA[c]	Control	T1	T2	T3	SEM[b]
4:0	2.49^{x}	3.17^{y}	3.00^{y,z}	2.73^{x,z}	0.116
6:0	1.97^{x}	1.85^{x}	2.31^{y}	1.63^{z}	0.066
8:0	1.35^{x}	1.12^{y}	1.59^{z}	1.07^{y}	0.049
10:0	3.47^{x}	2.71^{y}	4.02^{z}	2.88^{y}	0.134
12:0	4.55^{x}	3.54^{y}	5.04^{z}	3.95^{w}	0.110
14:0	12.03^{x}	12.88^{y}	13.05^{y}	13.01^{y}	0.218
cis-9 14:1	1.60^{x}	1.48^{x,y}	1.47^{x,y}	1.33^{y}	0.052
15:0	1.93^{x}	1.24^{y}	1.53^{z}	1.52^{z}	0.049
16:0	33.42^{x}	32.21^{y}	33.73^{x}	32.07^{y}	0.363
cis-9 16:1	2.68^{x}	2.24^{y}	2.34^{x,y}	2.24^{y}	0.111
17:0	0.94^{x}	0.73^{y}	0.82^{z}	0.83^{z}	0.009
18:0	6.30^{x}	9.73^{y}	6.92^{x}	9.45^{y}	0.364
18:1					
cis-9	14.67^{x,y}	15.60^{x}	13.14^{y}	14.53^{x,y}	0.470
cis-11	0.69^{x}	0.69^{x}	0.51^{y}	0.71^{x}	0.029
cis-12	0.21^{x}	0.20^{x}	0.19^{x}	0.24^{y}	0.006
cis-13	0.072	0.067	0.061	0.073	0.004
cis-15	0.11^{x}	0.12^{x,y}	0.11^{x}	0.14^{y}	0.005
cis-16	0.053	0.055	0.058	0.061	0.0032
trans-4	0.012^{x}	0.014^{y}	0.010^{z}	0.014^{y}	0.0004
trans-5	0.013	0.015	0.013	0.014	0.0014
trans-6+7+8	0.23^{x}	0.25^{x}	0.19^{y}	0.27^{x}	0.010
trans-9	0.19^{x}	0.17^{x,y}	0.15^{y}	0.19^{x}	0.007
trans-10	0.47^{x}	0.42^{x,z}	0.32^{y}	0.40^{z}	0.018
trans-11	0.95^{x,y}	0.87^{x,y}	0.70^{x}	1.09^{y}	0.078
trans-12	0.29^{x,y}	0.26^{x}	0.24^{x}	0.32^{y}	0.014
trans-13 + 14	0.36^{x}	0.43^{y}	0.30^{z}	0.52^{w}	0.015
trans-15	0.18	0.21	0.16	0.23	0.020
trans-16 + *cis*-14	0.24^{x}	0.25^{x,y}	0.21^{x}	0.29^{y}	0.013
Σ*cis*	15.80^{x}	16.73^{x}	14.07^{y}	15.75^{x,y}	0.499
Σ*trans*	2.94^{x}	2.89^{x}	2.31^{y}	3.34^{x}	0.139
Σ18:1	18.74^{x}	19.61^{x}	16.37^{y}	19.08^{x}	0.628
cis-9,*cis*-12 18:2	2.20^{x}	1.89^{y}	1.90^{y}	2.29^{x}	0.080
Σ18:2[d]	2.81^{x}	2.39^{y}	2.46^{y}	2.90^{x}	0.091
cis-10,*cis*-12 CLA	0.000^{x}	0.000^{x}	0.000^{x}	0.023^{y}	0.0008
cis-9,*trans*-11 CLA[e]	0.57^{x}	0.40^{x,y}	0.39^{y}	0.44^{x,y}	0.050
cis-10,*trans*-12 CLA	0.00^{x}	0.00^{x}	0.00^{x}	0.05^{y}	0.003
trans-11,*cis*-13 + *cis*-9,*cis*-11 CLA	0.013	0.011	0.010	0.013	0.0010
trans-9,*cis*-11 CLA	0.015^{x}	0.009^{y}	0.009^{y}	0.011^{y}	0.0009
trans-10,*cis*-12 CLA	0.012^{x}	0.181^{y}	0.007^{x}	0.074^{z}	0.0040
trans-11,*trans*-13	0.037	0.038	0.023	0.037	0.0045
trans,trans CLA[f]	0.023^{x}	0.036^{y}	0.200^{z}	0.093^{w}	0.0024
ΣCLA	0.67	0.68	0.64	0.74	0.043
18:3n-3	0.35^{x}	0.33^{x,y}	0.30^{y}	0.37^{x}	0.014
20:0	0.12^{x}	0.14^{y}	0.11^{x}	0.15^{y}	0.005
21:0	0.023	0.019	0.024	0.021	0.0033
Ratio					
cis-9 14:1/14:0	0.132^{x}	0.114^{y}	0.114^{y}	0.101^{y}	0.0049
cis-9 16:1/16:0	0.080	0.070	0.070	0.070	0.0033
cis-9 18:1/18:0	2.36^{x}	1.61^{y}	1.88^{z}	1.64^{y}	0.066
cis-9, *trans*-11 CLA/*trans*-11 18:1	0.627^{x}	0.456^{y}	0.532^{z}	0.449^{y}	0.0134

[a]Cows received 4-d abomasal infusions (3 kg/d) of skim milk (control), or skim milk containing emulsified supplements of *trans*-10,*cis*-12 CLA (T1), *trans*-10,*trans*-12 CLA (T2), or a mixture of geometric isomers of 10,12 CLA (T3). See Table 2 for the FA composition of CLA supplements.
[b]Error DF, 6. Means within row not sharing common roman superscripts differ significantly ($P < 0.05$).
[c]Values represent the mean for day 4 of infusion for four cows. FA composition was determined by GC using both a temperature gradient and isothermal conditions for the separation of 18:1, 18:2, and CLA isomers.
[d]Sum of 18:2 excluding isomers of CLA.
[e]Refers to unresolved *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA.
[f]Refers to unresolved *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA, *trans*-9,*trans*-11 CLA, and *trans*-10,*trans*-12 CLA.

current experiment. However, further studies would be required to confirm the assertion that *cis*-10,*cis*-12 CLA is not a potent inhibitor of milk fat synthesis in the dairy cow.

In addition to the 10,12 CLA isomers, the T3 supplement also contained four positional 9,11 isomers that accounted for proportionately 0.180 of total CLA content, and as such treatment T3 supplied 0.48 g *trans*-9,*trans*-11 CLA, 0.16 g *cis*-9,*cis*-11 CLA, 0.14 g *cis*-9,*trans*-11 CLA, and 0.11 g *trans*-

TABLE 6
Output of FA in Milk During Abomasal Infusions of CLA Supplements (g/d)

FA[c]	Treatment[a]				SEM[b]
	Control	T1	T2	T3	
4:0	12.2^{x}	13.1^{x}	16.4^{y}	11.0^{x}	0.93
6:0	10.4^{x}	8.0^{x,z}	13.1^{y}	6.8^{z}	0.92
8:0	7.4^{x}	5.0^{x,y}	9.3^{x}	4.6^{y}	0.73
10:0	20.0^{x}	12.4^{y}	24.0^{x}	12.9^{y}	2.02
12:0	27.2^{x}	16.5^{y}	30.3^{x}	18.3^{y}	2.31
14:0	73.4^{x,y}	61.2^{x}	80.6^{y}	62.3^{x}	5.11
cis-9 14:1	9.8^{x}	7.1^{y}	8.8^{x,y}	6.7^{y}	0.77
15:0	11.7^{x}	5.7^{y}	9.0^{x,y}	7.5^{y}	1.08
16:0	200^{x}	153^{y}	208^{x}	155^{y}	13.0
cis-9 16:1	15.6^{x}	10.6^{y}	13.8^{x}	10.7^{y}	0.83
17:0	5.8^{x}	3.5^{y}	5.0^{x,y}	4.0^{y}	0.48
18:0	37.0^{x}	45.4^{y}	42.1^{x,y}	42.1^{x,y}	2.35
Σ*cis* 18:1	96.7	80.4	85.5	74.1	6.73
Σ*trans* 18:1	17.0^{x}	14.1^{y}	14.1^{y}	15.1^{x,y}	0.70
Σ18:1	110.0	92.5	106.1	82.5	10.62
Σ18:2 [d]	16.8^{x}	11.7 ^{y}	15.0^{x,z}	13.5^{y,z}	0.65
cis-10,*cis*-12 CLA	0.00^{x}	0.00^{x}	0.00^{x}	0.11^{y}	0.009
cis-9,*trans*-11 CLA[e]	3.27^{x}	2.02^{y}	2.36^{y}	2.08^{y}	0.149
cis-10,*trans*-12 CLA	0.00^{x}	0.00^{x}	0.00^{x}	0.20^{y}	0.007
trans-9,*cis*-11 CLA	0.09^{x}	0.04^{y}	0.06^{z}	0.05^{y,z}	0.003
trans-10,*cis*-12 CLA	0.06^{x}	0.86^{y}	0.04^{x}	0.33^{z}	0.055
trans,trans CLA[f]	0.13^{x}	0.16^{x}	1.20^{y}	0.42^{z}	0.068
ΣCLA	3.90^{x}	3.31^{y}	3.85^{x}	3.44^{x,y}	0.135
18:3n-3	2.07^{x}	1.58^{y}	1.86^{x,z}	1.66^{y,z}	0.068
20:0	0.68	0.66	0.68	0.65	0.040
ΣFA	593^{x}	470^{y}	606^{x}	468^{y}	36.4
Summary					
Σ ≤C14	168^{x}	127^{y}	189^{x}	126^{y}	12.4
Σ C16	216^{x}	164^{y}	224^{x}	166^{y}	13.6
Σ ≥C18	188	167	177	161	10.4

[a]Cows received 4-d abomasal infusions (3 kg/d) of skim milk (control), or skim milk containing emulsified supplements of *trans*-10,*cis*-12 CLA (T1), *trans*-10,*trans*-12 CLA (T2), or a mixture geometric isomers of 10,12 CLA (T3). See Table 2 for the FA composition of CLA supplements.
[b]Error DF, 6. Means within a row not sharing common roman superscripts differ significantly ($P < 0.05$).
[c]Values represent the mean for day 4 of infusion for four cows. FA composition was determined by GC using both a temperature gradient and isothermal conditions for the separation of 18:1 and 18:2 isomers.
[d]Sum of 18:2 excluding isomers of CLA.
[e]Refers to unresolved *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA.
[f]Refers to unresolved *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA, *trans*-9,*trans*-11 CLA, and *trans*-10,*trans*-12 CLA.

9,*cis*-11 CLA/d. Postruminal infusions of *cis*-9,*trans*-11 CLA (4) or *trans*-9,*trans*-11 CLA (31), the predominant 9,11 isomer in treatment T3, are known to have no effects on milk fat synthesis in the dairy cow. Although there is evidence in the literature that *trans*-9,*cis*-11 CLA concentrations are increased in milk from cows fed diets causing milk fat depression (10,24) and may contribute to the reduction in milk fat synthesis when infused postruminally (31), the amount of this isomer supplied by the T3 infusion is very small. Furthermore, a lack of increase in the concentration of *trans*-9,*cis*-11 CLA in milk, which is known to be efficiently transferred in response to postruminal infusions (31), suggests that the amounts of *trans*-9,*cis*-11 CLA available to the mammary gland were extremely small and therefore unlikely to have had a significant role in the regulation of milk fat synthesis in the current experiment. Under the GC conditions used in this study, *cis*-9,*cis*-11 CLA and *trans*-11,*cis*-13 CLA are not resolved and elute as a single peak (22). However, there was no change in the concentration of these co-eluting isomers in milk fat from cows infused with treatment T3, suggesting that the concentration of *cis*-9,*cis*-11 CLA was not substantially increased. In light of the nonsignificant changes in milk fat *cis*-9,*cis*-11 and *trans*-11,*cis*-13 concentrations, it is probable that the amounts of *cis*-9,*cis*-11 CLA available to the mammary gland were not sufficiently large to contribute significantly to the decrease in milk fat yield in response to treatment T3.

It is evident that the decreases in milk fat synthesis in response to treatment T3 are not explained by the amounts of *trans*-10,*cis*-12 CLA supplied by this infusion. *Cis*-10,*trans*-12 was the second-most abundant CLA isomer in treatment T3, and was transferred from the abomasum into milk at a similar efficiency as the known inhibitor *trans*-10,*cis*-12 CLA. In view of the amount of *cis*-10,*trans*-12 CLA supplied by treatment T3 (1.19 g/d), comparable transfer characteristics to the known inhibitor *trans*-10,*cis*-12 CLA, and the relative amounts of other CLA isomers, effects of which can largely be accounted for, leads to the overall conclusion that *cis*-10,*trans*-12 CLA contributed to the reduction in milk fat synthesis in response to treatment T3.

TABLE 7
Apparent Transfer of CLA Isomers from the Abomasum into Milk Fat for Different CLA Supplements

Transfer efficiency[b] (%)	Treatment[a]		
	T1	T2	T3
cis-10,*cis*-12 CLA	—		39.6 ± 11.52
cis-10,*trans*-12 CLA	—		16.7 ± 1.69
trans-10,*cis*-12 CLA	19.2 ± 4.88		14.9 ± 2.03
trans,trans CLA[c]	—	23.0 ± 5.19[d]	

[a]Cows received 4-d abomasal infusions (3 kg/d) of skim milk (control) or skim milk containing emulsified supplements of *trans*-10,*cis*-12 CLA (T1), *trans*-10,*trans*-12 CLA (T2), or a mixture of 10,12 geometric isomers of CLA (T3). See Table 2 for the FA composition of CLA supplements.
[b]Values represent the apparent transfer efficiency (mean ± SD) from the abomasum into milk for four cows on day 4 of infusion.
[c]Transfer of *trans*-10,*trans*-12 CLA was unable to be calculated for treatment T3 owing to the contribution of *trans*-9,*trans*-11 in the infused CLA supplement and co-elution of *trans*-9,*trans*-11 with *trans*-10,*trans*-12 CLA under the GC conditions used to determine milk FA composition.
[d]Infused CLA supplement delivered only small amounts of *trans*-9,*trans*-11 CLA (Table 3), and therefore the increase in *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA, *trans*-9,*trans*-11 CLA, and *trans*-10,*trans*-12 CLA content in milk determined by GC was assumed to be *trans*-10,*trans*-12 CLA.

Park and Pariza (11) reported a dramatic reduction in the fat content of growing mice fed supplements containing a mixture of CNA isomers enriched with *cis*-10,*trans*-12 19:2 and *trans*-11,*cis*-13 19:2. Data from the present experiment suggest that, by structural analogy with *cis*-10,*trans*-12 CLA, the *cis*-10,*trans*-12 19:2 rather than *trans*-11,*cis*-13 19:2 was the isomer of CNA with antilipogenic properties. Furthermore, the effect of CNA on body fat in growing mice was significantly greater than a mixture of CLA isomers containing *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA. Milk fat responses observed in the current study tend to support an increased potency of 10,12 conjugated FA when the *trans*-10,*cis*-12 arrangement of double bonds is substituted for the *cis*-10,*trans*-12 structure. However, it is important to recognize that the difference in potency between the two 10,12 CLA isomers infused in this experiment was less dramatic in terms of milk fat synthesis compared with the differences in lipid accretion of growing mice fed a mixture of *cis*-10,*trans*-12 CNA and *trans*-11,*cis*-13 CNA relative to *trans*-10,*cis*-12 CLA (11).

Evaluation of several CLA cognates on glycerol release and TAG accumulation in cultured 3T3-L1 adipocytes has pointed toward a *trans*-10,*cis*-12 double bond arrangement in conjunction with a free carboxyl group on the first carbon atom as being an integral structural component for antilipogenic effects, with the implication that either a *trans*-10 or *cis*-12 double bond is an essential feature (2). Data from the present experiment tend to suggest that the antilipogenic effects of 10,12 geometric isomers of CLA containing both a *cis* and *trans* double bond are more pronounced when the double bond closest to the carboxyl group. In support of this, TAG accumulation in fully differentiated 3T3-L1 adipocytes has recently been shown to be reduced during incubation with *cis*-10 17:1 or *cis*-10 19:1, albeit to a lesser extent than *trans*-10,*cis*-12 CLA, whereas *trans*-10 17:1 and *trans*-10 19:1 have no effect (2). Overall these findings are consistent with the position and orientation of the double bond relative to the carboxyl group of the FA moiety being fundamental to the inhibitory effects on lipid accretion in cultured adipocytes or milk fat synthesis in lactating dairy cows.

Further support for the importance of an uninterrupted carbon chain between the *trans*-10,*cis*-12 double bond structure and the carboxyl group is provided by studies that have examined the effects of calendic acid (*trans*-8,*trans*-10,*cis*-12 18:3) on body composition of growing mice (32), conjugated 18:3 (*cis*-6,*trans*-10,*cis*-12 18:3) on milk fat synthesis in dairy cows (33), and methyl-branched CLA (α-methyl *trans*-10,*cis*-12 18:2) on fat accumulation in adipocytes and hepatocytes (34). In all experimental models the addition of the double bond or a methyl group between the terminal carboxyl group and the *trans*-10,*cis*-12 conjugated bond system abolished or markedly reduced the inhibitory effects on lipid synthesis associated with the *trans*-10,*cis*-12 carbon–carbon double bond structure.

A complex mixture of CLA isomers was used to examine the role of *cis*-10,*trans*-12 CLA in the regulation of milk fat synthesis in the current study. Even though the data suggest that *cis*-10,*trans*-12 CLA is a potent inhibitor of milk fat synthesis, these findings need to be confirmed in studies using higher-purity *cis*-10,*trans*-12 CLA preparations. Since the unique inhibitory effects of *trans*-10,*cis*-12 CLA on lipid synthesis have been demonstrated both in the lactating dairy cow (4,13) and the 3T3-L1 adipocyte cell culture model (2,34), a small amount of *cis*-10,*trans*-12 CLA was purified from the isomer mixture infused in this study (treatment T3) and used to examine the effects of this isomer on lipid accumulation of murine 3T3-L1 adipocytes. Adipocyte cells were cultured with *cis*-10,*trans*-12 CLA, *trans*-10,*cis*-12 CLA, *cis*-9,*trans*-11 CLA, or a control medium. Both *cis*-10,*trans*-12 CLA and *trans*-10,*cis*-12 CLA markedly reduced lipid accumulation (0.27 and 0.24 mg TAG/mg protein, respectively) compared with the control or *cis*-9,*trans*-11 CLA treatments (corresponding values 1.00 and 1.20, respectively) (35).

In conclusion, postruminal infusions of a mixture of 10,12 CLA isomers were found to result in similar decreases in milk fat output as relatively pure preparations of *trans*-10,*cis*-12 CLA. Comparable reductions in milk fat synthesis in response to 1.80 and 4.14 g *trans*-10,*cis*-12/d, supplied by treatments T3 and T1, indicate that other isomers of CLA have the potential to exert antilipogenic effects. *Trans*-10,*trans*-12 CLA had no effect on mammary lipid metabolism, and the evidence from this experiment points toward *cis*-10,*trans*-12 exerting at least as potent antilipogenic effects as the known inhibitor *trans*-10,*cis*-12 CLA.

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