***trans*-10, *cis*-12 Conjugated Linoleic Acid Decreases Lipogenic Rates and Expression of Genes Involved in Milk Lipid Synthesis in Dairy Cows¹**

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ABSTRACT

Feeding conjugated linoleic acid (CLA) reduces milk fat synthesis in lactating dairy cows, and the effect has been shown to be specific for the *trans*-10, *cis*-12 CLA isomer. Our objectives were to examine potential mechanisms by which *trans*-10, *cis*-12 CLA inhibits milk fat synthesis. Multiparous Holstein cows (n = 4) in late lactation were used in a balanced 2 × 2 crossover design. Treatments consisted of a 5 d abomasal infusion of either skim milk (control) or purified *trans*-10, *cis*-12 CLA (13.6 g/d) emulsified in skim milk. On d 5 of infusion, mammary gland biopsies were performed and a portion of the tissue analyzed for mRNA expression of acetyl CoA carboxylase, fatty acid synthetase, Δ^9 -desaturase, lipoprotein lipase, fatty acid binding protein, glycerol phosphate acyltransferase and acylglycerol phosphate acyltransferase. Lipogenic capacity was evaluated with another portion of the tissue. Infusion of *trans*-10, *cis*-12 CLA decreased milk fat content and yield 42 and 48%, respectively and increased the *trans*-10, *cis*-12 CLA content in milk fat from <0.1 to 4.9 mg/g. Reductions in milk fat content of C₄ to C₁₆ fatty acids contributed 63% to the total decrease in milk fat yield (molar basis). Analysis of the ratios of specific fatty acid pairs indicated *trans*-10, *cis*-12 CLA also shifted fatty acid composition in a manner consistent with a reduction in Δ^9 -desaturase. Mammary explant incubations with radiolabeled acetate established that lipogenic capacity was decreased 82% and acetate oxidation to CO₂ was reduced 61% when cows received *trans*-10, *cis*-12 CLA. Infusing *trans*-10, *cis*-12 CLA also decreased the mRNA expression of all measured enzymes by 39 to 54%. Over-

all, data demonstrated the mechanism by which *trans*-10, *cis*-12 CLA inhibits milk fat synthesis includes decreasing expression of genes that encode for enzyme involved in circulating fatty acid uptake and transport, de novo fatty acid synthesis, desaturation of fatty acids and triglyceride synthesis.

(Key words: conjugated linoleic acid, lactation, milk fat, milk fat depression)

Abbreviation key: ACC = acetyl CoA carboxylase, AGPAT = acylglycerol phosphate acyltransferase, CLA = conjugated linoleic acid, FAS = fatty acid synthetase, FABP = fatty acid binding protein, GPAT = glycerol phosphate acyltransferase, LPL = lipoprotein lipase, PPAR = peroxisome proliferator-activated receptor, SCD = stearoyl CoA desaturase, SREBP = sterol regulatory element-binding proteins.

INTRODUCTION

Dietary supplements of conjugated linoleic acid (CLA) reduce milk fat synthesis in lactating cows (Lor and Herbein, 1998; Chouinard et al., 1999a, 1999b), pigs (Harrell et al., 2000; Poulos et al., 2000), and humans (Masters et al., 2002) and decrease body fat content in several species of growing animals (see summary by Baumgard et al., 2000a). Most investigations have used commercial supplements containing a mixture of CLA isomers, and we recently demonstrated that the *trans*-10, *cis*-12 CLA isomer inhibited milk fat synthesis in dairy cows, whereas the *cis*-9, *trans*-11 CLA had no effect (Baumgard et al., 2000b). The *trans*-10, *cis*-12 CLA isomer has also been shown to be responsible for the reduction in body fat content of growing mice (Park et al., 1999).

Mechanisms by which *trans*-10, *cis*-12 CLA alters lipid metabolism are not well defined. Production of milk fat by bovine mammary cells utilizes fatty acids derived from de novo synthesis and fatty acids from circulation. Possible points of regulation include either of these processes as well as intracellular fatty acid

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transport, desaturation, triglyceride synthesis, and fat secretion. Abomasal infusion of 7.0, 10.0, and 14.0 g/d of *trans*-10, *cis*-12 CLA reduced milk fat yield by 35 to 50%, and alterations in fatty acid composition demonstrated that inhibition of de novo fatty acid synthesis accounted for the majority (>70% molar basis) of the reduction in milk fat (Baumgard et al., 2000b; Baumgard et al., 2001). In contrast, 3.5 g/d of *trans*-10, *cis*-12 CLA decreased milk fat yield by 25%, but de novo synthesized fatty acids were reduced in the same proportion as the fatty acids derived from circulation (Baumgard et al., 2001). Secretion of milk fat requires triglycerides to have a certain plasticity and Δ^9 -desaturase plays an important role in regulating the fluidity of milk fat. Based on changes in the fatty acid pattern, it appears that CLA reduces Δ^9 -desaturase (Lor and Herbein, 1998; Chouinard et al., 1999a), and this effect is also specific for the *trans*-10, *cis*-12 CLA isomer (Baumgard et al., 2000b). However, the effect of CLA on expression of key enzymes in mammary lipid synthesis has not been examined.

Our objective was to determine the mechanism(s) by which *trans*-10, *cis*-12 CLA reduces milk fat synthesis. This included analyzing milk fat composition to gain insight on the origin of milk fatty acids, and obtaining mammary tissue to quantify metabolic flux rates for lipogenesis and CO₂ production, and mRNA abundance for key enzymes involved in milk fat synthesis.

MATERIALS AND METHODS

Animals and Diets

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee. Four multiparous lactating Holstein cows (286 ± 54 d postpartum; mean ± SD) fitted with rumen fistulas were randomly assigned in a balanced 2 × 2 crossover design. Cows were housed in metabolic tie stalls in an environmentally controlled room (23°C) with artificial ventilation and 24 h lighting. Cows were fed a TMR formulated using the Cornell Net Carbohydrate and Protein System (Fox et al., 1992). Diet was formulated to meet or exceed the predicted requirements for energy, protein, minerals, and vitamins (NRC, 1989). Chopped alfalfa hay was the major forage component and cracked shelled corn the primary concentrate (Table 1). Cows were given ad libitum access to feed with equal portions of fresh feed given at 0600 and 1800 h. Orts were weighed and recorded daily. Feed was sampled on d 5 of each period, analyzed by wet chemistry methods (Dairy One, Ithaca, NY) and reported as an average (Table 1). Water was available at all times.

Table 1. Ingredient and chemical composition of total mixed diet.

Composition	Concentration
Ingredient, % DM ¹	
Chopped alfalfa hay	50.7
Cracked corn	40.6
Whole cottonseed	2.6
Expelled soybean meal	5.7
Trace mineralized salt and vitamins ²	0.4
Chemical analysis ³	
Crude protein, % DM	17.3
Neutral detergent fiber, % DM	39.5
Acid detergent fiber, % DM	32.3
NE _L ⁴ , Mcal/kg DM	1.55

¹Dietary DM averaged 88.7%.

²Contained 385 g of NaCl and 615 of a trace mineral and vitamin mix. Trace mineral and vitamin mix contained (g/kg of mix): Mn, 1.1; Zn, 1.4; Fe, 0.50; Cu, 0.25; I, 0.027; Co, 0.024; Se, 0.007; retinyl acetate, 0.258; cholecalciferol, 0.007; and *dl*- α -tocopheryl acetate, 2.56.

³Analyses by Dairy One (Ithaca, NY).

⁴Net energy for lactation.

Treatments consisted of abomasal infusion of *trans*-10, *cis*-12 CLA or skim milk (control). The *trans*-10, *cis*-12 CLA was provided by a supplement (Natural Lipids, Hovdebygda, Norway) in which *trans*-10, *cis*-12 represented 98% of CLA isomers as previously reported (Baumgard et al., 2001). The CLA supplement was emulsified in skim milk to obtain an adequate volume for a uniform and continuous infusion of CLA. An emulsion was prepared each experimental period using a microfluidizer as previously described (Chouinard et al., 1999a). A daily volume of 4 L of emulsion was infused with the concentrations of *trans*-10, *cis*-12 CLA in emulsions being 0 and 0.34% for the control and CLA treatment, respectively. Thus, the daily dose of *trans*-10, *cis*-12 CLA was 13.6 g. This daily dose was selected based on a previous dose-response study that established a maximum decrease in milk fat synthesis (Baumgard et al., 2001). Skim milk, which served as the control and the emulsified CLA supplement were stored at 4°C until infused.

Treatments were abomasally infused for a period of 5 d, with a 14-d interval between periods. Infusates passed through the rumen fistula and sulcus omasi into the abomasum via a 0.5 cm (i.d.) polyvinyl chloride tubing (Spires et al., 1975). Emulsions were continuously infused using pumps (Plum Infusion System XL 11555; Abbott Laboratories, North Chicago, IL) that were programmed to provide the infusion volume over a 24-h period.

Cows were milked in their tie stalls at 0600 and 1800 h daily. Yield was determined and samples taken from each milking. One aliquot was stored at 4°C with a preservative (bronopol tablet; D&F Control System, San Ramon, CA) until analyzed for fat and protein by

infrared analysis (Dairy One). We have previously established that this method of milk fat analysis is valid even with samples that have very low milk fat content due to treatment with CLA (Chouinard et al., 1999b). A second aliquot was stored at -20°C until analyzed for fatty acid composition.

Biopsies

Mammary gland biopsies were performed 4 to 5 h after the a.m. milking on d 5 of the treatment period. As a prophylactic measure cows were given intramuscular penicillin (6 million units, 2 $\times$ /d) on the day before and for 7 d after the biopsy. The biopsy procedure was according to the method of Farr et al. (1996) with modifications. Briefly, cows were administered xylazine (30 to 40 mg, intravenously) approximately 15 min before biopsy, and a 20-ml lidocaine HCl subdermal block was administered in a circular pattern surrounding the incision site. A 5- to 6-cm incision was made in the skin on the midpoint section of a rear quarter of the mammary gland and connective tissue was blunt dissected away revealing the gland capsule. The mammary tissue biopsy (~500 mg) was then obtained and divided into two portions; one was immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation, and the second portion was placed in ice cold isotonic sucrose and transferred to the laboratory for metabolic flux measurements. The gland capsule and connective tissue were then sutured with chromic gut (Ethicon, Inc., Somerville, NJ), and the skin was sutured using PDS II monofilament (Ethicon, Inc.). Cows were milked within 2 h after biopsy, again at the scheduled milking (1800) and again 3 to 4 h after the evening milking. Care was taken to remove all milk from the biopsied quarter. No mammary infections were encountered, and effects on milk yield and feed intake postsurgery were minimal and transitory (data not reported).

Tissue Incubations

Mammary tissue rates of acetate incorporation into fatty acids and oxidation to CO_2 commenced within 5 min after the biopsy. Mammary tissue explants were prepared using a Stadie-Riggs hand microtome, and the flux measurements were according to Bauman et al. (1973). Briefly, tissue explants (~100 mg) were incubated in triplicate in 3 ml of Krebs-Ringer bicarbonate buffer plus 25 μM HEPES. Each incubation also included 10 mM acetate, 0.5 μCi of 1- ^{14}C acetate (Amersham, Arlington Heights, IL), 10 mM glucose, and insulin (10 ng/ml). The incubation medium (pH 7.4) was gassed with a mixture of $\text{O}_2:\text{CO}_2$ (95:5), sealed with a rubber serum cap that contained a suspended plastic

well and incubated in a shaking water bath at 37°C for 3 h. Incubations were terminated with addition of 0.25 ml of 0.5 M H_2SO_4 and radioactive CO_2 was trapped in the suspended plastic well that contained a 2- $\times$ 2-cm filter paper saturated with 0.1 ml of 25% KOH as previously described (Bauman et al., 1973). Total lipids were extracted from the tissues as described by Folch et al. (1957) and the radioactivity determined.

RNA Isolation and Northern Blot Analysis

Total RNA was isolated from mammary tissue samples according to Chomczynski and Sacchi (1987). Samples of total RNA (15 to 20 μg) were separated in 1% agarose-formaldehyde gels, transferred to GeneScreen membranes (DuPont NEN, Boston, MA) by capillary blotting, and hybridized to the cDNA probes at 45°C in a solution containing 50% formamide (Fluka Chemical Corp., Ronconoma, NY), 10% dextran sulfate (Pharmacia Biotech, Inc., Piscataway, NJ); 5 $\times$ SSPE buffer (0.9 M NaCl, 5 mM EDTA, and 25 mM NaH_2PO_4 ; pH 6.8), 10 $\times$ Denhardt's solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% BSA), 100 $\mu\text{g}/\text{ml}$ salmon sperm DNA and 1% sodium dodecyl sulfate. Ovine cDNA for acetyl-CoA carboxylase (**ACC**) and Δ^9 -desaturase (stearoyl-CoA desaturase; **SCD**) were provided by M. T. Travers and M. C. Barber (Hannah Research Institute, UK). Ovine cDNA for fatty acid synthetase (**FAS**) was donated by C. Leroux (LGBC-INRA, France). Bovine expressed sequence tags with sequence homology to glycerol phosphate acyltransferase (**GPAT**), acylglycerol phosphate acyltransferase (**AGPAT**) and lipoprotein lipase (**LPL**) were obtained from J. C. Byatt (Monsanto Co., St. Louis, MO). cDNA for fatty acid binding protein (**FABP**) was purchased from American Type Culture Collection (Manassas, VA). Expression of all analyzed genes was quantified in a Fujix Bio-imaging Analyzer BAS 1000 (Fuji Medical Systems, Ltd., Stamford, CT) and normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase mRNA.

Fatty Acid Analysis

Milk fat was extracted according to Hara and Radin (1978) and base-catalyzed transesterified according to the method of Christie (1982) with modifications (Chouinard et al., 1999a). Fatty acid methyl esters were quantified using a gas chromatograph (Hewlett Packard GCD system HP 6890+; Avondale, PA) equipped with a Supelcowax-2560 fused silica capillary column (100 m $\times$ 0.25 mm (i.d.) with 0.2- μm film thickness; Supelco, Bellefonte, PA) as previously described (Baumgard et al., 2001). Each peak was identified using pure methyl ester standards (Nu Check Prep, Elysian,

Figure 1. Temporal pattern of milk fat content (A) and milk fat yield (B) in cows abomasally infused with control (●) or *trans*-10, *cis*-12 CLA (13.6 g/d) (■). Values are means, $n = 4$; SE ranged from 0.04 to 0.18% for milk fat percentage and 3 to 17 g/milking for milk fat yield. Animals were milked twice/d and biopsied after the ninth milking.

MN). A butter oil reference standard (CRM 164; Commission of the European Community Bureau of References, Brussels, Belgium) was used to determine recoveries and correction factors for individual fatty acids. The butter oil standard was also analyzed at regular intervals as part of quality control procedures. High-resolution nuclear magnetic resonance spectroscopy (^{13}C) verified the CLA supplement was comprised almost exclusively of the *trans*-10, *cis*-12 CLA isomer (M. Aursand and A. Saebo, Natural Lipids; personal communication).

Statistical Analysis

Animal production, milk fat composition, and metabolic flux data were analyzed with the Proc Mixed procedure of SAS (SAS Inst. Inc., Cary, NC, 1992) with treatment, period and cow included in the model and reported as least square means $\pm$ SEM. To verify

Table 2. Production parameters from cows abomasally infused with either control (skim milk) or *trans*-10, *cis*-12 CLA.

Variable	Treatment ¹		SEM	P
	Control	CLA ²		
DMI, kg/d ³	17.7	16.6	0.8	0.30
Milk yield, kg/d ⁴	19.9	17.1	0.9	0.06
Milk fat ⁴				
%	3.13	1.80	0.08	<0.01
g/d	618	320	19	<0.01
Milk protein ⁴				
%	3.20	3.36	0.07	0.01
g/d	639	536	13	<0.01

¹Treatments were abomasally infused for 5 d.

²Supplement of *trans*-10, *cis*-12 CLA provided 13.6 g/d.

³Dry matter intake value represents day 4 (biopsy was taken on d 5).

⁴Values represent last four milking before biopsy.

treatment effects and control for existing conditions, performance data were covariantly adjusted for preinfusion values (-2 and -1 d relative to initiation of infusion). Expression data were analyzed utilizing a paired *t*-test on the difference between treatments and presented as mean percentage from control $\pm$ SD.

RESULTS

Milk fat yield and fat percentage were noticeably reduced after 1 d of infusion of *trans*-10, *cis*-12 CLA and both progressively decreased until reaching nadir at approximately d 3 to 4 of infusion (Figure 1). After 5 d of abomasally infused *trans*-10, *cis*-12 CLA, milk fat content and yield decreased 42 and 48%, respectively (Table 2). As shown in earlier studies (Baumgard et al., 2000b), milk fat percent and yield returned to pretreatment values over a 4- to 5-d interval after treatment with *trans*-10, *cis*-12 CLA was terminated (data not presented). There was a trend for reduced milk yield (14%) and a slight but significant reduction in milk protein yield (16%).

Milk fat content of *trans*-10, *cis*-12 CLA increased from undetectable levels (<0.1 mg/g) during the control period to 4.9 mg/g of fatty acids when cows received *trans*-10, *cis*-12 CLA. Yields of all other milk fatty acids were decreased when cows were abomasally infused with *trans*-10, *cis*-12 CLA (data not presented). However, the magnitude of reduction differed among fatty acids and, as a consequence, the fatty acid pattern of milk fat was altered. The concentration of most fatty acids synthesized *de novo* decreased and those derived from preformed fatty acids increased (Table 3) when cows received *trans*-10, *cis*-12 CLA. In addition, the percentage of fatty acids that are either substrates or products of the Δ^9 -desaturase enzyme were altered.

Table 3. Composition of milk fat from cows abomasally infused with either control (skim milk) or *trans*-10, *cis*-12 conjugated linoleic acid (CLA).

Fatty acid	Treatment ¹			P
	Control	CLA ²	SEM	
– mg/g of fatty acid –				
C _{4:0}	36.2	29.5	0.4	<0.01
C _{6:0}	19.4	11.8	0.4	<0.01
C _{8:0}	7.9	4.6	0.3	<0.01
C _{10:0}	23.5	14.1	0.8	<0.01
C _{12:0}	26.1	21.1	0.6	<0.01
C _{14:0}	91.3	88.3	2.3	0.41
C _{14:1 cis-9}	7.5	5.2	0.3	<0.01
C _{15:0}	10.1	9.9	0.1	0.35
C _{16:0}	238.8	231.5	1.7	0.02
C _{16:1 cis-9}	11.8	13.4	0.8	0.18
C _{17:0}	5.6	6.4	0.2	0.02
C _{18:0}	142.3	171.4	5.4	<0.01
C _{18:1 trans 6-8}	3.6	4.7	<0.1	<0.01
C _{18:1 trans-9}	2.4	2.3	<0.1	0.06
C _{18:1 trans-10}	4.3	5.5	0.3	0.01
C _{18:1 trans-11}	15.2	16.4	0.2	<0.01
C _{18:1 trans-12}	4.2	4.0	0.1	0.21
C _{18:1 cis-9}	251.3	249.6	3.0	0.71
C _{18:2 cis-9, cis-12}	28.7	35.5	0.4	<0.01
C _{18:2 cis-9, trans-11}	5.4	4.4	0.2	<0.01
C _{18:2 trans-10, cis-12}	<0.1	4.9	0.2	<0.01
C _{18:3}	6.0	6.6	0.1	0.03
C _{20:0}	1.2	1.3	<0.1	0.04
Other	57.3	57.5	1.4	0.92
Ratios				
C _{14:0} /C _{14:1 cis-9}	12.7	18.8	1.2	<0.01
C _{16:0} /C _{16:1 cis-9}	20.5	18.8	0.9	0.20
C _{18:0} /C _{18:1 cis-9}	0.57	0.69	<0.1	<0.01
C _{18:1 t-11/c-9,t-11 CLA}	2.8	3.7	0.1	<0.01

¹Treatments were abomasally infused for 5 d and values represent a mean of the last four milkings before biopsy.

²Supplement of *trans*-10, *cis*-12 CLA was 13.6 g/d.

Milk fat ratios of myristic to myristoleic, stearic to oleic acid, and vaccenic acid to *cis*-9, *trans*-11 CLA were increased when cows received *trans*-10, *cis*-12 CLA (Table 3).

In vitro rates of lipogenesis (¹⁴C acetate incorporation into milk fat) in mammary tissue taken on d 5 of CLA infusion were decreased 82% compared with tissue taken from control cows (Figure 2). Likewise, rates of acetate oxidation to CO₂ were reduced 61% (Figure 2).

We examined the effect of treatment on mRNA abundance for a number of enzymes known to play key roles in the regulation of milk fat synthesis. The lengths of mRNA transcripts for all analyzed enzymes were close to values previously reported in literature (data not presented). Abomasally infusing *trans*-10, *cis*-12 CLA for 5 d altered enzymes involved in lipogenesis as indicated by the fact that mammary gland mRNA abundance of FAS, ACC, and SCD were decreased by 40, 39, and 48%, respectively (Figure 3). In addition, mRNA abundance for LPL and FABP was reduced by 48 and 54%, respectively (Figure 4). GPAT and AGPAT are

Figure 2. Rates of lipogenesis and oxidation by explants of mammary gland tissue biopsied from cows during the control period (gray bars) and when abomasally infused with *trans*-10, *cis*-12 CLA (black bars). Values are based on utilization rates of 1-¹⁴C acetate and represent means, n = 4; SE for lipogenic rates were 143 and 122 nmol/100 mg of tissue·3 h for control and *trans*-10, *cis*-12 CLA infusion periods, respectively (P < 0.001). SE for oxidation rates were 123 and 104 nmol/100 mg of tissue·3 h for control and *trans*-10, *cis*-12 CLA infused animals, respectively (P < 0.001).

involved in synthesis of milk fat triglycerides and their mRNA abundance was also decreased 42 and 41%, respectively, after 5 d *trans*-10, *cis*-12 CLA infusion (Figure 5).

DISCUSSION

CLA are known to have a range of beneficial effects in animal models and this has stimulated research on

Figure 3. mRNA abundance for the genes encoding acetyl CoA carboxylase (ACC), fatty acid synthetase (FAS) and Δ^9 -desaturase (SCD) from mammary tissue during abomasal infusion of control (skim milk; gray bars) or *trans*-10, *cis*-12 CLA (black bars). Representative Northern blot images of corresponding mRNA are in lanes 1 and 2 for the control period and 3 and 4 for the *trans*-10, *cis*-12 CLA infusion period, respectively. Values for the control period were set equal to 100 and values for the *trans*-10, *cis*-12 CLA period contrasted against these on an individual cow basis (n = 4); SD were 18, 22, and 10% (P < 0.01) for ACC, FAS, and SCD, respectively.

Figure 4. mRNA abundance for the genes encoding glycerol phosphate acyltransferase (GPAT) and acylglycerol phosphate acyltransferase (AGPAT) from mammary tissue during abomasal infusion of control (skim milk; gray bars) or *trans*-10, *cis*-12 CLA (black bars). Representative Northern blot images of corresponding mRNA are in lanes 1 and 2 for the control period and 3 and 4 for the *trans*-10, *cis*-12 CLA infusion period, respectively. Values for the control period were set equal to 100 and values for the *trans*-10, *cis*-12 CLA period contrasted against these on an individual cow basis ($n = 4$); SD were 12 and 18% ($P < 0.05$) for GPAT and AGPAT, respectively.

the biology of CLA in ruminants (Bauman et al., 2000; Bauman et al., 2001). Commercial supplements of CLA decreased milk fat synthesis (Loor and Herbein, 1998; Chouinard et al., 1999a, 1999b) and these effects were

Figure 5. mRNA abundance for the genes encoding for lipoprotein lipase (LPL) and fatty acid binding protein (FABP) from mammary tissue during abomasal infusion of control (skim milk; gray bars) or *trans*-10, *cis*-12 CLA (black bars). Representative Northern blot images of corresponding mRNA are in lanes 1 and 2 for the control period and 3 and 4 for the *trans*-10, *cis*-12 CLA infusion period, respectively. Values for the control period were set equal to 100 and values for the *trans*-10, *cis*-12 CLA period contrasted against these on an individual cow basis ($n = 4$); SD were 11 and 15% ($P < 0.05$) for LPL and FABP, respectively.

caused by the *trans*-10, *cis*-12 CLA isomer (Baumgard et al., 2000b; Baumgard et al., 2001). This was verified in the present study, and both the temporal pattern (Figure 1) and the magnitude of the reduction in milk fat yield (Table 2) were comparable to similar doses in previous studies. Milk fatty acids originate from two sources, de novo synthesis and uptake of preformed lipids from the circulation (Bauman and Davis, 1974; Moore and Christie, 1979). Examination of the milk fatty acid composition demonstrated that CLA causes a reduction in the secretion of all fatty acids, but those of de novo origin were more extensively affected (Loor and Herbein, 1998; Chouinard et al., 1999a; Baumgard et al., 2000b) this was also observed in the present study (Table 3). On a molar basis, fatty acids C_4 to C_{16} accounted for 63% of the reduction observed with *trans*-10, *cis*-12 CLA infusion.

Based on aforementioned results, we hypothesized the mechanism by which CLA reduces milk fat synthesis was via inhibiting the biochemical machinery associated with de novo fatty acid synthesis (Baumgard et al., 2000b). In the present study a tissue explant system was used to examine the effects of treatment on acetate incorporation into fatty acids, and, consistent with our hypothesis, an 82% reduction in lipogenic rates was observed when cows received *trans*-10, *cis*-12 CLA (Figure 2). This tissue system measures the capacity for fatty acid biosynthesis from acetate under conditions of saturating substrate supply, and it has been previously validated and shown to synthesize a lipid class and fatty acid pattern characteristic of de novo synthesis by the mammary gland (Bauman et al., 1973). mRNA abundance was compared for ACC and FAS, two key enzymes in de novo fatty acid synthesis, and both were reduced about 40% when cows received *trans*-10, *cis*-12 CLA (Figure 3). There have been no previous studies looking at the effect of *trans*-10, *cis*-12 CLA on enzymes involved in milk fat synthesis. However, diet-induced milk fat depression is a situation where ruminally produced *trans*-10, *cis*-12 CLA appears to be involved (see review by Bauman and Griinari, 2001), and Piperova et al. (2000) observed that mRNA abundance of ACC and activity of ACC and FAS in mammary tissue were reduced when lactating cows were fed a milk fat depressing diet. Dietary supplements of CLA also reduce body fat accretion, but studies of the lipogenic enzymes have given inconsistent results. Tsuboyama-Kasaoka et al. (2000) reported that dietary supplements of CLA caused a decrease in mRNA abundance for ACC and FAS in adipose tissue of growing mice whereas others observed no change in mRNA abundance of FAS in liver and adipose tissue of growing rats (Azain et al., 2000) and adipose tissue of growing pigs (Bee et al., 2000).

Circulating preformed fatty acids derived from the diet or body reserves are also important contributors to total milk fat. We recently demonstrated that a low level of *trans*-10, *cis*-12 CLA (3.5 g/d) which decreased milk fat yield by 25%, reduced the yield of longer chain fatty acids to the same extent as short and medium chain fatty acids (Baumgard et al., 2001). To investigate this, mammary tissue mRNA abundance was examined for two key enzymes in this process—LPL, which hydrolyzes triacylglycerol circulating in triglyceride-rich lipoprotein particles, and FABP, which is involved in the uptake and intracellular trafficking of fatty acids (Bauman and Davis, 1974; Bernlohr et al., 1999; Lehner and Kukis, 1996). Marked decreases in expression of both enzymes were observed when cows received *trans*-10, *cis*-12 CLA (Figure 3). Consistent with this, dietary supplements of CLA have also been shown to decrease milk fat content of lactating sows (Harrell et al., 2000; Poulos et al., 2000) and nursing women (Masters et al., 2002), two species, where the uptake of preformed circulating lipids is the predominant source of the fatty acids in milk fat. 3T3-L1 cells cultured with a variety of CLA isomers had reduced LPL activity (Park et al., 1997) and it was later shown this was specifically due to the *trans*-10, *cis*-12 CLA isomer (Park et al., 1999). These results are also consistent with studies showing fat accretion in adipose tissue was reduced in growing mice fed a high-fat diet supplemented with CLA (West et al., 1998), a dietary situation where uptake of circulating fatty acids would be the major carbon source for fat accretion. In contrast, Tsuboyama-Kasaoka et al. (2000) observed that a dietary supplement of CLA had no effect on LPL mRNA expression in adipose tissue of growing mice fed a low fat diet. However, the quantitative importance of uptake of circulating fatty acids and therefore expression or activity of adipose tissue LPL would be minimal in animals fed a low fat diet, and this may have limited detection of differences.

The Δ^9 -desaturase enzyme plays a critical role in regulating milk triglyceride fluidity by introducing a *cis*-double bond in fatty acids, which lowers their melting point (Parodi, 1982). Substrate to product ratios of $C_{14:0}:C_{14:1}$, $C_{18:0}:C_{18:1}$ and $C_{18:1}$ *trans*-11/ $C_{18:2}$ *cis*-9, *trans*-11 CLA represent a desaturase index and serve as a proxy for Δ^9 -desaturase activity. Based on changes in the desaturase index, we hypothesized that CLA reduces the mammary expression and/or activity of Δ^9 -desaturase (Chouinard et al., 1999a), and further demonstrated this effect occurred with abomasal infusions of *trans*-10, *cis*-12-CLA, whereas *cis*-9, *trans*-11 CLA had no effect on milk fat composition (Baumgard et al., 2000b). The present study also demonstrated that *trans*-10, *cis*-12 CLA altered the ratios for the fatty acid pairs that serve as a proxy for Δ^9 -desaturase (Table 3).

Furthermore, we showed that mRNA abundance for SCD in the mammary gland was reduced 54% for the *trans*-10, *cis*-12 CLA treatment (Figure 3). Others have shown that *trans*-10, *cis*-12 CLA decreased the expression of Δ^9 -desaturase in cultured 3T3-L1 cells whereas *cis*-9, *trans*-11 CLA had no effect (Choi et al., 2000). In addition, it has been demonstrated that *trans*-10, *cis*-12 CLA reduces hepatic SCD activity (Bretillon et al., 1999; Park et al., 2000), whereas *cis*-9, *trans*-11 CLA and a variety of *trans*- $C_{18:1}$ fatty acids (including *trans*-11 $C_{18:1}$) had no effect (Park et al., 2000).

The synthesis of milk fat triglycerides involves a high degree of positional specificity for the various fatty acids (Parodi, 1982). GPAT and AGPAT are two enzymes required for milk triglyceride synthesis (Moore and Christie, 1979). After five days of abomasal infusion, *trans*-10, *cis*-12 CLA significantly reduced the expression of both enzymes (Figure 4).

The present investigation demonstrates that the mechanism by which *trans*-10, *cis*-12 CLA markedly decreases milk fat production involves reduction in mRNA expression for key enzymes associated with fat synthesis by the mammary gland. The range of specific processes in milk fat synthesis that are affected and the similar magnitude of effects on expression of key enzymes in these processes emphasize the coordinated nature of the regulation. However, the specific intracellular signaling cascade has not been elucidated. Linoleic (n-6 fatty acid) and linolenic acid (n-3 fatty acid) are essential substrates for the synthesis of eicosanoids and other lipid mediators involved with intracellular signaling. Given the structural similarity between CLA isomers and linoleic acid, it is likely that some of the biological effects observed with CLA supplements may be mediated by modulation of eicosanoid synthesis and thus altered intracellular signaling (Pariza et al., 2000).

Investigations of the intracellular signaling mechanisms with polyunsaturated fatty acids may provide insight. The system by which n-3 and n-6 polyunsaturated fatty acids are able to markedly reduce lipid synthesis has been extensively investigated in hepatic and adipose tissue and involves coordinately suppressing genes that code for enzymes involved in lipid synthesis (Auwerx, 1999; Horton and Shimomura, 1999; Clark, 2001; Roche et al., 2001). Two families of transcription factors have been implicated, peroxisome proliferator-activated receptors (PPAR) and sterol regulatory element binding-proteins (SREBP). This work has not been extended to lipid synthesis in mammary tissue. However, PPAR γ has been reported in bovine mammary tissue (Sundvold et al., 1997), and CLA is a ligand for the PPAR (Moya-Camarena and Belury, 1999). CLA has also been shown to regulate white adipose tissue expression of both SREBP-1 and PPAR γ (Tsuboyama-

Kasaoka et al., 2000). The list of genes for which regulatory response elements for these transcription factors have been identified is growing and includes genes for most of the enzymes examined in our study (Horton and Shimomura, 1999; Clark, 2001; Roche et al., 2001).

CONCLUSIONS

The *trans*-10, *cis*-12 CLA isomer is a very potent inhibitor of milk fat production; in the present study a daily dose of 13.6 g (0.08% of the daily dry matter intake) resulted in a 42% decrease in milk fat content and a 48% reduction in milk fat yield. Results demonstrated that the mechanisms by which *trans*-10, *cis*-12 CLA decreased milk fat yield and content involves many aspects of milk fat synthesis. Specifically, this CLA isomer dramatically reduced the mammary gland's lipogenic capacity (rates of acetate incorporation into fatty acids) and decreased the expression of genes encoding enzymes involved in the uptake and transport of circulating fatty acids, de novo fatty acid synthesis, desaturation of fatty acids, and formation of triglycerides. Thus, treatment with *trans*-10, *cis*-12 CLA resulted in coordinated decreases in mRNA abundance for key enzymes involved in the production of milk fat and these effects were rescued when treatment ceased.

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