Effects of intravenous infusion of *trans*-10, *cis*-12 18:2 on mammary lipid metabolism in lactating dairy cows

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ABSTRACT

It has previously been established that supplementation of *trans*-10, *cis*-12 18:2 reduces milk fat content and fat deposition in several species. The objectives of the study were 1) to examine whether potential mechanisms by which *trans*-10, *cis*-12 18:2 is reported to affect lipid metabolism in adipose tissue of different species could be partly responsible for the inhibition in milk fat synthesis in lactating dairy cows; and 2) to investigate the effects of *trans*-10, *cis*-12 18:2 on the expression of a newly identified isoform of stearoyl-coenzyme A desaturase (SCD) in bovine mammary tissue. Four primiparous Holstein cows in established lactation, fitted with indwelling jugular catheters, were used in a balanced 2 × 2 crossover design. For the first 5 d of each period, cows were infused intravenously with a 15% lipid emulsion providing 10 g/d of either *cis*-9, *cis*-12 18:2 (control) or *trans*-10, *cis*-12 18:2 (conjugated linoleic acid; CLA). On d 5 of infusion, mammary gland biopsies were performed and tissues were analyzed for mRNA expression of acetyl-coenzyme A carboxylase, fatty acid synthetase, lipoprotein lipase, SCD1, SCD5, sterol regulatory element-binding protein-1, IL6, IL8, and tumor necrosis factor- α by real-time PCR. Compared with the control treatment, CLA reduced milk fat concentration and yield by 46 and 38%, respectively, and increased the *trans*-10, *cis*-12 18:2 content in milk fat from 0.05 to 3.54 mg/g. Milk yield, milk protein, and dry matter intake were unaffected by treatment. Infusion of the CLA treatment reduced the mRNA expression of acetyl-coenzyme A carboxylase and fatty acid synthetase by 46 and 57%, respectively, and tended to reduce the expression of SCD1 and lipoprotein lipase. Abundance of mRNA for sterol regulatory element-binding protein-1 was reduced by 59% in the CLA treatment group. However, infusing *trans*-10, *cis*-12 18:2 did not affect the expression of transcripts for SCD5, tumor necrosis factor- α , IL6, and IL8. Results

from the current study corroborate the idea that effects of *trans*-10, *cis*-12 18:2 reported on adipose tissue in animal models and humans are not part of the response in the inhibition of milk fat synthesis in lactating dairy cows. They also support the hypothesis that SCD1 and SCD5 present important differences in their regulation and physiological roles.

Key words: conjugated linoleic acid, lipid metabolism, stearoyl-coenzyme A desaturase

INTRODUCTION

The relation between *trans*-10, *cis*-12 18:2 and decreased milk fat output is well established (Baumgard et al., 2000). Over the last decade, results from many studies have shown that, in lactating dairy cows, *trans*-10, *cis*-12 18:2 has an inhibitory effect that is specific to milk fat and has no consequence on other milk components or milk yield (Bauman et al., 2008). The effect of *trans*-10, *cis*-12 18:2 on milk fat synthesis is dependent on dose and this relationship is curvilinear, with a maximum inhibition of milk fat secretion around 50% (de Veth et al., 2004). Many aspects of milk fat synthesis are affected by *trans*-10, *cis*-12 18:2. Indeed, dietary supplementation of this conjugated linoleic acid (CLA) isomer reduces expression of lipogenic enzymes and inhibits the desaturation of fatty acids. Results from previous studies raised the possibility that reduced milk fat synthesis reported when *trans*-10, *cis*-12 18:2 is provided to dairy cows can be partly explained by the effect of this CLA isomer on gene expression of stearoyl-coenzyme A desaturase-1 (SCD1; Baumgard et al., 2002b). Recently, a new isoform of this enzyme, namely, stearoyl-coenzyme A desaturase-5 (SCD5), has been identified in cattle (Lengi and Corl, 2007) but has never been identified in bovine mammary tissue. Therefore, the first objective of the current experiment was to study the presence of SCD5 in bovine mammary tissue and to evaluate the impact of *trans*-10, *cis*-12 18:2 on the mRNA abundance of this enzyme.

Trans-10, *cis*-12 18:2 has also been investigated for its effects on lipid metabolism in adipose tissue. *Trans*-10, *cis*-12 18:2 decreases body fat and increases

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Table 1. Composition of the TMR

Item	Amount, % (DM basis)
Ingredient	
Timothy silage	32.2
Corn silage	26.2
Soybean meal	11.2
Rolled barley	21.1
Cracked corn	7.4
Mineral and vitamin premix ¹	0.8
Iodized salt	0.3
Calcium carbonate	0.7
Chemical composition ²	
DM, % as fed	41.9
CP	16.2
NDF	38.7
ADF	20.1
Ca	0.7
P	0.4
NE _L , Mcal/kg	1.42

¹Contained 15% Ca, 5% P, 5% Mg, 25% NaCl, 40 mg/kg of Co, 3,500 mg/kg of Fe, 100 mg/kg of I, 850 mg/kg of Cu, 3,100 mg/kg of Mn, 30 mg/kg of Se, 3,400 mg/kg of Zn, 350 kIU/kg of vitamin A, 150 kIU/kg of vitamin D, and 2,000 IU/kg of vitamin E.

²Calculated from ingredient components using the average for intake and cow characteristics (NRC, 2001).

lean body mass in different animal models and in humans (Wang and Jones, 2004). Multiple potential antiobesity mechanisms of *trans*-10, *cis*-12 18:2 have been suggested (Whigham et al., 2007). Emerging data suggest that *trans*-10, *cis*-12 18:2 induces proinflammatory cytokines and chemokines, which lead to decreased adipogenesis and insulin resistance in adipose tissue (Brown et al., 2004).

In bovine mammary tissue, evidence suggests that transcription of genes encoding for lipogenic enzymes is coordinately downregulated when *trans*-10, *cis*-12 18:2 is provided to dairy cows and that sterol regulatory element-binding protein-1 (**SREBP1**) and spot 14 are both implicated in the transcriptional regulation (Harvatine and Bauman, 2006). However, multiple regulatory systems could be involved in the coordinated downregulation of lipogenic enzymes during CLA-induced milk fat depression. The second objective of the present trial was to examine whether potential mechanisms by which *trans*-10, *cis*-12 18:2 are reported to affect lipid metabolism in adipose tissue of different species could be partly responsible for the inhibition in milk fat synthesis observed in lactating dairy cows.

MATERIALS AND METHODS

Animals and Diets

All procedures involving dairy cows were conducted according to the regulations of the Canadian Council on Animal Care (1993) and were approved by the Université Laval Animal Care Committee. Four primiparous

Holstein cows (mean BW: 560 ± 24 kg) in midlactation (mean DIM: 195 ± 16 d) fitted with indwelling jugular catheters were intravenously infused with a lipid emulsion enriched with either *trans*-10, *cis*-12 18:2 (CLA) or *cis*-9, *cis*-12 18:2 (control) according to a 2 × 2 cross-over design. Infusion periods were 5 d in length, with a 23-d washout between periods. Animals were housed in a tie-stall facility. Throughout the experiment, cows were fed a TMR once daily (1000 h), which was formulated to meet or exceed NRC requirements (NRC, 2001; Table 1). Orts were weighed daily just before feeding, and the amount of feed offered was adjusted to ensure 10% feed refusal. Silage samples were taken every other day and pooled weekly, and DM content was measured by oven-drying at 65°C for 48 h. Proportions of TMR ingredients were adjusted based on DM content of forages before each infusion period. Free access to water was provided at all times. Cows were weighed on 2 consecutive days at the beginning of each period.

Infusion Procedures

Three days before initiation of infusion, an indwelling catheter was placed in each of the external jugular veins. Catheters were kept patent with physiological saline (sodium chloride 0.9% injection USP bag; Baxter Corporation, Toronto, Ontario, Canada) plus 200 IU/mL of heparin (Hepalean Injectable 10,000 U/mL; Organon Canada, CDMV, St-Hyacinthe, Québec, Canada) before the infusion period. Effects of *trans*-10, *cis*-12 18:2 were investigated using a supplement that contained 80% of an equal mixture of *cis*-9, *trans*-11 18:2 and *trans*-10, *cis*-12 18:2 as FFA (Clarinol A-95; Loders Crokiaan, Channahon, IL; Table 2). The amount infused for the first treatment (CLA) was calculated to provide 10 g/d of *trans*-10, *cis*-12 18:2. The second treatment consisted of a control in which CLA was replaced with linoleic acid (*cis*-9, *cis*-12 18:2; Sigma-Aldrich Canada, Oakville, Ontario, Canada). To equilibrate the amounts of total 18:1 and 18:2 among treatments, FFA supplements [oleic acid, *cis*-9 18:1: VWR International, Ville Mont-Royal, Québec, Canada; α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 18:3): Larodan Fine Chemicals, Malmö, Sweden] were included in experimental infusates (Table 3).

The experimental FFA mixtures were diluted in 164 or 173 g of 20% Intralipid (Baxter Corporation) for CLA and the control, respectively. For both treatments, 2.7 g of phosphatidylcholine (Sigma-Aldrich Canada Ltd.) and 13 g of glycerin (Fisher Scientific Company, Ottawa, Ontario, Canada) were added as emulsifiers, and the mixtures were brought to 450 mL with sterile water. Emulsions were obtained using a high-pressure homogenizer (Emulsi-flex-C50; Avestin Inc., Ottawa,

Table 2. Fatty acid composition of lipid supplements and amounts infused daily in each treatment group

Variable	Oleic acid ¹	Linoleic acid ²	Linolenic acid ³	Conjugated linoleic acid ⁴
Fatty acid, % (by wt)				
14:0	2.81	0.10	<0.01	0.06
16:0	3.40	7.11	<0.01	3.81
<i>Cis</i> -9 16:1	4.02	0.13	<0.01	0.09
18:0	0.83	4.08	<0.01	1.89
<i>Cis</i> -9 18:1	62.74	15.39	0.64	11.27
<i>Cis</i> -11 18:1	4.28	1.31	<0.01	1.01
<i>Cis</i> -9, <i>cis</i> -12 18:2	6.67	67.93	18.20	0.94
<i>Cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.34	0.14	80.89	0.15
<i>Cis</i> -9, <i>trans</i> -11 18:2	0.33	0.09	<0.01	37.02
<i>Trans</i> -10, <i>cis</i> -12 18:2	0.04	0.02	<0.01	37.02
Others	14.54 ⁵	3.70 ⁶	0.27 ⁷	3.41 ⁸
Treatment ⁹	Amount infused, g/d			
Control	0.00	30.01	0.01	0.00
CLA	2.52	0.00	0.00	27.00

¹VWR International (Ville Mont-Royal, Québec, Canada).²Sigma-Aldrich Canada (Oakville, Ontario, Canada).³Larodan Fine Chemicals (Malmö, Sweden).⁴Clarinol A-95 (Loders Croklaan, Channahon, IL).⁵Mainly other 18:1 isomers.⁶Mainly other 18:2 isomers (not conjugated) and other 18:1 isomers.⁷ γ -Linolenic acid.⁸Mainly other conjugated linoleic acid (CLA) isomers and 20:0.⁹Control = infusion of control fatty acid mixture; CLA = infusion of *trans*-10, *cis*-12 18:2 mixture (see Table 3 for composition).

Ontario, Canada), sterilized at 121°C for 20 min (MLS-3020U; Sanyo Electric Co. Ltd., Bensenville, IL), and subsequently cooled to 4°C until used. All treatments were infused intravenously, with a 23-d interval between periods. Lipid emulsions were continuously infused for 5 d by pumps (series 1.6 Plum Lifecare Pumps; Abbott Laboratories, Chicago, IL) so that the daily infusion volume (450 mL) was delivered over a 24-h period.

Sampling, Measurements, and Analyses

Cows were milked twice daily at 0700 and 1700 h. Milk was sampled and milk yield was recorded using calibrated milk meters (Flomaster Pro, DeLaval, Tumba, Sweden) at each milking. Milk samples were kept at 4°C, using bronopol as a preservative, before analysis for fat, protein, lactose, and SCC with a Foss MilkoScan 4000 instrument (Foss Electric, Hillerød, Denmark) combined with a Bentley 2000 instrument (Bentley Instruments, Chaska, MN). All milk composition analysis was conducted at the Québec Dairy Production Centre of Expertise (Ste-Anne-de-Bellevue, Québec, Canada). Additional milk samples were collected and stored at -20°C without preservative for subsequent analysis.

Lipid extraction of milk samples was performed and the extracted lipids were methylated according to the method of Chouinard et al. (1997). Composition

analyses of the fatty acids were carried out with a gas chromatograph (HP 5890A Series II; Hewlett-Packard, Palo Alto, CA) equipped with a 100-m CP-Sil 88 capillary column (0.25 μ m i.d., 0.20 μ m film thickness; Chrompack, Middleburg, the Netherlands) and a flame-ionization detector. At the time of sample injection, the column temperature was 80°C for 1 min, and was increased at 2°C/min to 215°C and maintained for 30 min. Inlet and detector temperatures were 220 and 230°C, respectively. The split ratio was 100:1. The flow rate for H₂ carrier gas was 1 mL/min. Most fatty acid peaks were identified and quantified using either a quantitative mixture or pure methyl ester standards (Larodan Fine Chemicals; Sigma-Aldrich Canada Ltd.; Matreya LLC, Pleasant Gap, PA; Nu-Chek Prep, Elysian, MN; Naturia, Sherbrooke, Québec, Canada). Standards for *trans*-5 18:1, *trans*-10 18:1, and *trans*-16 18:1 were not available commercially and were identified by order of elution according to the method of Precht et al. (2001). The response factor for *cis*-9 18:1 was used to quantify these *trans* 18:1 isomers.

Mammary biopsies were performed 3 to 4 h after the a.m. milking on d 5 of the treatment period. Mammary tissue was obtained under a local anesthesia (2 mL of Lidocaine HCl 2% and Epinephrine; Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) in the midpoint section of a rear quarter of the mammary

Table 3. Fatty acids provided on a daily basis during intravenous infusion

Fatty acid, g/d	Treatment ¹	
	Control	CLA
FFA ²		
18:1		
<i>Cis</i> -9	4.6	4.6
18:2		
<i>Cis</i> -9, <i>cis</i> -12	20.4	0.4
<i>Cis</i> -9, <i>trans</i> -11	0.0	10.0
<i>Trans</i> -10, <i>cis</i> -12	0.0	10.0
Total	20.4	20.4
18:3		
<i>Cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.1	0.1
Others	5.0	4.4
Esterified fatty acids ³		
16:0	4.4	4.4
<i>Cis</i> -9 18:1	7.9	8.0
<i>Cis</i> -9, <i>cis</i> -12 18:2	19.2	19.5
<i>Cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	2.1	2.1
Others	3.8	3.9

¹Control = infusion of the control fatty acid mixture; CLA = infusion of the *trans*-10, *cis*-12 18:2 mixture.
²Fatty acids from the commercial supplements that were provided to balance total 18:1, 18:2, and 18:3 concentrations among treatments.
³Fatty acids in triacylglycerol and phospholipid from the commercial lipid emulsion (20% Intralipid; Baxter Corporation, Toronto, Ontario, Canada) in which the FFA were emulsified.

gland using a 14-gauge Tru-cut biopsy needle (Source Medical, Pointe-Claire, Québec, Canada). Biopsy specimens (30 mg) were immediately rinsed with PBS (Applied Biosystems/Ambion, Austin, TX) to remove excess blood and stored at −20°C in RNAlater (Applied Biosystems/Ambion).

For RNA extraction, tissue was homogenized in TRI Reagent (Molecular Research Center Inc., Cincinnati, OH) using a glass homogenizer, followed by isolation of total RNA according to the instructions of the manufacturer. The RNA pellets were resuspended in RNase-free water and quantified at 260 nm using a ND-1000 spectrophotometer (Nano-Drop, Wilmington, DE). Total RNA was reverse transcribed (500 ng per reaction) into cDNA using the Omniscript reverse transcription kit (Qiagen, Valencia, CA) according to the instructions of the manufacturer, with oligo(dT) (Roche Applied Science, Indianapolis, IN) as the primer.

Real-time PCR reactions were performed using the Quantitect SYBR Green PCR kit (Qiagen) and an Applied Biosystems 7300 Real-Time PCR machine (Applied Biosystems, Foster City, CA). Quantification of gene transcripts for acetyl-coenzyme A carboxylase-α (ACC), fatty acid synthetase (FAS), lipoprotein lipase (LPL), SREBP1, SCD1, SCD5, tumor necrosis factor-α (TNFα), IL6, IL8, and α-LA (negative control) was completed using gene-specific primers (Table 4). β-Actin was used as the endogenous control. Fold change was

calculated using the 2^{−ΔΔC_T} method (Livak and Schmittgen, 2001), with the control group serving as the comparator. The 2^{−ΔΔC_T} values were used to determine statistical treatment differences.

Reaction conditions were as follows: one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min. Each reaction was performed in duplicate wells.

Statistical Analysis

Data were analyzed as a 2 × 2 crossover design using the PROC MIXED procedure (SAS Institute, Cary, NC) according to the model

Y_{ijk} = μ + T_i + P_j + C_k + ε_{ijk},

where Y_{ijk} is the individual observation, μ is the overall mean, T_i is the effect of treatment (i = 1, 2), P_j is the effect of period (j = 1, 2), C_k is the effect of cow (k = 1, 2, 3, and 4; treated as a random effect), and ε_{ijk} is the residual error term.

Cow effect was included in the model as a random effect. The subject of the repeated statement was the effect of period.

To study the temporal pattern of milk fat (content and yield) and FCM during intravenous infusion of fatty

Table 4. Summary of genes, primers, and product sizes

Gene ¹	Primer sequence	Product size, bp
ACC	F 5'GGGTGAAAGACTGGGTTGGAA'3 R 5'GACAGAGCACGGATGTGATG'3	172
FAS	F 5'AATGACCACTTTGCCGATGT'3 R 5'AACACCATGTGGCAGGAAGT'3	152
LPL	F 5'GAGCCAAAAGAAGCAGCAAG'3 R 5'AGGCAGGGTAAAAGGGATGT'3	181
SREBP1	F 5'ATGCCATCGAGAAACGCTAC'3 R 5'GTCCGCAGACTCAGTTCTC'3	180
SCD1	F 5'CCCTTTCCCTTGAGCTGTCTG'3 R 5'ATGCTGACTCTCTCCCCTGA'3	180
SCD5	F 5'AGAAGGGGAGGAAGCTTGAC'3 R 5'GGAGGCCAGGAAGTAGGAGT'3	166
TNFα	F 5'TAACAAGCCGGTAGCCACG'3 R 5'GCAAGGGCTCTTGATGGCAGA'3	175
IL6	F 5'TGCAGTCTTCAAACGAGTGG'3 R 5'TAAGTTGTGTGCCCCAGTGA'3	181
IL8	F 5'GCCTAAACCCCAAGGAAAAG'3 R 5'CACCAGACCCACACAGAACA'3	196
α-LA	F 5'TACTGGTTGGCCCATAAAGC'3 R 5'CAAGGGGGTACAAAGAAGCA'3	157
β-Actin	F 5'CTCTTCCAGCCTTCCTTCCT'3 R 5'GGGCAGTGATCTCTTTCTGC'3	178

¹ACC = acetyl coenzyme A carboxylase; FAS = fatty acid synthetase; LPL = lipoprotein lipase; SREBP1 = sterol regulatory element-binding protein-1; SCD1 = stearoyl-coenzyme A desaturase-1; SCD5 = stearoyl-coenzyme A desaturase-5; TNFα = tumor necrosis factor-α.

Table 5. Dry matter intake, BW, milk yield, and milk composition from lactating cows during intravenous infusion of fatty acid supplements¹

Variable	Treatment		SEM	P-value
	Control	CLA ²		
DMI, kg/d	16.3	16.3	1.2	0.92
Milk yield, kg/d	24.3	27.1	3.2	0.15
FCM, kg/d	24.4	19.8	1.9	0.05
Milk protein				
Content, %	3.35	3.28	0.14	0.39
Yield, g/d	800	884	90	0.18
Milk fat				
Content, %	4.18	2.26	0.33	0.01
Yield, g/d	975	601	50	0.01
Milk lactose				
Content, %	4.61	4.61	0.07	0.71
Yield, g/d	1,120	1,245	142	0.15
SCS ³	2.2	3.0	0.7	0.16

¹Values represent means from d 4 and 5 of infusion of fatty acid supplements, providing 10 g/d of *cis*-9, *cis*-12 18:2 (control) or *trans*-10, *cis*-12 18:2 (conjugated linoleic acid; CLA); n = 4.

²See Table 3 for composition.

³SCS = $\log_2(\text{SCC}/100,000) + 3$.

acid supplements, data were analyzed as repeated measures within the crossover by using a mixed model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k + D_l + (T \times D)_{il} + \varepsilon_{ijkl},$$

where D_l is the effect of day of infusion ($l = 0$ to 5), $(T \times D)_{il}$ is the effect of the interaction between treatment and day of infusion, and other terms are as described above.

The subject of the repeated statement was the cow $\times$ period interaction. When an effect of day of infusion or a treatment $\times$ day of infusion interaction was detected, linear and quadratic contrasts for a time effect were performed.

Significance was declared at $P \leq 0.05$ and trends were declared at $0.05 < P < 0.15$. The values reported are least squares means and standard errors.

RESULTS AND DISCUSSION

Intravenous infusion of *trans*-10, *cis*-12 18:2 was provided to dairy cows in an effort to gain insight into the mechanisms by which this CLA isomer affects milk fat synthesis in dairy cows. *Trans*-10, *cis*-12 18:2 supplementation did not alter DMI ($P = 0.92$; Table 5). In addition, no differences were observed in SCS between the 2 treatments ($P = 0.16$; Table 5) or between periods ($P = 0.16$; data not shown), with the latter indicating that biopsies did not affect udder health. Infusion of *trans*-10, *cis*-12 18:2 had no effect on milk yield, milk protein (content and yield), or milk lactose (content and yield; $P > 0.15$). In contrast, intravenous infusion of *trans*-10, *cis*-12 18:2 decreased milk fat content and yield by 46 and 38%, respectively ($P < 0.05$). These

results are in agreement with those from several experiments in which *trans*-10, *cis*-12 18:2 had no effect on milk components other than fat, when supplemented as an abomasal infusion (Chouinard et al., 1999b; Baumgard et al., 2001), as calcium salts (Perfield et al., 2002; Gervais et al., 2005), or as an intravenous infusion (Viswanadha et al., 2003). However, some experiments in which *trans*-10, *cis*-12 18:2 was supplemented to dairy cows reported a decrease in milk fat that was accompanied by an increase in milk protein (Bauman et al., 2008). Temporal patterns of milk fat content and yield, and FCM showed that infusion of *trans*-10, *cis*-12 18:2 led to a linear decrease in milk fat synthesis ($P < 0.05$) until reaching a steady state after 3 d of treatment (Figure 1). de Veth et al. (2004) combined results from previous studies and found a strong relationship between the reduction in milk fat yield and abomasal dose of *trans*-10, *cis*-12 18:2 in dairy cows. The extent of decline observed in the current study with intravenous infusion of 10 g/d of *trans*-10, *cis*-12 18:2 was in the range of what would be predicted based on the correlation established with abomasal infusions. *Trans*-10, *cis*-12 18:2 was transferred from blood to the mammary gland and incorporated into milk fat with an efficiency of 21%, which is comparable with what was observed previously (de Veth et al., 2004; Gervais and Chouinard, 2008).

Many studies have reported a shift in the respective proportions of de novo and preformed fatty acids in milk from dairy cows after abomasal infusion of CLA (Loor and Herbein, 1998; Chouinard et al., 1999a). Indeed, *trans*-10, *cis*-12 18:2 decreased proportions of short- and medium-chain fatty acids in milk fat and, consequently, increased proportions of long-chain fatty

acids. These results led to the hypothesis that the mechanism by which CLA decreases milk fat synthesis involves inhibition of de novo fatty acid synthesis in the mammary gland. In a later study, Baumgard et al. (2002b) demonstrated that abomasal infusion of *trans*-10, *cis*-12 18:2 decreased abundance of mRNA of ACC and FAS, 2 key enzymes involved in de novo synthesis. However, in the same experiment, mRNA abundance of LPL and fatty acid-binding protein, enzymes related to the uptake of preformed fatty acids and their incorporation into milk fat, was also reduced. These results were in accordance with the fact that, despite effects being primarily on de novo fatty acids, abomasal infusion of *trans*-10, *cis*-12 18:2 resulted in a reduction in the yields of most fatty acids. Other trials have established that when low doses of *trans*-10, *cis*-12 18:2 (≤ 5 g/d) are used to inhibit milk fat synthesis in dairy cows, the preferential inhibition of de novo fatty acid synthesis does not occur and the effects on milk fatty acid yield are distributed equally between preformed and de novo fatty acids (Baumgard et al., 2001; Peterson et al., 2002). In the current experiment, intravenous administration of 10 g/d of *trans*-10, *cis*-12 18:2 for 5 d to dairy cows decreased yields of de novo fatty acids and, to a lesser extent, preformed fatty acids and resulted in a shift in the proportions of fatty acids based on their origin (Table 6). Intravenous infusion of *trans*-10, *cis*-12 18:2 affected mRNA abundance of lipogenic enzymes of the mammary gland (Figure 2). Indeed, abundance of mRNA of ACC and FAS was reduced by 46 and 57% ($P < 0.05$), respectively, and expression of the transcript for LPL showed a tendency to decrease (-36% ; $P = 0.14$) with infusion of *trans*-10, *cis*-12 18:2. Therefore, as observed when dietary *trans*-10, *cis*-12 18:2 was provided to dairy cows, intravenous infusion of this CLA isomer inhibited the synthesis of milk fatty acids of both origins, with a more pronounced effect on milk fatty acids synthesized de novo by the mammary gland.

The fluidity of milk fat is a prerequisite for the secretion of milk fat by mammary epithelial cells, and this characteristic is largely dependent on the acylation of oleic acid (*cis*-9 18:1) in the *sn*-3 position of milk triglycerides (Hawke and Taylor, 1983). In the mammary tissue of dairy cows, SCD1 activity regulates the availability of oleic acid by introducing a *cis*-9 double bond on the saturated chain of stearic acid (18:0; Cook et al., 1976). However, SCD1 is also able to add a *cis*-9 double bond in a large spectrum of medium- and long-chain fatty acids (Ntambi and Miyazaki, 2004). The primary substrates of SCD1 in the mammary gland are 14:0, 16:0, 18:0, and *trans*-11 18:1, which are, respectively, converted into *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1, and *cis*-9, *trans*-11 18:2. Many studies have

Figure 1. Temporal pattern of milk fat content (A), milk fat yield (B), and FCM (C) in lactating cows during intravenous infusion of 10 g/d of *cis*-9, *cis*-12 18:2 (control; □) or *trans*-10, *cis*-12 18:2 (CLA; ○); $n = 4$. P -values represent the interaction between treatment (trt) and linear (lin) and quadratic (quad) effects of time during the infusion (from d 0 to d 5).

investigated the enzymatic activity of SCD1 in the mammary tissue of lactating dairy cows by measuring desaturase indexes, which generally correspond to 4 product:substrate ratios related to the aforementioned milk fatty acids (Kelsey et al., 2003). Baumgard et al.

(2002b) demonstrated that abomasal infusion of 10 g/d of *trans*-10, *cis*-12 18:2 decreased the desaturase indexes in milk fat from dairy cows. These results raised the possibility that the reduced milk fat synthesis reported when *trans*-10, *cis*-12 18:2 is provided to dairy cows

can be partly explained by the effect of this isomer on SCD1. However, when lower doses of *trans*-10, *cis*-12 18:2 are supplemented to dairy cows, a lower but significant inhibition of milk fat synthesis occurs, without any change in the SCD1 index (Baumgard et al.,

Table 6. Fatty acid composition of milk fat from lactating cows after a 5-d intravenous infusion of fatty acid supplements¹

Item	Treatment		SEM	P-value
	Control	CLA		
Fatty acid, mg/g				
4:0	31.0	26.7	1.3	0.05
6:0	21.3	13.3	0.7	0.001
8:0	14.6	8.5	0.5	0.002
10:0	36.0	21.0	1.6	0.004
12:0	43.8	30.0	2.1	0.004
14:0	117.2	115.6	3.3	0.63
<i>Cis</i> -9 14:1	9.9	8.4	1.2	0.36
15:0	11.4	9.6	0.7	0.03
16:0	291.4	266.6	8.9	0.008
<i>Cis</i> -9 16:1	13.4	12.7	1.1	0.52
17:0	24.5	21.3	1.0	0.21
18:0	84.6	108.4	7.7	0.011
<i>Trans</i> -5 18:1	0.9	0.5	0.2	0.18
<i>Trans</i> -6–8 18:1	1.7	2.7	0.2	0.001
<i>Trans</i> -9 18:1	1.2	1.4	0.1	0.007
<i>Trans</i> -10 18:1	1.8	2.4	0.1	0.02
<i>Trans</i> -11 18:1	6.7	9.0	0.8	<0.001
<i>Trans</i> -12 18:1	2.2	2.9	0.1	0.008
<i>Cis</i> -9 18:1	136.7	122.7	10.3	0.22
<i>Cis</i> -11 18:1	3.7	4.9	0.3	0.03
<i>Cis</i> -12 18:1	1.5	1.8	0.1	0.03
<i>Cis</i> -13 18:1	0.31	0.52	0.02	0.002
<i>Trans</i> -16 18:1	2.0	2.6	0.2	0.07
<i>Cis</i> -15 18:1	1.2	1.4	0.1	0.001
<i>Cis</i> -9, <i>cis</i> -12 18:2	33.1	36.0	1.1	<0.001
20:0	1.4	1.6	0.1	0.21
<i>Cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	4.2	6.8	0.6	0.15
<i>Cis</i> -9, <i>trans</i> -11 18:2	2.9	8.9	0.4	0.001
<i>Trans</i> -10, <i>cis</i> -12 18:2	0.05	3.54	0.46	0.04
<i>Cis</i> -9, <i>trans</i> -11, <i>cis</i> -15 18:3	0.60	0.60	0.03	0.96
Glycerol ²	125.8	120.4	0.7	0.007
Summation				
De novo ³	268.1	229.3	7.3	<0.001
16:0 + <i>cis</i> -9 16:1	305.2	282.2	9.4	0.007
Preformed ⁴	306.1	366.2	14.3	<0.001
SCD1 index				
Specific ratio ⁵				
<i>Cis</i> -9 14:1	0.078	0.067	0.009	0.43
<i>Cis</i> -9 16:1	0.043	0.046	0.003	0.39
<i>Cis</i> -9 18:1	0.57	0.54	0.04	0.44
<i>Cis</i> -9, <i>trans</i> -11 18:2	0.303	0.500	0.018	0.009
Overall index ⁶	0.21	0.27	0.02	0.14

¹Values represent means from d 4 and 5 of infusion of fatty acid supplements, providing 10 g/d of *cis*-9, *cis*-12 18:2 (control) or *trans*-10, *cis*-12 18:2 (conjugated linoleic acid; CLA); n = 4.

²Glycerol in milk was calculated as described by Schauff et al. (1992).

³Fatty acids with chain lengths from 6 to 14.

⁴Fatty acids with chain lengths of 15, 17, or longer.

⁵Specific ratios for stearoyl-coenzyme A desaturase-1 (SCD1) activity are ratio of the SCD1 product divided by the sum of the SCD1 product and substrate, as described by Kelsey et al. (2003): *cis*-9 14:1 = (*cis*-9 14:1)/(*cis*-9 14:1 + 14:0); *cis*-9 16:1 = (*cis*-9 16:1)/(*cis*-9 16:1 + 16:0); *cis*-9 18:1 = (*cis*-9 18:1)/(*cis*-9 18:1 + 18:0); *cis*-9, *trans*-11 CLA = (*cis*-9, *trans*-11 CLA)/(*cis*-9, *trans*-11 CLA + *trans*-11 18:1).

⁶Overall SCD1 index was calculated as follows: (*cis*-9 14:1 + *cis*-9 16:1 + *cis*-9 18:1 + *cis*-9, *trans*-11 CLA)/(*cis*-9 14:1 + 14:0 + *cis*-9 16:1 + 16:0 + *cis*-9 18:1 + 18:0 + *cis*-9, *trans*-11 CLA + *trans*-11 18:1).

Figure 2. Relative mRNA abundance of genes encoding for acetyl coenzyme A carboxylase (ACC), fatty acid synthetase (FAS), lipoprotein lipase (LPL), and sterol regulatory element-binding protein-1 (SREBP1) in mammary tissue of lactating dairy cows after a 5-d intravenous infusion of fatty acid supplements providing 10 g/d of *cis*-9, *cis*-12 18:2 (control; black bars) or *trans*-10, *cis*-12 18:2 (conjugated linoleic acid; white bars). Data are least squares means and their standard errors.

2001; Peterson et al., 2002). The dose of *trans*-10, *cis*-12 18:2 used in the current trial caused an acute inhibition in milk fat synthesis. Therefore, a significant impact on SCD1 activity was expected. However, milk fatty acid composition of the treatment groups showed no effect of *trans*-10, *cis*-12 18:2 on desaturase indexes (Table 6). The ratio of *cis*-9, *trans*-11 18:2 to (*cis*-9, *trans*-11 18:2 + *trans*-11 18:1) was significantly higher for the CLA treatment group, but this effect was attributable to the exogenous *cis*-9, *trans*-11 18:2 intravenously infused with the CLA emulsion (Table 3). In agreement with the present results, Kay et al. (2007) observed no effect of CLA on the desaturase index when supplementing grazing dairy cows with doses of *trans*-10, *cis*-12 18:2 similar to the one used in the current study. Dietary supplementation of *trans*-10, *cis*-12 18:2 has been proven to affect bovine mammary mRNA abundance of SCD1 in vitro (Peterson et al., 2003) and in vivo (Baumgard et al., 2002b). Furthermore, Keating et al. (2006) demonstrated that this effect of *trans*-10, *cis*-12 18:2 on SCD1 mRNA could be a result of direct transcriptional downregulation at the SCD1 transcriptional enhancer element of the SCD1 gene promoter. Accordingly, in the present experiment, intravenous infusion of *trans*-10, *cis*-12 18:2 tended ($P = 0.06$) to cause a 26% reduction in mRNA abundance of SCD1 (Figure 3). This lack of correlation between SCD1 expression and the desaturase indexes has been observed previously in bovine mammary (Bionaz and Looor, 2008) and interfascicular adipose tissues (Archibeque et al., 2005). Bauman et al. (2003) noted that many factors could result in variations in SCD1 ratios

among cows. These include differences in the primary or tertiary structure of the enzyme caused by gene polymorphisms, posttranslational modifications, and factors that could interfere with the synthesis and secretion of fatty acids related to SCD1 activity into milk fat. Further research is needed to establish the relationship between desaturase indexes, SCD1 enzymatic activity, and mRNA abundance of SCD1, and to clarify the effects of *trans*-10, *cis*-12 18:2 on these parameters in bovine mammary tissue.

Homologs of the SCD genes have been found in many mammalian species, and multiple isoforms of the enzyme have been observed in mice (Zheng et al., 2001) and rats (Mihara, 1990). Before the recent identification and characterization of SCD5 by Lengi and Corl (2007), SCD1 was believed to be the only isoform present in cattle. In the current experiment, expression of the transcript for SCD5 was detected, but no effect of treatment on the mRNA abundance of this isoform was observed (Figure 3). To our knowledge, this is the first study to report expression of SCD5 in bovine mammary tissue. The regulation of SCD1 and SCD5 most likely differ significantly, as illustrated by the profound differences in expression profile (Lengi and Corl, 2007). Differences in promoter composition sequence also highlight potential differences in transcriptional regulators for each gene (Lengi, A. J., and B. A. Corl, unpublished results). Posttranslational control of each enzyme could differ as well. The relatively short half-life (3 to 4 h) of the mammalian SCD1 protein enables its rapid transcriptional regulation (Heinemann and Ozols, 1998). The N terminus of the SCD1 protein contains peptide

Figure 3. Relative mRNA abundance of genes encoding for stearoyl-coenzyme A desaturase-1 (SCD1) and stearoyl-coenzyme A desaturase-5 (SCD5) in mammary tissue of lactating dairy cows after a 5-d intravenous infusion of fatty acid supplements providing 10 g/d of *cis*-9, *cis*-12 18:2 (control; black bars) or *trans*-10, *cis*-12 18:2 (conjugated linoleic acid; white bars). Data are least squares means and their standard errors.

sequences rich in proline, glutamic acid, serine, and threonine (PEST sequences; Kato et al., 2006). These specific AA sequences target proteins for rapid degradation and are thereby considered to be at the origin of the protein instability (Rechsteiner and Rogers, 1996). The lack of PEST sequences on the SCD5 protein suggests that this isoform exhibits a greater stability; therefore, activity of this isoform may not be as sensitive as SCD1 to *trans*-10, *cis*-12 18:2 supplementation (Lengi and Corl, 2007). The results from the present investigation support the hypothesis that SCD1 and SCD5, despite the fact that they are both expressed in mammary tissue, present important differences in their regulation and physiological roles.

Supplementing lactating dairy cows with *trans*-10, *cis*-12 18:2 resulted in a 59% decrease in mRNA abundance of SREBP1 ($P < 0.05$; Figure 2). Sterol regulatory element-binding protein-1 is a transcription factor that regulates transcription of key enzymes in the synthesis and secretion of milk fat by the mammary gland (Bauman et al., 2008). Supplementing lactating dairy cows with *trans*-10, *cis*-12 18:2 is reported to decrease mRNA abundance of SREBP1 and SREBP1 regulatory proteins in mammary tissue (Harvatine and Bauman, 2006). Taken together, these observations support the concept that SREBP1 plays a key role in the inhibition of milk fat synthesis by *trans*-10, *cis*-12 18:2. However, under the conditions of the current trial, the temporal pattern of milk fat content and yield (Figure 1) showed that inhibition of milk fat synthesis reached a nadir at d 3 of the intravenous infusion of *trans*-10, *cis*-12 18:2. The mammary tissue biopsies were collected 2 d after the maximum decrease in milk fat synthesis had occurred. Therefore, one should not ignore the fact that the effects observed on mRNA abundance of the different enzymes could also be due to the feedback of reduced milk fat synthesis on their respective mRNA expression.

Conjugated linoleic acids have also been investigated for their effects on lipid metabolism in adipose tissue, first in the mouse and subsequently in other animal models and in humans (see review by Wang and Jones, 2004). Dietary CLA are reported to decrease body fat and increase lean body mass in rodents (Park et al., 2007), chickens (Zhang et al., 2007), and pigs (Corl et al., 2008). Even though most of the aforementioned results were obtained with CLA mixtures, evidence suggests that *trans*-10, *cis*-12 18:2 is the CLA isomer causing changes in fat accretion and lipid metabolism and not *cis*-9, *trans*-11 18:2 (Mersmann, 2002). Multiple potential antiobesity mechanisms of *trans*-10, *cis*-12 18:2 have been suggested, and these include increasing energy expenditure, modulating adipocyte metabolism, increasing fatty acid β -oxidation, and modulating

adipokines and cytokines. Emerging data propose that *trans*-10, *cis*-12 18:2 induces proinflammatory cytokines and chemokines, which, through autocrine-paracrine signaling, lead to decreased adipogenesis in adipose tissue (Brown et al., 2004).

Among the proinflammatory molecules, the cytokine IL6 and the chemokine IL8 are reported to have effects on adiposity and to be increased in response to CLA supplementation (LaRosa et al., 2007). Inflammatory cytokines inhibit adipogenesis through downregulation of peroxisome proliferator-activated receptor- γ (PPAR γ) expression (Tanaka et al., 1999). Peroxisome proliferator-activated receptor- γ is a transcription factor highly expressed in extra mammary tissue and its key role in metabolism of adipose tissue is well established (Tang et al., 2008). Expression of PPAR γ has also been reported in bovine mammary tissue (Sundvold et al., 1997), and recent data suggest that this transcription factor could be implicated in the regulation of milk fat synthesis based on the small but constant upregulation of PPAR γ mRNA abundance throughout lactation (Bionaz and Looor, 2008). In adipose tissue, downregulation of PPAR γ results in downregulation of adipogenic enzymes such as LPL and SCD1. Observations from the current experiment combined with results from other trials (Baumgard et al., 2002b; Harvatine and Bauman, 2006) have determined that mRNA expression of many enzymes involved in milk fat synthesis, including LPL and SCD1, is also reduced in mammary tissue when lactating cows are supplemented with *trans*-10, *cis*-12 18:2. In the current study, mRNA abundance of IL6 and IL8 was not affected by treatment (Figure 4). Therefore, if downregulation of PPAR γ is implicated in CLA-mediated inhibition in milk fat synthesis, this effect does not appear to originate from an inflammatory response of mammary tissue orchestrated by cytokines, as reported in adipose tissue.

Tumor necrosis factor- α , another proinflammatory cytokine, is known to reduce mRNA expression of glucose transporter 4, stimulate hormone-sensitive lipase expression, and decrease LPL activity, and, as a consequence, induce insulin resistance and lipid mobilization in adipose tissue (Coppack, 2001). Previous studies demonstrated that glucose transporter 4 is not present in the mammary tissue of lactating dairy cows (Zhao et al., 1996), which is consistent with the fact that insulin is reported to have no effect on mammary uptake of glucose (McGuire et al., 1995). In addition, in contrast to adipose tissue, where it stimulates lipid synthesis and inhibits lipolysis, insulin has no effect on the uptake by the mammary gland of the other precursors of milk fat synthesis, such as acetate, BHBA, and circulating fatty acids. In the current study, a 5-d intravenous infusion of 10 g/d of *trans*-10, *cis*-12 18:2

Figure 4. Relative mRNA abundance of genes encoding for tumor necrosis factor- α (TNF α), IL6, and IL8 in mammary tissue of lactating dairy cows after a 5-d intravenous infusion of fatty acid supplements providing 10 g/d of *cis*-9, *cis*-12 18:2 (control; black bars) or *trans*-10, *cis*-12 18:2 (conjugated linoleic acid; white bars). Data are least squares means and their standard errors.

had no effect on mRNA abundance of TNF α (Figure 4) in mammary tissue. Furthermore, Baumgard et al. (2002a) demonstrated, in lactating dairy cows, that at a dose sufficient to induce severe milk fat depression, *trans*-10, *cis*-12 18:2 did not affect the response of the animal to homeostatic signals regulating lipolysis and glucose uptake. Therefore, the *trans*-10, *cis*-12 18:2-induced milk fat depression is apparently not a result of an increase in the insulin resistance of mammary tissue. This is consistent with the lack of effect of insulin on milk fat content and yield observed during a 96-h hyperinsulinemic-euglycemic clamp in lactating dairy cows (McGuire et al., 1995). Moreover, the decrease in mRNA abundance of LPL observed when lactating dairy cows received *trans*-10, *cis*-12 18:2 (current experiment; Baumgard et al., 2002b) is not mediated through signaling involving TNF α .

Recently, Harvatine et al. (2009) provided *trans*-10, *cis*-12 18:2 to dairy cows at a dose sufficient to induce a 38% reduction in milk fat yield and observed that transcriptional downregulation was occurring for most of the lipogenic enzymes in mammary tissue, whereas abundance of mRNA of LPL, FAS, SCD1, and fatty acid-binding protein-4 were increased in adipose tissue.

This combination of results corroborates the idea that mechanisms underlying *trans*-10, *cis*-12 18:2-induced milk fat depression have to be different from the effects of this CLA isomer in extramammary tissue. However, studies investigating the effects of *trans*-10, *cis*-12 18:2 on extramammary tissue were carried out using mostly nonruminant animal models or humans. Therefore, the lack of effects of *trans*-10, *cis*-12 18:2 on proinflamma-

tory cytokines could also be due to species variation related to lipid metabolism.

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