Mammary Lipogenic Enzyme Activity, *trans* Fatty Acids and Conjugated Linoleic Acids Are Altered in Lactating Dairy Cows Fed a Milk Fat–Depressing Diet[1]

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ABSTRACT The objectives of the present study were to examine the effect of a milk fat–depressing (MFD) diet on: *1*) the activity of mammary acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), *2*) ACC mRNA relative abundance and *3*) distributions of conjugated linoleic acids (CLA) and *trans*-18:1 fatty acids (tFA) in milk fat. Twelve lactating Holstein cows were used in a single reversal design. Two diets were fed: a control diet (60:40% forage/concentrate) and an MFD diet (25:70% forage/concentrate, supplemented with 5% soybean oil). The MFD diet decreased ($P < 0$ 0.001) milk fat by 43% and ACC and FAS activity by 61 and 44%, respectively. A reduced ACC mRNA relative abundance ($P < 0.001$) corresponded with the lower ACC activity. The fatty acids synthesized de novo were decreased ($P < 0.002$), whereas tFA were increased from 1.9 to 15.6% due predominantly to a change in *trans*-10-18:1 isomer ($P < 0.001$). With the MFD diet, the *trans*-7, *cis*-9 and *trans*-10, *cis*-12 CLA isomers were elevated ($P < 0.001$), in contrast to the decrease in *trans*-11-18:1 ($P < 0.001$) and *cis*-9, *trans*-11-18:2. The data were consistent with a dietary effect on mammary de novo FA synthesis mediated through a reduction in ACC and FAS activity and in ACC mRNA abundance. The results were compatible with a role of *trans*-10, *cis*-12 CLA in milk fat depression, but alterations noted in tFA and other CLA isomers suggest that they also may be important during diet-induced milk fat depression. J. Nutr. 130: 2568–2574, 2000.

KEY WORDS: • *acetyl-CoA carboxylase* • *fatty acid synthase* • trans *fatty acids* • *conjugated linoleic acid* • *lactating dairy cows*

In lactating dairy cows, various dietary conditions (Grummer 1991, Kalscheur et al. 1997a, Selner and Shultz 1980) result in milk fat depression. A reduction in milk fat content has also been associated (Chouinard et al. 1999a, Loor and Herbein 1998, Romo et al. 1996) with conjugated linoleic acids (CLA)[3] and *trans*-18:1 fatty acids (tFA), produced during rumen biohydrogenation of polyunsaturated fatty acids (PUFA) (Harfoot et al. 1973, Tanaka and Shigeno 1976).

Using feeding (Kalscheur et al. 1997a and 1997b) and infusion (Gaynor et al. 1994 and 1995, Romo et al. 1996) experiments with lactating cows, we were able to demonstrate that an increase in milk tFA was associated with milk fat depression. However, the lack of a consistent reduction in milk fat despite an elevation in total tFA (Griinari et al. 1997, Kalscheur et al. 1997b) led Griinari et al. (1997) to suggest a role of specific *trans*-isomers in milk fat depression. Subsequently, Griinari et al. (1998) reported that an increase in the *trans*-10-18:1 isomer was associated with milk fat depression. Although we did not emphasize a connection, we observed a decrease in milk fat and an increase in the *trans*-10 and *trans*-12-18:1 isomers in milk of lactating cows fed a milk fat–depressing (MFD) diet (Piperova et al. 1997).

The abomasal infusion of commercially available CLA mixtures has also resulted in reduced milk fat in lactating cows (Chouinard et al. 1999a, Loor and Herbein 1998). Recently, Baumgard et al. (2000) provided convincing data that the *trans*-10, *cis*-12 CLA isomer is responsible for milk fat depression. Nevertheless, a decrease in milk fat was also observed when a CLA mixture containing the *cis*-8, *trans*-10 isomer (Chouinard et al. 1999b) was infused, suggesting that other CLA isomers may also be involved. Detailed studies of CLA isomer distribution may shed further light on this subject.

During dietary milk fat depression, fatty acids (FA) synthesized de novo in the mammary gland were disproportionately reduced (Banks et al. 1984, Loor and Herbein 1998, Wonsil et al. 1994), suggesting possible effects on lipogenic enzyme activity. Acetyl-CoA (ACC) carboxylase is a likely candidate

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[3] Abbreviations used: CLA, conjugated linoleic acid(s); MFD, milk fat–depressing; ACC, acetyl-CoA carboxylase; FA, fatty acid(s); FABE, fatty acid butyl esters; FAME, fatty acid methyl esters; FAS, fatty acid synthase; PUFA, polyunsaturated fatty acids; GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography; tFA, *trans*-18:1 fatty acid(s).

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because it is the rate-limiting enzyme for de novo FA synthesis (Mellenberger et al. 1973) and has been reported to be inhibited by long chain FA (Wakil et al. 1983). Also, dietary PUFA affect the gene expression of various lipogenic enzymes (Clarke 1993, Clarke and Jump 1994) and have been shown (Toussant et al. 1981) to coordinately suppress the hepatic content of FAS and ACC proteins. If specific tFA and/or CLA isomers are involved in reducing de novo milk FA synthesis, it is possible that they exert effects similar to those described above.

In view of this, we wondered whether an MFD diet would result in increases in specific CLA and tFA isomers in milk fat and in decreases in mammary ACC and FAS activity and ACC mRNA relative abundance. Consequently, the objectives of the present study were to determine the effect of an MFD diet on the activities of ACC and FAS and relative abundance of ACC mRNA in cow mammary gland and to examine the milk CLA and *trans*-18:1 isomer profiles during milk fat depression.

MATERIALS AND METHODS

Animals and diets. All procedures for this study were carried out under Protocol R-95-33A approved by the University of Maryland Animal Care and Use Committee. Twelve lactating Holstein cows (84 ± 13 d postpartum) were used. The control diet had a forage/concentrate ratio of 60:40 and was formulated to meet the NRC requirements for milk production at 40 kg/d and 3.5% milk fat. Cows were fed the control diet during the 2-wk preliminary period and randomly assigned to either the control or an MFD diet that contained 25% forage and 70% concentrate, supplemented with 5% soybean oil (Wesson vegetable oil, ingredient: soybean oil). Diets were fed in a single reversal design during 3-wk experimental periods.

The ingredients and chemical composition of the diets are presented in **Table 1**. Forage and concentrate dry matter were determined weekly, and the total mixed ration was adjusted accordingly to maintain a constant forage/concentrate ratio on a dry matter basis during the experiment. Cows were housed in tie stalls and bedded with wood shavings except when they were turned out to be milked in a milking parlor at 0200 and 1400 h. Diets were fed as total mixed ration once daily at 0800 h. Feed refusals were recorded once daily at 0600 h.

Milk sample collection and analyses. Milk production was recorded daily, and milk samples were collected for six consecutive milking sessions at the end of each experimental period. Milk fat, protein and somatic cell count were analyzed with infrared analysis (Foss Milkoscan; Foss Food Technology Corp., Eden Prairie, MN). FA composition of milk fat was determined as described below.

Total FA pattern. FA methyl esters (FAME) were prepared according to a direct transesterification method with anhydrous methanolic HCl (Gaynor et al. 1995). Resulting FAME were separated and quantified with a 30 m × 0.25 mm fused silica capillary column coated with SP-2380 (Supelco Inc., Bellefonte, PA), essentially as described by Wong et al. (1993). A variety of FAME standard mixtures, including GLC-60 (Nu Check Prep; Elysian, MN), were used to help identify components and assist in the calculation of response factors. Correction factors for total *trans*-18:1 values were obtained as described previously (Atal et al. 1994, Sampugna et al. 1982).

Short and medium chain FA were analyzed as butyl esters (FABE), which were mathematically converted to FAME and normalized to the FAME chromatogram (Gander et al. 1962). The original FABE procedure was modified as follows. Milk samples (200 μL), in screw-capped test tubes, were heated at 100°C for 1 h in the presence of 1 mL of butanol and 200 μL of acetyl chloride. Aliquots of the upper layer were analyzed using a Hewlett-Packard 5880 gas-liquid chromatograph equipped with a split injector, a flame ionization detector and a 25 m × 0.2 mm fused silica capillary column coated with HP1 (Hewlett Packard, Avondale, PA). Helium was used as the carrier gas at a flow rate of 2 mL/min with a split ratio of 45:1. After 5 min at 90°C, the column temperature was raised (4°C/min) to 106°C, and at 10 min, it was programmed at 5°C/min to a final temperature of 250°C. Standard mixtures, including GLC-60, were converted to FABE to aid in the identification and quantification of components.

TABLE 1

Ingredients and chemical composition of the total mixed ration

	Diet	
	Control	MFD[1]
	g/100 g dry matter	
Ingredient		
Corn silage	45.2	25.0
Alfalfa haylage	14.8	0.0
Ground corn	22.8	52.2
Soybean meal	13.8	15.4
Soybean oil	0.0	5.0
Soyplus[2]	1.6	0.0
Urea	0.0	0.6
Dicalcium phosphate	0.5	0.7
Limestone	0.3	0.9
NaCl	0.4	0.4
Potassium-magnesium sulfate[3]	0.6	0.0
KCl	0.5	0.0
Trace minerals and vitamins[4]	0.2	0.2
MgO	0.2	0.3
Chemical composition		
Crude protein	17.5	17.3
UIP[5]	6.3	6.4
Acid detergent fiber	18.1	8.2
Neutral detergent fiber	31.2	17.5
Nonfiber carbohydrate	40.7	53.1
Net energy for lactation, *Mcal/kg* (*MJ/kg*)	0.7 (2.9)	0.9 (3.8)
Fat	3.0	7.2
Calcium	0.75	0.75
Phosphorus	0.37	0.37
Sodium	0.31	0.22
Magnesium	0.30	0.21
Sulfur	0.15	0.15
Potassium	1.32	1.15
Chlorine	0.41	0.61

1 Milk fat–depressing diet.
2 Soyplus/West Central (Ralston, IA).
3 Pitman-Moore, Inc. (Chicago, IL); guaranteed analysis: 22% S, 18% K, and 11% Mg.
4 Combined 549 g/kg calcium carbonate and 451 g/kg trace mineral and vitamin mix. Trace mineral and vitamin mix combined (per kg mix): 24 g Mn, 24 g Zn, 6.5 g Cu, 0.29 g I, 0.048 g Co, 0.144 g Se, 0.95 g retinyl acetate, 0.018 g cholecalciferol, and 12.67 g DL-α-tocopheryl acetate.
5 Undegraded intake protein.

trans-18:1 isomer distribution. Butyl esters, prepared as described above, were separated by preparative Ag^{+}-thin layer chromatography to obtain a *trans* monoene fraction (Sampugna et al. 1982) using FABE standards of 14:0, *cis*-9-18:1 and *trans*-9-18:1 to help locate fractions of interest. A GLC system similar to that previously described (Wong et al. 1993) was used to separate isomers, except that a 100 m × 0.25 mm fused silica capillary column (SP-2560; Supelco Inc.) was used at a column temperature of 173°C and a split ratio of 100:1 and with nitrogen as make-up gas (25 mL/min). Isomers of *cis* and *trans* FA with double bonds in the 6, 7, 9, 11, 12, 13 and 15 positions (Sigma Chemical Co., St. Louis, MO), as well as a sample of *trans*-10-18:1 and *cis*- and *trans*-18:1 fractions isolated from a shortening sample (Sampugna et al. 1982), were converted to FABE to assist in the identification and quantification of the *trans*-18:1 isomers.

Conjugated FA. The milk fat was extracted using a modified Folch procedure (Christie 1982). FAME were prepared as described by Sehat et al. (1998a) and were analyzed using GLC and Ag^+–high performance liquid chromatography (HPLC) to obtain estimates of total CLA and to determine isomer distribution patterns. The GLC system, as described previously (Eulitz et al. 1999), used a fused silica capillary column (CP-Sil 88; 100 m × 0.25 mm i.d. × 0.2 μm film thickness; Chrompack), which was held at 70°C for 4 min after injection, temperature programmed (13°C/min) to 175°C, held at 175°C for 27 min and then temperature programmed (4°C/min) to 215°C and maintained at 215°C for 31 min. Hydrogen was used as the carrier gas, at a split ratio of 20:1. The detector and injector were set at 250°C.

Argentation–HPLC separation (Sehat et al. 1998b) of CLA methyl esters was carried out with an HPLC (Waters 510 solvent delivery system; Waters Associates, Milford, MA), equipped with a 100-μL injection loop (Waters 600E System Controller), a photodiode array detector (Waters 996) operated at 233 nm and a Waters software program (Millennium Version 2.15). Three ChromSpher 5 Lipids analytical silver-impregnated columns (each 4.6 mm i.d. × 250 mm stainless steel, 5-μm particle size; Chrompack, Bridgewater, NJ) were used in series. The mobile phase was 0.1% acetonitrile in hexane and operated isocratically at a flow rate of 1.0 mL/min. The column head pressure was 1050 psi for the three columns in series. The flow commenced for 0.5 h before sample injection. Typical injection volumes were 5–15 μL, representing ~40–120 μg of FAME. Details on the identification and quantification of the CLA isomers have been described elsewhere (Eulitz et al. 1999, Sehat et al. 1998a and 1998b).

Biopsy procedure. Mammary tissue biopsy was performed (Farr et al. 1996) on 10 cows during the 3rd wk of each experimental period to obtain a core sample of 0.7–0.8 g. For the first three or four milking sessions after biopsy, care was taken to milk the cows thoroughly by hand to remove any blood clots lodged in the gland.

Enzyme analysis. After the biopsy procedure, tissue samples were homogenized as described by Mellenberger et al. (1973). The enzyme activities were assayed in the cytosolic fraction (105,000 × *g*) at 37°C in the linear range of activity. Acetyl-CoA carboxylase (EC 6.4.1.2) activity was determined using the ^{14}C-labeled bicarbonate fixation method (Mellenberger et al. 1973) and expressed as nanomoles of bicarbonate incorporated into acid-stable products per minute per milligram of cytosolic protein. FA synthase activity was determined spectrophotometrically (Hardie et al.1981) by measuring malonyl-CoA–dependent oxidation of NADPH at 37°C and was expressed as nanomoles of NADPH oxidized per minute per milligram of cytosolic protein. Protein concentrations were measured according to the bicinchoninic acid procedure (Pierce Chemical, Rockford, IL) with bovine serum albumin as the standard.

Northern blot analysis of total RNA. Immediately after biopsy, the mammary tissue (250 mg) was homogenized in 2.5 volumes of ice-cold denaturing solution (4 mol/L guanidinium thiocyanate, 25 nmol/L sodium citrate, pH 7, 0.5% sarcosyl, 0.1 mol/L 2-mercaptoethanol) and kept at −80°C until analyzed. The total RNA was isolated as described by Chomczynski and Sacchi (1987) and assessed for purity. Concentrations were determined spectrophotometrically at A_{260}. Only samples with $A_{260}/_{280}$ ratios of >1.80 were analyzed. The RNA integrity was confirmed visually on a 1% agarose-formaldehyde minigel. Total RNA (50 μg), separated as described by Sambrook et al. (1989), was transferred overnight to a positively charged nylon membrane (Hybond-N^+; Amersham International, Buckinghamshire, U.K.) via capillary action. The RNA was cross-linked to the membrane (40 s at 1200 μJ × 100) in a UV Stratalinker 1800 (Stratagene, La Jolla, CA). The acetyl-CoA carboxylase cDNA (730 bp) was amplified from pig adipose tissue RNA, and the sequence corresponding to nucleotides 642-1370 of the rat ACC cDNA (Lopez-Casillas et al. 1988) was confirmed by Liu (1992). The membrane was prehybridized (5–6 h) and hybridized (16–18 h with 2.45–2.95 × 10^9 dpm/L [α-^{32}P]dCTP-labeled ACC cDNA) in a solution consisting of 50% formamide, 50 mmol/L NaH_2PO_4, pH 6.5, 5 mmol/L EDTA, pH 7.4, 25% 20× standard saline citrate, pH 7.0, 5× Denhardt's solution, 10% SDS and 200 μg of denatured salmon sperm DNA, except that the hybridization solution contained 1× Denhardt's solution. Subsequently, the membrane was stripped in 5 g/L SDS at 100°C for 1 h and rehybridized with 25 ng of bovine ribosomal protein S4 (accession no. U31305; 638 bp) cDNA (kindly provided by Dr. S. L. Ogg, University of Maryland, College Park, MD) to correct for sample loading and transfer variation during Northern blotting.

Autoradiograms were obtained through the exposure of membranes to Kodak XAR-5 film for 7 d at −80°C. A single band of ~9.5 kb for ACC mRNA was visible (**Fig. 1**), and the size was verified by an RNA marker ladder (0.24–9.5 kb; Sigma Chemical). Autoradiograms were scanned twice and analyzed with ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The bovine ribosomal protein S4 mRNA was detected through exposure to Kodak XAR-5 film for 6 h at −80°C. Values were normalized across samples using bovine ribosomal protein S4 mRNA. The results are presented as the relative abundance of ACC mRNA in densitometric units.

Statistical analysis. Statistical analyses were conducted using the General Linear Models Procedure in the Statistical Analysis System (Version 6.12, 1998; SAS Institute, Cary, NC). The statistical model included the effect of cow, experimental period and treatment. Probability values of <0.05 were considered to be statistically significant. All data are reported as least-squares means.

RESULTS

Animal health. The repeated biopsies caused minimal animal discomfort. The mammary glands healed without infection and showed no evidence of clinical mastitis, and there were no long-term adverse effects on milk production or composition.

Milk production and composition. Dietary treatment did not affect either dry matter intake or daily milk production (**Table 2**). Milk protein was increased by 0.34 percentage units ($P < 0.003$), resulting in higher milk protein production ($P < 0.002$) when the cows were fed the MFD diet. The milk fat percent and yield were reduced by 43% ($P < 0.001$), and the fat-corrected milk yield was decreased by 18% ($P < 0.002$) compared with the control.

The milk fat of the cows that were fed the MFD diet contained 62% less total saturated FA ($P < 0.001$) than that of the control animals (**Table 3**). The relative proportion of short and medium chain FA (6:0 to 14:0) was decreased by 27% ($P < 0.002$), and the amount (g/d) in the milk was reduced by 60% ($P < 0.001$). Palmitic acid, which is partially derived from de novo synthesis in the mammary gland, was decreased by 32%($P < 0.001$). Although the percentage of

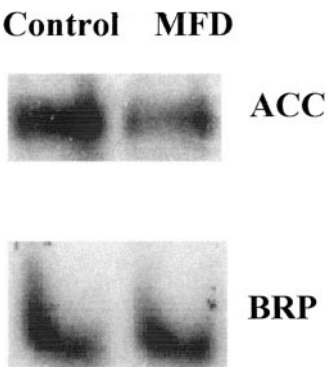

FIGURE 1 A representative Northern blot of mammary tissue mRNA for acetyl-CoA carboxylase (ACC) from lactating cows fed a control or a milk fat–depressing (MFD) diet. Total RNA (50 μg) was hybridized to a [^{32}P]-labeled ACC cDNA probe. The intensity of a single transcript (7-d exposure) of ~9.5 kb was compared for both diets. The bovine ribosomal protein S4 cDNA (BRP) transcript was visualized after 6-h exposure.

TABLE 2

Dry matter intake, milk production, and milk composition of cows fed control, or milk fat–depressing (MFD) diets[1]

Item	Control	MFD	SED[2]	*P*[3]
		kg/d		
DMI[4]	20.4	19.7	0.21	<0.090
Milk	27.6	27.6	0.98	<0.987
3.5% FCM[5]	28.6	23.4	0.98	<0.002
		g/100 g		
Milk components				
Fat	3.28	1.88	0.09	<0.001
Protein	3.24	3.58	0.06	<0.003
		g/d		
Milk production				
Fat	945	536	36.8	<0.001
Protein	930	1,055	52.8	<0.002
SCC[6]/L, *n*	2098	1986	722	<0.914

[1] Values are least square means, $n = 10$.
[2] Standard error of the difference.
[3] Probability that treatments are not different.
[4] Dry matter intake.
[5] Energy corrected yield corresponding to milk containing 3.5% fat.
[6] Somatic cell count.

stearic acid and *cis*-18:1 was not affected by the milk fat depression treatment, their daily yield in milk was significantly reduced ($P < 0.001$). The percentage of linoleic acid [18:2 (n-6)] and linoleic acid isomers (18:2i), some of which also contain one or two *trans* double bonds, was higher ($P < 0.001$) in the milk fat of cows when fed the MFD diet. The daily amounts (g/d) of 18:2 and 18:2i secreted in the milk were not significantly different from those of the controls. Due to the dietary change, milk tFA percentage was increased from 1.9 to 15.6% ($P < 0.001$), and the amount of tFA secreted daily in milk was 16.9 g in the control animals versus 75.6 g in the cows that were fed the MFD diet.

The major component in the *trans*-18:1 FA fraction of milk from cows fed the control diet was the *trans*-11 isomer (**Table 4**). However, sizable amounts of *trans*-13+14, as well as *trans*-10 and -12, isomers were found (Table 4). In contrast, the *trans*-10-18:1 was the predominant isomer in the milk fat of the cows fed the MFD diet, representing ~60% of the total *trans*-18:1 FA. Except for the *trans*-6+7+8 and the *trans*-9, the concentration of all other *trans*-18:1 components was reduced ($P < 0.001$) compared with that of the control cows. The amounts of most of the individual *trans*-isomers secreted in the milk fat (g/d) were higher with the MFD diet. The yields of *trans*-15 and *trans*-16 isomers were least affected by the diet.

The total CLA content (**Table 5**) measured in the milk fat of the control cows was similar to data reported by others (Dhiman et al. 1999). The percentage of CLA in milk of cows fed the MFD diet increased from 5.6 to 9.5 mg/g fat ($P < 0.001$), whereas the yield of total CLA (g/d) did not differ due to the marked decrease in milk fat production. The major CLA isomers in the milk of the control cows were *cis*-9, *trans*-11, and *trans*-7, *cis*-9. The individual CLA isomer profile was altered by the MFD diet. Although the predominant isomer was still *cis*-9, *trans*-11 CLA, the percentage was decreased from 79.7 to 56.7% ($P < 0.001$) of the total CLA. A 10-fold increase in the *trans*-10, *cis*-12 ($P < 0.001$) CLA and a significantly greater proportion of *trans*-7, *cis*-9 CLA ($P < 0.001$) were found in milk from cows fed the MFD diet. The *cis*-8, *trans*-10 isomer was <2% of the total CLA and was not affected by diet.

Enzyme activity and ACC mRNA abundance. The ACC activity in the cows fed the control diet averaged 9.8 nmol/(min · mg protein), and FAS activity was 13.2 nmol of oxidized NADPH/(min · mg protein) (**Table 6**). These activities were similar to results reported in other studies (Akers et al. 1981, Mellenberger et al. 1973) for mammary tissue from

TABLE 3

Milk fatty acid composition of cows fed control or milk fat–depressing (MFD) diets[1]

Fatty acid	Control	MFD	SED[2]	*P*[3]
		g/100 g FAME		
4:0	3.8	3.2	0.17	0.051
6:0	2.3	1.4	0.08	0.001
8:0	1.6	0.9	0.14	0.002
10:0	3.5	2.4	0.15	0.001
12:0	4.2	3.2	0.18	0.002
14:0	12.0	9.3	0.40	0.001
14:1	1.0	1.0	0.09	0.884
16:0	30.7	21.3	0.59	0.001
16:1	2.1	1.9	0.09	0.235
18:0	9.7	8.8	0.56	0.238
trans-18:1	1.9	15.6	0.81	0.001
cis-18:1	19.7	19.3	0.63	0.607
18:2 (n-6)	3.1	6.3	0.39	0.001
18:2i[4]	0.9	1.5	0.08	0.001
18:3	0.6	0.5	0.04	0.251
Total PUFA[5]	4.5	8.3	0.49	0.001
Total SFA[6]	68.1	50.4	1.46	0.001
Total de novo FA	39.2	27.7	1.03	0.002
Total LCFA[7]	68.7	75.2	1.48	0.010
		g/d		
4:0	34.8	16.3	1.61	0.001
6:0	21.4	7.3	0.92	0.001
8:0	15.6	4.9	1.40	0.001
10:0	34.0	12.5	1.51	0.001
12:0	40.5	16.7	1.71	0.001
14:0	112.3	48.5	3.90	0.001
14:1	8.6	5.1	0.92	0.023
16:0	279.8	107.7	9.95	0.001
16:1	18.2	9.5	0.95	0.001
18:0	88.0	44.4	5.42	0.001
trans-18:1	16.9	75.6	6.04	0.001
cis-18:1	170.1	95.7	1.18	0.001
18:2 (n-6)	26.5	31.1	1.90	0.126
18:2i[4]	7.2	7.4	0.05	0.724
18:3	5.1	2.5	0.30	0.001
Total PUFA[5]	38.8	41.0	9.40	0.864
Total SFA[6]	626.4	242.0	22.65	0.001
Total de novo FA	223.8	89.9	12.03	0.001
Total LCFA[7]	611.8	373.9	21.00	0.001

[1] Values are least square means ($n = 10$). Other FA, including odd and branch chain, representing less than 3% of the total FAME are not included.
[2] Standard error of the difference.
[3] Probability that treatments are not different.
[4] Conjugated and nonconjugated.
[5] Total polyunsaturated fatty acids.
[6] Total saturated fatty acids.
[7] Long-chain fatty acids (16 carbon and greater).

TABLE 4

trans-18:1 content and isomer distribution in milk fat of cows fed control or milk fat–depressing (MFD) diets[1]

	Control	MFD	SED[2]	*P*
	g/100 g FAME			
Total *trans*-18:1	1.9	15.6	0.81	<0.001
	g/100 g total trans-18:1			
Double bond position				
6 + 7 + 8	2.6	6.9	0.7	<0.002
9	5.5	6.1	0.7	NS[3]
10	13.9	59.2	1.7	<0.001
11	28.5	10.9	0.8	<0.001
12	12.2	4.3	0.6	<0.001
13 + 14	22.6	8.6	0.9	<0.001
15	7.1	2.2	0.3	<0.001
16	7.4	1.4	0.6	<0.001
	g/d			
6 + 7 + 8	0.5	6.1	0.9	<0.002
9	1.0	5.4	0.9	<0.001
10	2.5	50.0	0.6	<0.001
11	5.1	8.4	0.8	<0.001
12	2.2	3.1	0.2	<0.02
13 + 14	4.0	7.2	0.7	<0.01
15	1.3	1.6	0.9	NS
16	1.3	1.0	0.6	NS

[1] Values are least square means ($n = 10$).
[2] Standard error of the difference.
[3] NS, $P \geq 0.05$.

TABLE 5

Conjugated linoleic acids (CLA) content and isomer distribution in milk fat of lactating cows fed control or milk fat–depressing (MFD) diets[1]

	Control	MFD	SED[2]	*P*
	g/100 g FAME			
Total CLA	0.56	0.95	0.11	<0.001
	g/100 g total CLA			
CLA isomers				
trans-7,*cis*-9	7.8	23.4	1.66	<0.001
cis-8,*trans*-10	1.5	1.8	0.06	<0.01
cis-9,*trans*-11	79.7	56.7	1.23	<0.001
trans-10,*cis*-12	1.0	10.1	0.49	<0.001
cis-11,*trans*-13	0.2	0.1	0.05	NS[3]
trans-11,*cis*-13	0.7	0.2	0.05	<0.001
cis-12,*trans*-14	0.7	0.7	0.19	NS
	g/d			
Total CLA	5.1	4.5	1.13	NS
trans-7,*cis*-9	0.4	1.1	0.04	<0.001
cis-8,*trans*-10	0.1	0.1	0.01	NS
cis-9,*trans*-11	4.1	2.6	0.36	<0.01
trans-10,*cis*-12	0.05	0.5	0.04	<0.001
cis-11,*trans*-13	0.01	0.004	0.001	<0.03
trans-11,*cis*-13	0.04	0.01	0.004	<0.001
cis-12,*trans*-14	0.04	0.03	0.005	NS

[1] Values are least square means ($n = 10$); *trans*/*trans*- and *cis*/*cis*-isomers are not included.
[2] Standard error of the difference.
[3] NS, $P \geq 0.05$.

lactating cows. Acetyl-CoA carboxylase and FAS activities were reduced by the MFD diet, and the effect was more pronounced for the ACC activity, which was inhibited by 62% ($P < 0.001$) compared with a 44% ($P < 0.001$) decrease in FAS activity. A parallel decrease ($P < 0.001$) in the mammary ACC mRNA relative abundance was observed in cows fed the MFD diet (Table 6), and the depressions of both ACC activity and ACC mRNA relative abundance were of a similar magnitude.

DISCUSSION

A high concentrate diet supplemented with soybean oil was used in the present study to provide PUFA for rumen biohydrogenation and a large amount of carbohydrate to lower rumen pH (Kalscheur et al. 1997a, Kennelly et al. 1999, Van Nevel and Demeyer 1996). These conditions lead to changes in rumen environment, incomplete biohydrogenation of PUFA and milk fat depression (Griinari et al. 1998, Harfoot and Hazlewood 1988, Kalscheur et al. 1997a).

Increased tFA formation in the rumen (Kalscheur et al. 1997a) and higher incorporation of tFA in the milk fat (Gaynor et al. 1995, Griinari et al. 1998, Kalscheur et al. 1997a, Romo et al. 1996) are some of the changes associated with milk fat depression. Indeed reduced milk fat and increased tFA were observed in the cows fed the MFD diet in this study. When we examined the isomer distributions of milk CLA and tFA, specific changes were observed in cows fed the MFD diet. Based on the changes in the isomer profiles, we postulate that the isomerization of PUFA to conjugated dienes and the conversion of CLA to *trans* monoenes in the rumen were affected by the diet.

An analysis of the CLA showed a decrease in the major *cis*-9, *trans*-11 isomer and an increase in *trans*-10, *cis*-12 and *trans*-7, *cis*-9 CLA in cows fed the MFD diet. The *trans*-10-18:1 was the predominant *trans* monoene in the milk fat during milk fat depression compared with a preponderance of

TABLE 6

Effect of a milk fat–depressing (MFD) diet on acetyl-CoA carboxylase and fatty acid synthase activity and ACC mRNA abundance in cow mammary gland tissue[1]

	Diet			
	Control	MFD	SED[2]	*P*[3]
Acetyl-CoA-carboxylase[4]	9.8	3.8	1.1	<0.001
Fatty acid synthase[5]	13.2	7.4	1.9	<0.001
ACC mRNA[6]	0.22	0.07	0.01	<0.001

[1] Values are least square means ($n = 10$).
[2] Standard error of the difference.
[3] Probability that treatments are not different.
[4] Values are expressed as nmol $[^{14}C]HCO_3$ fixed · min^{-1} · mg $protein^{-1}$.
[5] Values are expressed as nmol NADPH oxidized · min^{-1} · mg $protein^{-1}$.
[6] Values are expressed as densitometric units, normalized against bovine ribosomal protein S4 mRNA.

the *trans*-11-18:1 in the control cows. The changes in the *cis*-9, *trans*-11 CLA and *trans*-11-18:1 isomers were in agreement with the positive correlation found between these isomers in cow's milk (Jiang et al. 1996). The reduction observed in cows fed the MFD diet showed that it is unlikely that these isomers are involved in reducing milk fat. Data from studies with lactating mice fed *trans*-11-18:1 (unpublished data) and abomasal infusion of *cis*-9, *trans*-11 CLA in lactating cows (Baumgard et al. 2000) are consistent with the lack of an effect of these isomers on milk fat depression.

We found 3- and 10-fold increases, respectively, in tFA and CLA isomers containing a *trans*-10 double bond in the milk fat of cows fed the MFD diet. The *trans*-10, *cis*-12 CLA is probably produced in the rumen via action of a specific isomerase and further hydrogenated to *trans*-10-18:1 (Griinari and Bauman 1999a). The presence of *trans*-10, *cis*-12-18:2 has been reported or tentatively identified in studies in vitro with rumen fluid (Fellner et al. 1997) and in rumen and milk samples from cows (Griinari et al. 1997 and 1999b). Based on infusion studies with CLA isomers, Baumgard et al. (2000) concluded that the *trans*-10, *cis*-12 CLA isomer is responsible for milk fat depression, and our findings are consistent with this hypothesis. Nevertheless, the observation of milk fat depression during the infusion of a CLA mixture containing the *cis*-8, *trans*-10, isomer (Chouinard et al. 1999b) suggests that other CLA isomers may also be involved. Although we did not observe an increase in the *cis*-8, *trans*-10 isomer (Table 5), we did find a significant increase in the *trans*-7, *cis*-9 CLA in the milk of cows fed the MFD diet. However, its importance in eliciting milk fat depression is unclear.

The identity of *trans*-7, *cis*-9 CLA was elucidated by Yurawecz et al. (1998), and it has been described as the most abundant minor CLA in bovine and human milk. Formation of this isomer by the action of Δ^9-desaturase on *trans*-7-18:1 has been reported to occur in microsomal preparations of rat liver (Pollard et al. 1980), and Corl et al. (1998) showed that cow mammary gland can synthesize *cis*-9, *trans*-11 CLA from *trans*-11-18:1. Thus it is possible that *trans*-7, *cis*-9 CLA could originate from Δ^9-desaturation of *trans*-7–18:1 in mammary tissue. Further studies are required to determine whether this isomer is produced in the rumen.

Based on the FA composition (Table 3) in the milk of cows fed the MFD diet, the decrease in milk fat content was mostly related to a reduction in FA synthesized de novo in mammary tissue. Lower yields of these FA were consistent with observations of reduced ACC and FAS activity and ACC mRNA relative abundance. From the changes in ACC mRNA relative abundance, it is likely that the reduction in enzyme activities is due to a decreased content of these enzymes in the milk fat–depressed state.

PUFA can suppress lipogenic gene expression (Clarke and Jump 1994, Jump and Clark 1999, Jump et al. 1994). Although some dietary PUFA escapes rumen biohydrogenation, we do not believe that PUFA were directly involved in milk fat depression in this work. In previous studies (Gaynor et al. 1994, Romo et al. 1996) in lactating cows postruminally infused with identical amounts of PUFA, we observed a reduction in milk fat only when the infusion mixture contained *trans*-FA. Loor and Herbein (1998) reported that compared with abomasal infusion of linoleic acid alone, CLA was required before milk fat depression was demonstrated. In addition, when cows were fed diets supplemented with oils rich in PUFA (Kalscheur et al. 1997b), milk fat depression was not observed, and when cows were fed identical levels of dietary PUFA, milk fat was reduced only when rumen pH was lowered (Kalscheur et al.1997a). These studies suggest the conclusion that dietary PUFA per se are not sufficient to induce milk fat depression and that alterations in the rumen environment are also required. Such alterations were undoubtedly responsible for the increased levels of specific isomers observed in the present study, and we believe that one or more of these isomers are involved in milk fat depression.

In summary, the feeding of an MFD diet to lactating cows decreased de novo FA synthesis in the mammary gland. Changes in mammary ACC mRNA relative abundance were consistent with decreased de novo FA synthesis, indicating that the mechanism of milk fat depression may involve, at least in part, the regulation of gene expression of lipogenic enzymes. The alterations in the CLA and *trans*-18:1 isomer patterns were an indication of specific modifications in the rumen environment induced by the MFD diet. The results were in agreement with an effect of the *trans*-10, *cis*-12-CLA on milk fat depression, but the changes observed in other tFA and CLA isomers may also be important.

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