

Conjugated Linoleic Acid Is Synthesized Endogenously in Lactating Dairy Cows by Δ^9 -Desaturase^{1,2}

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ABSTRACT Conjugated linoleic acid (CLA) is a naturally occurring anticarcinogen found in milk fat and body fat of ruminants. Although CLA is an intermediate in ruminal biohydrogenation of linoleic acid, we hypothesized that its primary source was from endogenous synthesis. This would involve Δ^9 -desaturase and synthesis from *trans*-11 18:1, another intermediate in ruminal biohydrogenation. Our first experiment supplied lactating cows ($n = 3$) with *trans*-11 18:1 by abomasal infusion and examined the potential for endogenous synthesis by measuring changes in milk fat CLA. By d 3, infusion of *trans*-11 18:1 resulted in a 31% increase in concentration of *cis*-9, *trans*-11 CLA in milk fat, demonstrating that an active pathway for endogenous synthesis of CLA exists. Our second experiment examined the quantitative importance of endogenous synthesis of CLA in lactating cows ($n = 3$) by abomasally infusing a putative stimulator (retinol palmitate) or an inhibitor (sterculic oil) of Δ^9 -desaturase. Infusion of retinol palmitate had no influence on milk fatty acid desaturation, and yield of CLA in milk fat was not altered. However, sterculic oil infusion decreased the concentration of CLA in milk fat by 45%. Consistent with Δ^9 -desaturase inhibition, the sterculic oil treatment also altered the milk fat concentration of other Δ^9 -desaturase products as indicated by the two- to threefold increase in the ratios of 14:0 to 14:1, 16:0 to 16:1 and 18:0 to *cis*-18:1. Using changes in the ratio of 14:0 to 14:1 as an indication of the extent of Δ^9 -desaturase inhibition with the sterculic oil treatment, an estimated 64% of the CLA in milk fat was of endogenous origin. Overall, results demonstrate that endogenous synthesis of CLA from *trans*-11 18:1 represented the primary source of CLA in milk fat of lactating cows. J. Nutr. 130: 2285–2291, 2000.

KEY WORDS: • conjugated linoleic acid • Δ^9 -desaturase • lactation • milk fat • ruminants

Conjugated linoleic acid (CLA) has a wide range of physiologic effects in animal models [see reviews by Banni and Martin (1998) and Pariza (1999)]. Many of these may represent positive health benefits of dietary CLA. Dairy products are the major dietary source of CLA, and *cis*-9, *trans*-11 octadecadienoic acid is the predominant CLA isomer in natural

lipids (Parodi 1997). A trivial name, ruminic acid, was proposed for this isomer on the basis of its ruminant origin (Kramer et al. 1998). The sequence of ruminal biohydrogenation of linoleic acid involves isomerization to form *cis*-9, *trans*-11 CLA followed by successive reductions to *trans*-11 octadecenoic acid (vaccenic acid) and stearic acid (Harfoot and Hazlewood 1988). On this basis, the CLA in milk fat and body fat of ruminants has been assumed to be CLA that has escaped complete biohydrogenation in the rumen (Chin et al. 1992, Parodi 1997 and 1999).

Concentrations of CLA in milk fat can be enhanced by changes in the diet, especially utilization of diets with greater linoleic acid content [see review by Griinari and Bauman (1999)]. However, certain diets that have low levels of linoleic acid, e.g., pasture or fish oil feeding, also increase the concentration of CLA in milk fat. These diets contain high levels of other polyunsaturated fatty acids (PUFA) that do not yield CLA as an intermediate in rumen biohydrogenation (Griinari and Bauman 1999, Harfoot and Hazelwood 1988). This raises the possibility of alternative sources of milk fat CLA. In the ruminal biohydrogenation of linoleic acid, CLA is a transient intermediate, whereas *trans*-11 18:1 accumulates (Harfoot and Hazlewood 1988). Furthermore, *trans*-11 18:1 is an intermediate in the biohydrogenation of several PUFA (Griinari and Bauman 1999). On this basis, we hypothesized that CLA could

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be produced by endogenous synthesis from *trans*-11 18:1 by Δ^9 -desaturase (Griinari et al. 1997). Consistent with this, mammary gland and adipose tissue of ruminants have substantial Δ^9 -desaturase activity (Kinsella 1972, Martin et al. 1999, St. John et al. 1991, Ward et al. 1998).

The objective of this investigation was to examine the endogenous synthesis of CLA in lactating dairy cows. The first experiment supplied *trans*-11 18:1 by abomasal infusion and examined the potential for endogenous synthesis by measuring changes in milk fat CLA. In the second experiment, we examined the quantitative importance of endogenous synthesis of CLA by inhibiting the activity of Δ^9 -desaturase with sterculic oil. In this latter study, we also included a treatment with retinol palmitate, a compound that has been reported to enhance gene expression of Δ^9 -desaturase in mouse liver.

MATERIALS AND METHODS

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals. Experiments utilized multiparous Holstein cows fitted with rumen fistulas. Cows were maintained in metabolism stalls at the University's Large Animal Research and Teaching Unit. Total mixed diets were formulated using the Cornell Net Carbohydrate and Protein System (Fox et al. 1992) to meet or exceed predicted requirements (NRC 1989). Ingredients and chemical composition of the diet are presented in Table 1. Cows were fed for ad libitum intake with fresh feed being offered twice daily. The amount of feed consumed was measured daily and water was available at all times.

Cows were milked at 0600 and 1800 h each day. At each milking, yield was recorded and milk was sampled. One aliquot of milk was stored at 4°C with a preservative (Bronopol tablet; D&F Control System, San Ramon, CA) until analyzed by infrared analysis for fat and protein content (Northeast DHI, Ithaca, NY). A second aliquot without preservative was stored at -20°C until fatty acid analysis.

Treatments were infused into the abomasum. This is a convenient

experimental method to simulate dietary supply of compounds while avoiding possible alterations by rumen bacteria. The abomasum was accessed by passing a polyvinyl chloride tube (0.5-cm i.d.) through the rumen fistula, rumen compartments and sulcus omasi, and into the abomasum as described previously (Spires et al. 1975).

Experiment 1. The three cows averaged 152 ± 25 d postpartum (mean \pm SD) at the start of the study. The 11-d experiment consisted of a pretreatment period (d 1-3), a treatment period (d 4-6), and a post-treatment period (d 7-11). Skim milk (vehicle) was infused abomasally during the pre- and post-treatment periods. During the treatment period, a *trans* fatty acid emulsion in skim milk was used. A mixture containing equal amounts of *trans*-11 and *trans*-12 octadecenoic acids (Lot #7363:10; Larodan Fine Chemicals, Malmö, Sweden) was used due to availability and cost. Company specifications indicated that the *trans*-11 and *trans*-12 18:1 were in equal ratio and comprised >99% of the fatty acids in the chemical mixture; this was confirmed by our own analysis. The *trans*-18:1 mixture was added to heated skim milk and an emulsion prepared using a microfluidizer as described by Chouinard et al. (1999). The final concentration of the *trans*-octadecenoic acid mixture was 0.5% in the skim milk emulsion.

Infusions used a peristaltic pump (Harvard Apparatus, South Natick, MA) calibrated to infuse continuously at a rate of 5 kg/d. This resulted in a delivery rate of 25.0 g/d of the *trans*-18:1 mixture during the treatment period. Sanitized carboys served as reservoirs for infusates, and were changed every 12 h.

Experiment 2. Three cows, 144 ± 94 d postpartum, were randomly assigned to a 3×3 Latin square design. Treatments were administered by abomasal infusion and included the following: 1) control (200 mL water/d), 2) retinol palmitate (4.8 g/d) and 3) sterculic oil (10 g/d). Equal volumes of the infusates were administered at 6-h intervals for 4 d with a 7-d interval between infusion periods.

For the retinol palmitate treatment, infusions were prepared as a suspension in water. Retinol palmitate (825,000 retinol equivalents/g) was obtained from Sigma-Aldrich (St. Louis, MO), and the final suspensions contained 24 g/L retinol palmitate. Cows were infused 4 times/d with 50 mL/infusion, resulting in a daily dosage of 4.8 g of retinol palmitate.

Sterculic oil was extracted from the seeds of the *Sterculia foetida* tree. Seeds were dehulled, crushed and the meats refluxed in diethyl ether to extract the oil (method 963.15; AOAC 1998). The yield of extracted oil was 49.6% of the seed meat by weight. The sterculic oil was prepared for abomasal infusion by making a 2% emulsion in skim milk as described for Experiment 1. Emulsions were stored at 4°C until infused, with fresh emulsions prepared for each treatment period. Cows receiving the sterculic oil treatment were infused with an equal amount 4 times/d. The daily dose averaged 9.7 g of sterculic oil and 468 mL of emulsion.

Fatty acid analysis. Lipid extraction of milk fat was performed according to Hara and Radin (1978). Methyl esters of the fatty acids were prepared by transesterification with sodium methoxide according to the method of Christie (1982) as detailed by Chouinard et al. (1999).

Fatty acid methyl esters were quantified by gas chromatography techniques. Two methods were used to allow complete separation of *trans*-11 and *trans*-12 octadecenoic acids and their respective desaturase products. One method used a CP-Sil 88 column (cyanopropyl polysiloxane; 100 m \times 0.25 mm i.d. with 0.20- μ m film thickness; Chrompack, Middlebury, The Netherlands) with two temperature-programmed gas chromatography runs. The first involved a temperature gradient program (70-240°C) and the second was an isothermal run at 160°C as described by Griinari et al. (1998). This method separated *trans*-11 and *trans*-12 octadecenoic acids into single-component peaks. The second method used a Supelcowax-10 column (fused silica, 60 m \times 0.32 mm i.d. with 0.25- μ m film thickness; Supelco, Bellefonte, PA) as described by Chouinard et al. (1999). This method provided data for *cis*-9, *trans*-12 octadecadienoic acid concentration, the general fatty acid composition of milk fat and the fatty acid composition of sterculic oil.

For both gas chromatography methods, fatty acids were identified using pure standards (Nu-Chek-Prep, Elysian, MN). A butter reference standard (CRM 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to

TABLE 1

Ingredient and chemical composition of experimental diets

Composition	Experiment 1	Experiment 2
Ingredient	g/100 g dry matter	
Chopped alfalfa hay	47.0	45.1
Cracked corn	31.0	22.8
Soybean meal	12.6	7.0
Extruded soybeans	—	8.4
Soy plus	4.6	—
Citrus pulp	—	9.9
Whole cottonseed	—	4.4
Beet pulp	2.3	—
Mineral-vitamin supplement ¹	2.5	2.4
Chemical analysis ²		
Crude protein	17.9	16.5
Crude fat	2.9	4.7
NDF ³	29.5	41.1
	MJ/kg dry matter	
NE _L ⁴	4.36	6.52

¹ Contained 385 g NaCl and 615 g of a trace mineral and vitamin mix per kg of supplement. Trace mineral and vitamin mix contained (g/kg 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.

² Analyses were by Northeast DHI (Ithaca, NY).

³ Neutral detergent fiber.

⁴ Net energy for lactation.

determine recoveries and correction factors for individual fatty acids in milk fat.

Statistical analysis. Data from Experiment 1 were analyzed using the general linear models procedure of SAS (1989) according to the following model:

$$Y_{ij} = \mu + T_i + C_j + E_{ij}$$

where Y_{ij} is the observation, μ is the overall mean, T_i is the treatment ($i = 1$ and 2), C_j is the cow ($j = 1, 2$ and 3) and E_{ij} is the residual error. Data from d 1 through 3 plus d 9 through 11 of the experimental period constituted the control values and data from d 6 (d 3 of treatment infusion) represented treatment values.

For Experiment 2, data were analyzed as a 3×3 Latin square design using the PROC MIXED procedure of SAS (1989) according to the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + E_{ijk}$$

where Y_{ijk} is the observation, μ is the overall mean, T_i is the treatment ($i = 1, 2$ and 3), P_j is the period ($j = 1, 2$ and 3), C_k is the cow ($k = 1, 2$ and 3) and E_{ijk} is the residual error. Data from d 3 and 4 from each treatment period were used in the analysis.

RESULTS

Experiment 1. Our initial experiment infused *trans*-11 18:1 abomasally to examine the potential for endogenous synthesis of CLA by Δ^9 -desaturase. Due to availability and cost we used a 50:50 mixture of *trans*-11 18:1 and *trans*-12 18:1. The Δ^9 -desaturase could catalyze the formation of *cis*-9, *trans*-11 CLA and *cis*-9, *trans*-12 18:2 from *trans*-11 18:1 and *trans*-12 18:1, respectively. Cows maintained constant feed intake during the 11 d of the study (data not presented). The yields of milk and milk fat were also relatively constant throughout the study (Fig. 1). However, there were alterations in the pattern of milk fatty acids over the treatment period. Abomasal infusion of the mixture of *trans*-11 and *trans*-12 octadecenoic acids resulted in the appearance of these fatty acids in milk fat (Fig. 2). In addition, the respective *cis*-9, *trans*-*n* octadecadienoic acids formed from *trans*-11 and *trans*-12 octadecenoic acids by the action of Δ^9 -desaturase

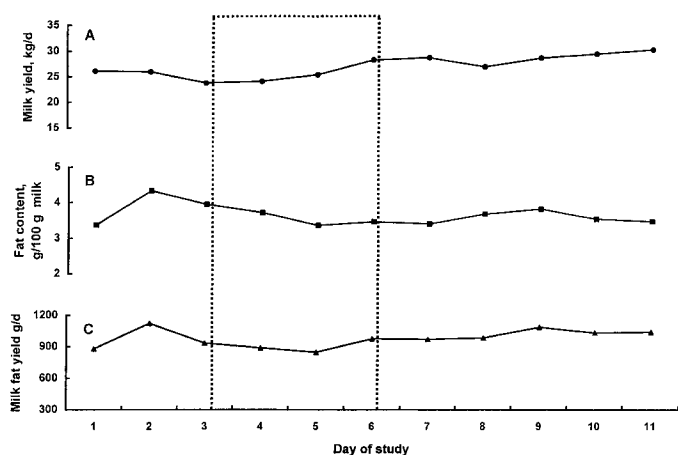


FIGURE 1 Temporal patterns for milk yield (Panel A), milk fat content (Panel B) and milk fat yield (Panel C) in lactating dairy cows receiving abomasal infusion of *trans*-11 and *trans*-12 18:1. The treatment period (indicated by dotted line) involved a 3-d abomasal infusion of *trans*-11 and *trans*-12 18:1 emulsified in skim milk. Vehicle was infused abomasally for the 3-d pretreatment and 5-d post-treatment periods. Values represent mean for 3 cows; the pooled SEM was 0.8 kg/d for milk yield, 0.08% for milk fat content and 26 g/d for milk fat yield.

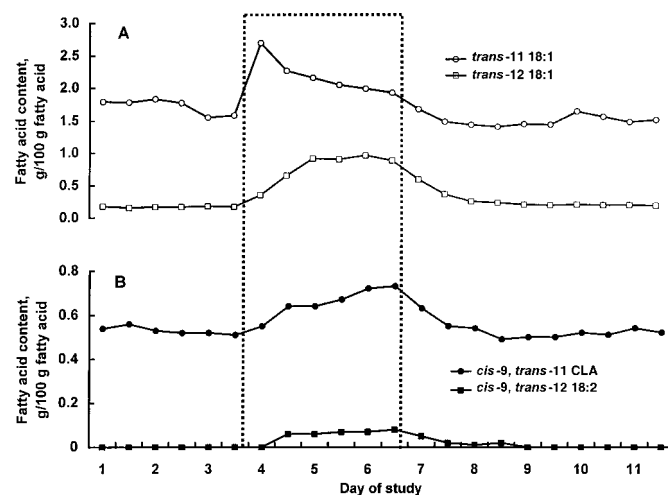


FIGURE 2 Temporal pattern of *trans*-11 and *trans*-12 18:1 (Panel A) and their Δ^9 -desaturated dienes (Panel B) in lactating dairy cows receiving abomasal infusion of *trans*-11 and *trans*-12 18:1. The treatment period (indicated by dotted lines) involved a 3-d abomasal infusion of *trans*-11 and *trans*-12 18:1 (25 g/d) that commenced on d 4 and continued through d 6. Vehicle was infused abomasally during the pretreatment and post-treatment periods. Values represent mean for 3 cows; the pooled SEM was 0.12 g/100 g fatty acid for *trans*-11 18:1, 0.03 g/100 g fatty acid for *trans*-12 18:1, 0.02 g/100 g fatty acid for *cis*-9, *trans*-11 CLA and 0.01 g/100 g for *cis*-9, *trans*-12 18:2. The analytical methods involved the use of pure standards to identify these individual fatty acid isomers.

were increased in milk fat. The *trans*-11 18:1 and *cis*-9, *trans*-11 18:2 had not reached constant concentrations in milk fat by d 3 of the fatty acid infusion. In contrast, the increase in *trans*-12 18:1 and its desaturase product (*cis*-9, *trans*-12 18:2) approached maximum concentrations in milk fat over the first 36 h, and these were maintained for the remaining 36 h of the abomasal infusion.

Estimates of *trans*-isomer recovery in milk fat components are preliminary because steady-state concentrations cannot be ensured during short-term infusions. This was especially evident for *trans*-11 18:1 and its desaturase product, *cis*-9, *trans*-11 CLA (Fig. 2). Using the mean of the pretreatment period plus the last 3 d of the post-treatment period for the baseline concentration, we made comparisons with d 3 of the fatty acid infusion period. Changes in milk fat yields of *trans*-11 18:1 and *cis*-9, *trans*-11 CLA accounted for $40 \pm 8\%$ (mean \pm SEM) of the abomasally infused *trans*-11 18:1, of which $31 \pm 9\%$ was due to an increase in *cis*-9, *trans*-11 CLA. An average of $64 \pm 9\%$ of the abomasally infused *trans*-12 18:1 was accounted for by milk fat changes in yields of *trans*-12 18:1 and *cis*-9, *trans*-12 18:2, but in this case only $10 \pm 2\%$ of the incorporation was due to the increase in *cis*-9, *trans*-12 18:2 content of milk fat. Thus, slightly more than one half of the abomasally infused *trans* 18:1 fatty acids were accounted for by increases in related fatty acids in milk; in the case of *trans*-11 18:1, however, a substantially greater portion was represented by an increase in the specific fatty acid formed by the action of Δ^9 -desaturase.

Experiment 2. To evaluate the quantitative importance of endogenous synthesis of CLA, we infused retinol palmitate and stercorolic oil abomasally. In this case, we utilized a diet containing extruded full-fat soybeans (Table 1); this type of diet results in increased milk fat concentrations of *trans*-11 18:1 and CLA (Chouinard et al. 1997, Dhiman et al. 1999). Dry matter intake and milk yield were not influenced by treatments (Table 2).

TABLE 2

Performance of lactating dairy cows during abomasal infusion of retinol palmitate or sterculic oil

Variable	Treatment ¹			SEM	P
	Control	Retinol palmitate	Sterculic oil		
Dry matter intake, kg/d	23.8	24.3	24.3	0.4	0.64
Milk yield, kg/d	35.2	34.9	34.9	0.4	0.85
Fat					
%	3.49	3.19	3.42	0.09	0.08
kg/d	1.22 ^a	1.10 ^b	1.18 ^a	0.03	0.03
Protein					
%	2.82 ^a	2.72 ^b	2.77 ^a	0.02	0.02
kg/d	0.97 ^a	0.92 ^b	0.95 ^a	0.01	0.01

¹ Treatments involved a 4-d abomasal infusion of water (control), retinol palmitate (4.8 g/d) or sterculic oil (9.7 g/d). Values ($n = 3$ cows) represent means of d 3 and 4 of infusion. Values in a row with different superscripts differ, $P < 0.05$.

Infusion with retinol palmitate resulted in minor decreases in milk content and yield of fat and protein (Table 2). Retinol palmitate had minimal effects on fatty acid composition of milk, although concentrations of CLA and palmitic acid were increased slightly (Table 3). However, yield of CLA was not affected, and the activity of Δ^9 -desaturase appeared unaltered on the basis of the constant ratios of relevant saturated fatty acids and their Δ^9 -desaturase products (Fig. 3).

Infusion of sterculic oil did not alter the content or yield of

milk components, but distinct changes in the fatty acid composition of milk occurred. Consistent with an inhibition of Δ^9 -desaturase, the ratios of fatty acid pairs dependent on this enzyme were altered. Milk fat ratios of 14:0 to 14:1, 16:0 to 16:1 and 18:0 to *cis*-9 18:1 were increased two- to threefold by treatment with sterculic oil (Fig. 3).

Infusion of sterculic oil also altered the relationship between *trans*-11 18:1 and *cis*-9, *trans*-11 CLA as shown by the temporal pattern over the infusion period (Fig. 4). By d 4 of

TABLE 3

Composition of milk fat during abomasal infusion of retinol palmitate and sterculic oil in lactating dairy cows

Fatty acid	Treatment ¹			SEM	P
	Control	Retinol palmitate	Sterculic oil		
	g/100 g fatty acids				
4:0	5.38 ^{ab}	5.05 ^b	5.61 ^a	0.12	0.02
6:0	2.54	2.53	2.60	0.03	0.24
8:0	1.31	1.33	1.29	0.02	0.34
10:0	2.49	2.66	2.66	0.06	0.14
12:0	2.63	2.84	2.72	0.07	0.13
14:0	9.10 ^c	9.83 ^b	10.49 ^a	0.22	0.004
14:1 ²	0.72 ^a	0.79 ^a	0.22 ^b	0.04	0.001
15:0	0.88 ^b	0.95 ^a	0.98 ^a	0.02	0.004
16:0	27.74 ^b	29.80 ^a	30.51 ^a	0.43	0.002
16:1 ³	1.24 ^a	1.25 ^a	0.48 ^b	0.07	0.001
17:0	0.52	0.51	0.51	0.02	0.96
18:0	12.67 ^b	11.40 ^b	19.08 ^a	0.47	0.001
18:1, <i>cis</i> ⁴	22.06 ^a	20.44 ^a	12.25 ^b	0.66	0.001
18:1, <i>trans</i> ⁵	3.64 ^b	3.54 ^b	4.09 ^a	0.10	0.007
CLA ⁶	0.42 ^b	0.50 ^a	0.25 ^c	0.03	0.001
18:2 ⁷	2.69	2.71	2.64	0.04	0.34
18:3 ⁸	0.39	0.43	0.41	0.01	0.10
Others	3.58 ^a	3.44 ^a	3.21 ^b	0.05	0.001

¹ Treatments involved a 4 d abomasal infusion of water (control), retinol palmitate (4.8 g/d) and sterculic oil (9.7 g/d). Values ($n = 3$ cows) represent average of d 3 and 4 of infusion. Values in a row with different superscripts differ, $P < 0.05$.

² Identification based on myristoleic acid. Procedure does not preclude the presence of other 14:1 isomers.

³ Identification based on palmitoleic acid. Procedure does not preclude the presence of other 16:1 isomers.

⁴ Identification based on oleic acid. Procedure does not preclude the presence of other 18:1 isomers.

⁵ Identification based on *trans*-11 and *trans*-12 18:1 isomers. Procedure does not preclude the presence of other *trans* isomers.

⁶ Conjugated linoleic acid. Identification based on *cis*-9, *trans*-11 CLA. Procedure allows for identification of 8/10, 9/11, 10/12 and 11/13 CLA isomers (*cis/trans* or *trans/cis*).

⁷ Identification based on linoleic acid. Procedure does not preclude the presence of other 18:2 isomers.

⁸ Identification based on linolenic acid. Procedure does not preclude the presence of other 18:3 isomers.

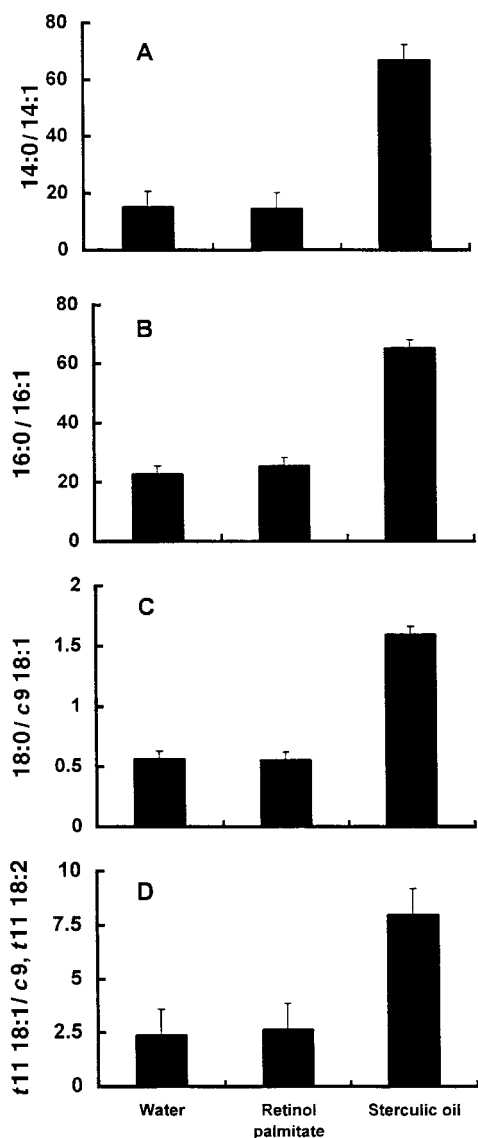


FIGURE 3 Ratio of the fatty acids to their Δ^9 -desaturated products in milk fat of lactating cows ($n = 3$) receiving abomasal infusions of water, retinol palmitate and sterculic oil. Ratios are based on fatty acid mean values (\pm SEM) of d 3 and 4 of the treatment period. The analytical methods involved the use of pure standards to identify these individual fatty acid isomers. In all cases, sterculic oil treatment differed from the other two treatments ($P < 0.001$).

infusion, the ratio of *trans*-11 18:1 to CLA was increased twofold (Fig. 3), and the concentration and yield of CLA in milk fat were reduced 45%. A similar temporal pattern was also observed for concentration changes of the other desaturase pairs, i.e., 14:0 vs. 14:1, 16:0 vs. 16:1 and 18:0 vs. *cis*-9 18:1 (data not presented).

DISCUSSION

Conjugated linoleic acid is found predominantly in food products derived from ruminants, and it has been generally assumed that the CLA was of rumen origin [see for example, Chin et al. (1992), Parodi (1997 and 1999)]. Indeed, CLA is an intermediate in the biohydrogenation of linoleic acid by rumen bacteria, and the CLA content of ruminant fats can be increased by formulating diets that contain more linoleic acid

(Dhiman et al. 1999, Kelly et al. 1998). However, kinetic studies of rumen biohydrogenation of linoleic acid to stearic acid have shown that *cis*-9, *trans*-11 CLA is a transient intermediate, whereas *trans*-11 18:1 is the intermediate that accumulates (Harfoot and Hazlewood 1988, Keeney 1970). Furthermore, dietary addition of plant oils containing α -linolenic acid also increase the CLA content of ruminant fat, and intermediates in its pathway of biohydrogenation include *trans*-11 18:1 but not CLA [see review by Griinari and Bauman (1999)]. Accordingly, we hypothesized that the CLA in ruminant tissues originated in part from endogenous synthesis.

Our initial experiment examined whether lactating cows could produce CLA from *trans*-11 18:1. Results clearly demonstrated that endogenous synthesis occurred. By d 3 of abomasal infusion of *trans*-11 18:1 (12.5 g/d), milk fat content of *cis*-9, *trans*-11 CLA had increased by 31%. The CLA concentration in milk fat had not reached a plateau by d 3 of infusion, indicating that studies of longer duration will be required to allow for definitive estimates of transfer efficiency, and these should also involve a range of *trans*-11 18:1 doses. Nevertheless, by d 3 of infusion, the increase in CLA concentration in milk fat accounted for 12% of the abomasally infused *trans*-11 18:1. The infused *trans*-octadecenoic acids consisted of a mixture of *trans*-11 18:1 and *trans*-12 18:1. Thus, *trans*-12 18:1 also provided a test for endogenous synthesis by Δ^9 -desaturase with the product of the reaction being *cis*-9, *trans*-12 18:2. We observed the appearance of *cis*-9, *trans*-12 18:2 in milk fat during the infusion period, although the amount was substantially less than observed for conversion of *trans*-11 18:1 to *cis*-9, *trans*-11 CLA (Fig. 2).

The precursor for the endogenous synthesis of CLA in ruminants would be *trans*-11 18:1, which originates in the rumen from incomplete biohydrogenation of PUFA. Several studies have demonstrated that substantial amounts of *trans*-18:1 fatty acids (60–300 g/d) reach the duodenum in lactating cows (Kalscheur et al. 1997a and 1997b, Wonsil et al. 1994). Other investigations have established that *trans* fatty acids were absorbed efficiently from the digestive tract and utilized by different ruminant tissues, including the mammary gland (Bickerstaffe et al. 1972, Thompson and Christie 1991). Methods used in the above investigations did not allow for

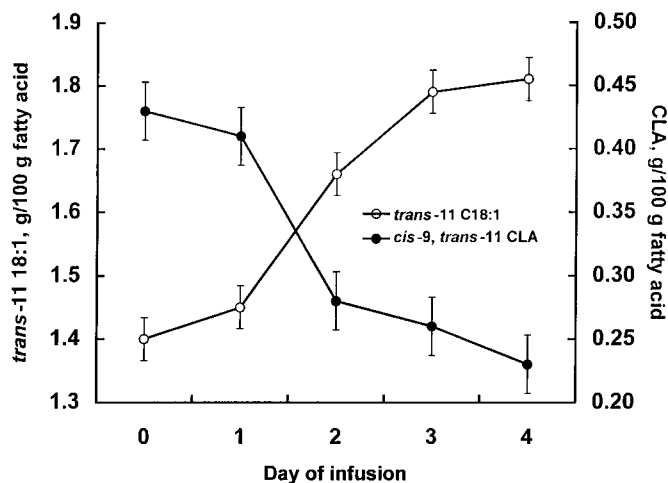


FIGURE 4 Temporal pattern of milk fat content of *trans*-11 18:1 and *cis*-9, *trans*-11 conjugated linoleic acid (CLA) in lactating cows ($n = 3$) before treatment (d 0) and during the 4 d of abomasal infusion of sterculic oil (9.7 g/d). Bars for each data point indicate SEM. The analytical methods involved the use of pure standards to identify these individual fatty acids.

separation of specific *trans*-18:1 isomers, but *trans*-11 has been shown to be the major *trans* octadecenoic acid isomer produced by rumen biohydrogenation under typical dietary conditions (Griinari and Bauman 1999).

In lactating cows, *trans*-18:1 fatty acids have been proposed to cause an inhibition of milk fat synthesis (Davis and Brown 1970, Erdman, 1996). Consistent with this, a decrease in milk fat yield occurs when partially hydrogenated vegetable oils were infused abomasally (Erdman 1996) and increases in milk fat content of *trans*-18:1 were highly correlated with reductions in the fat content of milk across a wide range of diets (Griinari et al. 1998). However, this effect appears to be related to specific *trans* isomers. We observed that abomasal infusion of 25 g/d of an equal mixture of *trans*-11 18:1 and *trans*-12 18:1 had no effect on milk fat yield or content (Fig. 1). Similarly, Rindsig and Schultz (1974) observed no reduction in milk fat when 25 g/d of *trans*-9 18:1 was infused abomasally. Other *trans*-18:1 isomers have not been examined, but we have shown that dietary-induced reductions in milk fat yield were closely related to specific increases in milk fat content of *trans*-10 18:1 and *trans*-10, *cis*-12 CLA (Griinari et al. 1998 and 1999). We further demonstrated that a dramatic reduction in milk fat secretion occurs in dairy cows with abomasal infusion of as little as 10 g/d of *trans*-10, *cis*-12 CLA, whereas infusion of *cis*-9, *trans*-11 CLA had no effect on milk fat synthesis (Baumgard et al. 2000).

The oxidative reaction catalyzed by Δ^9 -desaturase for endogenous synthesis of CLA involves cytochrome b_5 , NADH(P)-cytochrome b_5 reductase and molecular O_2 (Ntambi 1999). Palmitoyl-CoA and stearoyl-CoA are the primary substrates for the microsomal enzyme (Enoch et al. 1976), but Δ^9 -desaturase can also use the CoA esters of *trans* fatty acids, including *trans*-11 18:1 (Mahfouz et al. 1980, Pollard et al. 1980). In rodents, the enzyme was located predominantly in the liver (Ntambi 1995). In contrast, adipose tissue was the major site for Δ^9 -desaturase in growing ruminants, and mammary gland the major tissue site in lactating ruminants (Kinsella 1972, Martin et al. 1999, St. John et al. 1991, Ward et al. 1998). Studies with rodents have demonstrated that hepatic mRNA levels and enzyme activity of Δ^9 -desaturase are regulated by many factors, including physiologic state, diet and hormonal balance (Ntambi 1995 and 1999, Tocher et al. 1998). Investigations with ruminants are more limited. Martin et al. (1999) characterized the ontogeny of Δ^9 -desaturase gene expression in adipose tissue of growing cattle, and Ward et al. (1998) demonstrated that the onset of lactation in sheep resulted in a dramatic increase in mRNA for Δ^9 -desaturase in mammary tissue and a reciprocal reduction in adipose tissue.

Our second experiment evaluated the quantitative significance of endogenous synthesis in the production of CLA found in milk fat. For this objective, treatments were designed to alter tissue activity of Δ^9 -desaturase. One treatment involved administration of retinol palmitate. Administration of retinol palmitate to mice dramatically increased hepatic expression of Δ^9 -desaturase in both vitamin A-deficient and normal mice. Miller et al. (1997) demonstrated that liver desaturase mRNA levels were increased approximately three- and sevenfold in vitamin A-deficient and normal mice, respectively, when mice were fed 0.1% vitamin A in the diet. In our study, this treatment resulted in a significant increase in the concentration of CLA, but had no effect on the yield of CLA in milk fat. Overall, effects of retinol palmitate were relatively minor (Tables 2 and 3), and this treatment did not alter the milk fat ratio for any of the fatty acid pairs related to Δ^9 -desaturase activity (Fig. 3).

A second treatment involved abomasal infusion of sterculic

oil. As in the work of Kai and Pryde (1982), the sterculic oil contained 55.9% sterculic acid (8-[2-octyl-1-cyclopropenyl] octanoic acid) and 6.3% malvalic acid (7-[2-octyl-1-cyclopropenyl] heptanoic acid), fatty acids with a cyclopropene ring at the 9–10 position. These two cyclopropenoid fatty acids are very specific and highly potent inhibitors of Δ^9 -desaturase (Jeffcoat and Pollard 1977). We observed that infusion with sterculic oil resulted in decreased *cis*-9, *trans*-11 CLA concentration and a reciprocal increase in the *trans*-11 18:1 content of milk fat (Fig. 4). This clearly demonstrates the critical role of Δ^9 -desaturase as a source of the CLA in milk fat. Similar dramatic shifts were observed for the milk fat content of other fatty acid pairs that are affected by desaturase activity, i.e., 14:0:14:1, 16:0:16:1 and 18:0:*cis*-9 18:1. Thus, our experiment also confirms the important role of Δ^9 -desaturase in the production of oleic acid and provides the first evidence that this enzyme reaction is a major source of the myristoleic acid and palmitoleic acid found in milk fat.

Sterculic oil has been used previously to inhibit Δ^9 -desaturase, generally to study the role of this enzyme in the conversion of stearic acid to oleic acid. Previous investigations have included rodents, chickens and other species [see for example, Fan et al. (1982) and Phelps et al. (1965)]. Investigations have also included lactating goats and cows; these single-animal studies have reported increases in the 18:0:18:1 ratio in milk fat when sterculic oil was given by abomasal infusion (Bickerstaffe and Johnson 1972, Porter 1984) or by dietary addition of a rumen-protected form (Cook et al. 1976). Our specific interest was to evaluate the importance of endogenous synthesis of CLA, and we observed a 45% reduction in the milk fat content with the sterculic oil treatment. Thus, under the dietary conditions of the present experiment, a minimum of one half of the CLA in milk fat was of endogenous origin involving Δ^9 -desaturase.

The above estimate is a minimum based on the assumption that the sterculic oil dose inhibited Δ^9 -desaturase completely. Complete inhibition is unlikely, but the extent of Δ^9 -desaturase inhibition can be evaluated by comparing results from other fatty acid pairs that represent substrate:product ratios for the enzyme. A portion of the palmitoleic acid and oleic acid in milk fat could originate from mammary gland uptake of these fatty acids. However, comparison of 14:0 with 14:1 is ideal because 14:0 originates from mammary gland synthesis, and essentially the only source for myristoleic acid in milk fat is desaturation by Δ^9 -desaturase. During the sterculic oil treatment, the secretion of 14:1 in milk fat was reduced to 30% of the control period, indicating that inhibition of Δ^9 -desaturase was ~70%. Applying this adjustment to the relationship between *trans*-11 18:1 and CLA gives an estimate that ~64% of the CLA in milk fat originated via Δ^9 -desaturase. This is a maximum estimate, which assumes that all of the 14:1 is *cis*-9 14:1 that originates from endogenous synthesis. In addition, the kinetics for sterculic acid and malvalic acid inhibition of Δ^9 -desaturase have not been compared for different substrates, making this a limitation in extending inhibition estimates across substrates. Nevertheless, it is clear that endogenous synthesis via Δ^9 -desaturase represents the major source of CLA in milk fat.

A close linear relationship between *trans*-11 18:1 fatty acid and CLA has been observed for milk fat in a number of studies and across a wide range of diets [see review by Griinari and Bauman (1999)]. This relationship has been generally attributed to a common source for these two fatty acids as intermediates in ruminal biohydrogenation. However, our studies demonstrate that the close relationship between *trans*-11 18:1 and CLA in milk fat is related to the formation of *cis*-9, *trans*-11 CLA from *trans*-11 18:1 via Δ^9 -desaturase. This close

relationship has also been observed over a wide range of *trans*-11 18:1 concentrations (Griinari and Bauman 1999), suggesting a high capacity for endogenous synthesis of CLA. This is an important consideration in developing feeding strategies for the production of CLA-enriched milk. The focus should be on ruminal formation of *trans*-11 18:1 rather than CLA. In practical terms, this means that the most feasible options to enhance milk fat CLA concentrations may be to feed supplements containing *trans*-11 18:1 or dietary management of rumen biohydrogenation to increase the formation of *trans*-11 18:1 (Griinari and Bauman 1999).

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