Duodenal and Milk *Trans* Octadecenoic Acid and Conjugated Linoleic Acid (CLA) Isomers Indicate that Postabsorptive Synthesis Is the Predominant Source of *cis*-9-Containing CLA in Lactating Dairy Cows

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ABSTRACT Duodenal and milk samples obtained from lactating cows in a previous study were analyzed to compare the content and isomer distribution of conjugated linoleic acids (CLA) and *trans*-18:1 fatty acids (tFA). Four diets containing either low [25 g/100 g dry matter (DM)] or high (60 g/100 g DM) forage were fed with or without 2% added buffer to four multiparous Holstein dairy cows in a 2 × 2 factorial, 4 × 4 Latin square design with 3-wk experimental periods. Duodenal flows of CLA were low (1.02–1.84 g/d), compared with that of tFA (57–120 g/d), regardless of diet. The greatest amounts of CLA and tFA, as well as the greatest proportions of *trans*-10–18:1 ($P < 0.02$), and *cis*-9, trans-11 ($P < 0.01$) and *trans*-10, *cis*-12 CLA ($P < 0.01$) were in the duodenal flow of cows fed the low forage unbuffered diet. In milk fat, tFA were increased by the low forage unbuffered diet and the *trans*-10–18:1 ($P < 0.02$) replaced *trans*-11–18:1 as the major 18:1 isomer. Milk CLA secretion (7.2–9.1 g/d) was greater ($P < 0.001$) than that in the duodenal flow with each diet. This was due to the increase in *cis*-9, trans-11–18:2 and *trans*-7, *cis*-9 CLA, resulting most likely from endogenous synthesis via Δ9-desaturation of ruminally derived tFA. For other CLA isomers, duodenal flow was always greater than milk secretion, suggesting that they essentially were produced in the rumen. J. Nutr. 132: 1235–1241, 2002.

KEY WORDS: • *conjugated linoleic acids* • *trans fatty acids* • *lactating dairy cows*

Conjugated linoleic acids (CLA)[2] and *trans* fatty acids (tFA) are formed during biohydrogenation of dietary PUFA, predominantly linoleic acid, in the rumen (1,2). Studies on the biohydrogenation of linoleic acid have shown that the process begins with isomerization mainly to a *cis*-9,*trans*-11–18:2, followed by hydrogenation to *trans*-11–18:1 (3). It has been suggested that the occurrence of the variety of 18:1 isomers in the rumen involves double-bond migration (4,5). The formations of CLA and tFA in the rumen are interrelated and can be influenced by the rate of biohydrogenation. Dietary supply of PUFA and manipulation of rumen pH can alter rumen biohydrogenation (6,7,8), resulting in an increase in CLA and tFA secretion in milk fat (9–11). Experiments with lactating cows have shown that substantial amounts of tFA can reach the duodenum and are secreted in milk fat of lactating cows (6,12), while the levels of CLA that escape rumen biohydrogenation likely are minor (4). Until recently, it was accepted that CLA present in ruminant milk and tissues originates entirely from the rumen. However, Griinari et al. (13) examined the potential for endogenous synthesis of CLA in lactating cows by measuring changes in milk fat CLA in a study involving abomasal infusion of *trans*-11–18:1. They reported an increase in the concentration of *cis*-9, *trans*-11 CLA in milk and demonstrated that an active pathway for endogenous synthesis exists via Δ9-desaturation of ruminally derived *trans* fatty acids. Previously we observed that *trans*-7, *cis*-9–18:2 was the second most abundant CLA isomer in milk fat of lactating cows and we speculated that this CLA isomer may also have originated from Δ9-desaturation of *trans*-7–18:1 (11). All these findings suggested that tissue levels of CLA may not be a simple reflection of the portions that escaped rumen biohydrogenation. To our knowledge, the amounts of CLA produced in the rumen that reach the small intestine have not been directly measured and the effects of diet on rumen biohydrogenation have been evaluated primarily by the changes observed in milk and tissue CLA content (8,11,14–17). Only a few studies (18–20) have quantified CLA content in the digestive tract of ruminants. Analysis of CLA and tFA isomers in rumen or duodenal flow could help to evaluate the postruminal availability of individual isomers and their contribution to the CLA content in milk and other tissues.

The objective of this study was to evaluate the extent to which CLA and tFA isomers in milk fat reflected the amounts found in the duodenal flow of lactating cows fed diets with two levels of forage with or without buffer.

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[2] Abbreviations used: Ag^+, silver ion; B, buffer, CLA, conjugated linoleic acids; DM, dry matter; FABE, fatty acid butyl esters; GLC, gas-liquid chromatography; HF, high forage diet; LF, low forage diet; NB, without buffer; tFA, *trans* fatty acids.

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MATERIALS AND METHODS

Milk and duodenal samples from a previous experiment (6) were analyzed in this study. Four multiparous lactating Holstein cows were fed four diets consisting of two levels of forage, high [HF; 60 g/100 g dry matter (DM)] and low (LF; 25 g/100 g DM) with (B) or without (NB) buffer (1.5% $NaHCO_3$ and 0.5% MgO) addition. The forage portion of each diet consisted of 60% corn silage and 40% alfalfa haylage, and the total mixed diets were adjusted accordingly to maintain a constant forage to concentrate ratio on a DM basis. Ingredients and nutrient composition of the diets have been described previously (6). The diets were applied in a 2 × 2 factorial arrangement of treatments, in 4 × 4 Latin square design with 3-wk experimental periods. This experiment was carried out under Protocol R-94-20 approved by the University of Maryland Institutional Animal Care and Use Committee.

Analytical procedures. Milk samples for analysis were collected during the last 3 d (six consecutive milkings) of each treatment period. Duodenal contents were obtained every 4 or 6 h during d 19–21 such that a composite of 12 samples represented sampling every 2 h over a 24-h period. The samples were frozen at −20°C, thawed, composited and freeze-dried before being ground through a 1-mm screen. The nutrient flow in the duodenum was measured using chromium oxide, mixed with the concentrate portion of the diet, fed at the rate of ~20 g/d based on previous DM intake. Methods for measurement of duodenal flow of digesta have been described previously (6). Total tFA were analyzed by gas liquid chromatography (GLC) (6). *Trans* fatty acid isomers were analyzed as butyl esters (FABE), prepared from milk fat and duodenal samples by direct transesterification (11). Total tFA butyl ester was obtained after separation of FABE on silver ion (Ag^+)- thin layer chromatography (21), using FABE of 14:0, *cis*-9–18:1, and *trans*-9–18:1 standards to locate the fractions of interest. The tFA isomers were separated on a 100-m × 0.25-mm fused silica capillary column (SP-2560; Supelco Inc., Bellefonte, PA) in a GLC system and conditions previously described (11). The individual isomers were identified using *cis* and *trans*-18:1 fatty acids with double bonds in the 6, 7, 9, 11, 12, 13, and 15 positions (Sigma Chemical Co., St Louis, MO), a *trans*-10–18:1 (synthesized by Dr. E. Lehmann, at Hamburg University, Germany and obtained by Dr. Y. Ku), and *cis* and *trans*-18:1 fractions isolated from a shortening (21). Capillary gas-liquid chromatography confirmed that at least 98% of each standard was the expected isomer.

Total CLA content and isomer distributions were determined by a combination of GLC and Ag^+-HPLC as previously described (11). Dry duodenal samples (0.5 g) with no previous lipid extraction, and milk fat samples extracted as previously described (11) were methylated overnight at room temperature, using 0.04 mol/L H_2SO_4 in methanol, essentially as described by Christie (22). The FAME were extracted with hexane and passed through a small bed of anhydrous $KHCO_3$:Na_2SO_4 (9:1) to trap any remaining H_2SO_4. The extracts were dried using N_2 and purified by thin layer chromatography (21) before HPLC analysis. Aliquots (100 μL) of each sample diluted in 900 μL hexane were used in HPLC analysis. For milk samples, typical injection volumes were 15 μL, containing 40–120 μg of FAME and for the duodenal samples 25–50 μL, containing 20–80 μg of FAME. Details on the identification and quantification of the CLA isomers by HPLC analysis were reported elsewhere (23,24).

Statistical analysis. Data were analyzed as a 2 × 2 factorial arrangement of treatments in 4 × 4 Latin Square design using General Linear Models Procedure in the Statistical Analysis System of SAS, Version 6.12 (25). The statistical model included the effect of cow, experimental period, proportion of forage, level of buffer and forage × buffer interaction. Data were reported as least-squares means. Differences were considered significant at $P < 0.05$.

RESULTS

Duodenal flows (g/d) of total CLA and isomers are presented in **Table 1**. Twelve individual isomers with double bonds in positions 12,14; 11,13; 10,12; 9,11; 8,10; and 7,9 were separated and quantified in the *trans, trans* and *cis, trans*/*trans, cis* region of the CLA profile. Traces of *cis, cis* isomers were detected during the analysis but were not included in Table 1. Total CLA were highest (1.84 g/d) in cows fed the LF + NB diet compared with both HF diets (1.07 g/d, 1.02 g/d) and the

TABLE 1

Conjugated linoleic acid (CLA) isomers in duodenal flow of lactating dairy cows fed diets that contained high (HF) or low (LF) proportions of forage with (B) or without (NB) buffer

	HF		LF		Probability[1]			
CLA isomers	NB	B	NB	B	SEM[2]	F[3]	B[3]	F × B[3]
			g/d					
cis/trans, trans/cis[4]								
c-11, t-13	0.021	0.020	0.035	0.021	0.002	0.02	0.83	0.19
t-11, c-13	0.009	0.007	0.013	0.012	0.004	0.43	0.81	0.73
t-10, c-12	0.086	0.054	0.256	0.078	0.030	0.01	0.02	0.03
c-9, t-11	0.330	0.276	0.529	0.244	0.030	0.01	0.03	0.02
t-8, c-10	0.009	0.013	0.022	0.017	0.006	0.06	0.28	0.31
t-7, c-9	0.005	0.007	0.005	0.006	0.003	0.42	0.74	0.98
Total *cis/trans, trans/cis*	0.460	0.377	0.860	0.380	0.014	0.01	0.01	0.03
trans/trans[4]								
12, 14	0.088	0.107	0.082	0.067	0.007	0.17	0.34	0.11
11, 13	0.156	0.179	0.154	0.141	0.038	0.38	0.30	0.21
10, 12	0.109	0.140	0.234	0.121	0.018	0.03	0.12	0.01
9, 11	0.200	0.130	0.391	0.291	0.006	0.01	0.24	0.19
8, 10	0.038	0.073	0.096	0.049	0.009	0.01	0.04	0.20
7, 9	0.014	0.013	0.021	0.016	0.008	0.06	0.32	0.78
Total *trans/trans*	0.605	0.641	0.978	0.685	0.036	0.001	0.02	0.06
Total CLA	1.065	1.018	1.838	1.066	0.281	0.21	0.06	0.09

1 Probability that treatments effects are not different.
2 Four observations per treatment.
3 Effect of level of forage (F), buffer (B) and forage by buffer (F × B).
4 Geometric configuration of the double bond.

LF + B diet (1.07 g/d). The *cis*-9, *trans*-11–18:2 was the predominant CLA isomer in all duodenal samples. The amount of duodenal *trans*-10, *cis*-12–18:2 was increased ($P < 0.05$) with the LF + NB diet (0.26 g/d), compared with the other three treatments (0.09, 0.05 and 0.08 g/d). Small quantities (<0.02 g/d) of *trans*-8, *cis*-10, and *trans*-7, *cis*-9–18:2 were also found in the duodenal lipid. Only traces of *cis*-8, *trans*-10 were detected (data not shown). Isomers with double bonds in positions 10, 12 and 9, 11 in the *trans, trans* and *cis, trans* region of the CLA were most abundant (76% of the total CLA) in the cows fed LF + NB diet. Regardless of the diet, 50–70% of the duodenal CLA contained the *trans, trans* double bond configuration. Compared with the other diets, the amount of duodenal *trans, trans* isomers was greater with the LF + NB diet, due mostly to the isomers with double bonds in positions 9, 11 and 10, 12.

Duodenal flows of tFA were much greater than that of CLA in cows fed each diet **(Table 2)**. Although amounts of total tFA were similar among cows fed the LF + B diet and both HF diets, tFA flow was higher ($P < 0.01$) in cows fed the LF + NB diet, reflecting an increase in the amounts of all isomers. *Trans*-11–18:1 was the predominant isomer among individual duodenal tFA with all treatments; however, the amount of *trans*-10–18:1 was significantly increased ($P < 0.02$) and approached that of the *trans*-11–18:1 in cows fed the LF + NB diet.

Amounts of CLA secreted in milk were substantially greater **(Table 3)** than in the duodenal flow (Table 1). The difference was due to the increase in *cis*-9, *trans*-11–18:2 ($P < 0.001$) and *trans*-7, *cis*-9–18:2 ($P < 0.001$) isomers, which accounted for at least 93% of the total CLA in milk of cows fed each of the four experimental diets. *Cis*-9, *trans*-11–18:2 in the duodenal flow was 4–7% and duodenal *trans*-7, *cis*-9–18:2 was ~1% of the amounts in milk. The *trans*-10, *cis*-12 CLA in milk fat was increased ($P < 0.03$) with the LF + NB diet (Table 3), reflecting the higher quantity found in the duodenum (Table 1). Milk secretion of all other individual CLA isomers, including those with *trans*, *trans* double bonds, were proportional to the duodenal flows of individual CLA isomers, yielding ratios of milk to duodenal CLA between 0.46 to 0.85.

Milk secretion of tFA isomers **(Table 4)** paralleled the amounts observed in the duodenal flow (Table 2). Total tFA tended to be highest ($P < 0.07$) in cows fed the LF + NB diet, with increased levels of all individual isomers. In contrast to the other treatments, *trans*-10–18:1 was the predominant monoene in milk fat of cows fed the LF + NB diet.

DISCUSSION

In this study the amounts of individual CLA and tFA isomers in the duodenal flow were compared to their secretion in milk fat of lactating cows. Regardless of dietary treatment, duodenal flows of CLA were considerably less than that of tFA. If we assume that all of the duodenal CLA were generated in the rumen, amounts that escaped further rumen biohydrogenation were < 2 g/d. The studies of Harfoot and Hazlewood (26) and Katz and Keeney (27) have shown that *cis*-9, *trans*-11–18:2 is rapidly metabolized, while *trans*-11–18:1 accumulates during PUFA biohydrogenation in the rumen. If the isomerase and reductases involved in biohydrogenation are linked and free CLA is not readily released (28), we would expect high ratios of *trans*-18:1 to CLA. In fact, the ratio of *trans*-18:1 to CLA (ca. 60:1) was high in the duodenal flow of cows fed all diets, indicating an overwhelming production of *trans*-monoenes in the rumen. These data were in general agreement with other studies that have reported *trans*-18:1/CLA ratios of 26 to 32 in reticulo-ruminal contents and rumen bacteria (29). Recently, Kucuk et al. (20) studied the duodenal lipids in mature ewes and demonstrated much greater duodenal flows of *trans*-11–18:1 than flows of CLA.

It has been known for some time that tFA in ruminant fats are mixtures of several individual isomers (27,30,31). More recently, ruminant milk and tissue CLA also have been shown to contain a wide variety of isomers (15,24,32). In this study, in addition to the array of tFA isomers, we quantified 12 CLA isomers in both duodenal and milk fat. As has been noted herein and by others, in general, *trans*-11–18:1 is the major tFA isomer (8,11,27,30) and *cis*-9,*trans*-11–18:2 is the predominant CLA isomer in ruminant milk and tissue fat (9,11,15,33,34). Isomers containing the *trans*-10 double bond were also found in cows fed all diets, indicating that they are normal constituents of CLA and tFA. The isomer *trans*-10, *cis*-12–18:2 has been found in rumen fluid (35), bovine milk

TABLE 2

Trans octadecenoic fatty acid (tFA) isomers in duodenal flow of lactating dairy cows fed diets that contained high (HF) or low (LF) proportions of forage with (B) or without (NB) buffer

	HF		LF		Probability[1]			
tFA isomers	NB	B	NB	B	SEM[2]	F[3]	B[3]	F × B[3]
			g/d					
Double bond position								
6–8	1.02	0.4	3.72	2.52	0.53	0.001	0.25	0.71
9	2.41	1.61	4.55	2.57	0.42	0.02	0.03	0.33
10	5.73	4.96	29.13	8.11	3.64	0.02	0.06	0.07
11	20.76	20.03	33.61	20.37	2.51	0.09	0.07	0.10
12	5.68	5.79	9.52	6.33	0.43	0.001	0.03	0.02
13 + 14	14.11	14.04	22.86	15.52	0.87	0.001	0.01	0.01
15	5.74	5.21	8.53	6.07	0.42	0.01	0.03	0.12
16	5.45	4.97	7.98	5.63	0.81	0.13	0.19	0.38
Total tFA	61	57	120	66	10	0.01	0.02	0.04

[1] Probability that treatments effects are not different.
[2] Four observations per treatment.
[3] Effect of level of forage (F), buffer (B) and forage by buffer (F × B).

TABLE 3

Conjugated linoleic acid (CLA) isomers in milk fat of lactating dairy cows fed diets that contained high (HF) or low (LF) proportions of forage with (B) or without (NB) buffer

	HF		LF		Probability[1]			
CLA isomers	NB	B	NB	B	SEM[2]	F[3]	B[3]	F × B[3]
			g/d					
cis/trans, trans/cis[4]								
c-11, t-13	0.015	0.013	0.020	0.016	0.005	0.01	0.47	0.15
t-11, c-13	0.006	0.005	0.006	0.009	0.004	0.80	0.18	0.52
t-10, c-12	0.062	0.042	0.138	0.064	0.010	0.03	0.02	0.35
c-9, t-11	7.294	6.597	7.606	5.981	0.160	0.31	0.12	0.33
t-8, c-10	0.005	0.008	0.018	0.008	0.005	0.86	0.29	0.53
t-7, c-9	0.729	0.601	0.812	0.669	0.070	0.18	0.06	0.47
Total *cis/trans, trans/cis*	8.111	7.266	8.600	6.747	0.210	0.13	0.01	0.02
trans/trans[4]								
12, 14	0.030	0.051	0.036	0.031	0.014	0.09	0.69	0.77
11, 13	0.124	0.080	0.094	0.076	0.019	0.07	0.21	0.41
10, 12	0.095	0.073	0.120	0.102	0.012	0.03	0.37	0.35
9, 11	0.168	0.112	0.262	0.191	0.020	0.11	0.14	0.51
8, 10	0.022	0.029	0.017	0.028	0.011	0.28	0.57	0.57
7, 9	0.004	0.003	0.002	0.003	0.002	0.29	0.38	0.88
Total *trans/trans*	0.443	0.348	0.531	0.431	0.11	0.41	0.14	0.91
Total CLA	8.554	7.614	9.131	7.178	0.28	0.21	0.06	0.09

[1] Probability that treatments effects are not different.
[2] Four observations per treatment.
[3] Effect of level of forage (F), buffer (B) and forage by buffer (F × B).
[4] Geometric configuration of the double bond.

and tissue fat (9,11,15) and recently in duodenal contents of mature ewes (20).

Our analyses revealed that large amounts of duodenal CLA isomers were in the *trans, trans* configuration. Because conditions used to generate methyl esters can influence the isomerization of CLA (36), we were concerned that the high *trans, trans*-CLA content in the duodenal samples might be a reflection of sample matrix, sample preparation (freeze drying and grinding) or transesterification methodology. These concerns did not seem to be a factor because methylation of lipid previously extracted with methylene chloride/methanol and methylation of lipid from a fresh duodenal sample yielded similar proportions (data not shown) of *trans, trans*-18:2 isomers (Table 1). Additionally, the milk fat samples were methylated using the same procedure, yet these samples yielded low amounts of *trans, trans* isomers (Table 3). When the amounts of *trans, trans* isomers in milk fat were compared with the same isomers found in the duodenum, the ratios obtained were similar to those observed for other minor CLA isomers. For the above reasons we contend that the high levels of *trans, trans*

TABLE 4

Trans octadecenoic fatty acid (tFA) isomers in milk fat of lactating dairy cows fed diets that contained high (HF) or low (LF) proportions of forage with (B) or without (NB) buffer

	HF		LF		Probability[1]			
tFA isomers	NB	B	NB	B	SEM[2]	F[3]	B[3]	F × B[3]
			g/d					
Double bond position								
6–8	0.18	0.25	1.43	0.20	0.05	0.03	0.04	0.02
9	1.29	1.53	2.97	1.36	0.16	0.001	0.002	0.01
10	4.10	3.37	16.71	3.73	1.68	0.02	0.01	0.02
11	10.90	10.95	14.42	9.68	0.94	0.41	0.10	0.09
12	3.92	3.92	5.15	4.10	0.23	0.05	0.13	0.13
13+14	7.45	7.36	9.40	7.72	0.36	0.04	0.11	0.15
15	2.39	2.57	2.98	2.39	0.15	0.35	0.37	0.12
16	2.85	3.01	2.93	3.18	0.20	0.69	0.52	0.80
Total tFA	33	33	56	33	5	0.07	0.07	0.06

[1] Probability that treatments effects are not different.
[2] Four observations per treatment.
[3] Effect of level of forage (F), buffer (B) and forage by buffer (F × B).

CLA found in duodenal lipids reflect amounts actually produced in the rumen.

Feeding the LF + NB diet altered the total amounts and isomer distributions of CLA and tFA in duodenal and milk lipid. This was expected because the LF/high concentrate ratio in the diet provided high dietary PUFA and lowered rumen pH (6), conditions that alter rumen biohydrogenation (6,7,8,37,38). The LF + NB diet substantially increased the *trans*-10, *cis*-12–18:2 and *trans*-10–18:1 in the duodenal flow. However, despite the low pH (5.83) in the rumen of the LF + NB-fed cows, *cis*-9,*trans*-11–18:2 was the predominant duodenal CLA and there were also large amounts of *trans*-11–18:1 in the tFA. Kepler and Tove (39) reported that optimum isomerization of linoleic acid by a partially purified linoleate isomerase occurred between pH 7.0 and 7.2; however, the enzyme preparation was active over a wider range. The results of this study suggested that this enzyme may have considerable activity at a lower pH as well. In this regard, Kim et al. (28) have observed CLA formation at pH values ranging from 5.5 to 8.5 in washed suspensions of *Butyrivibrio fibrisolvens*.

Changes in isomer distribution resulting from the LF + NB diet were alleviated by the addition of buffer (LF + B), suggesting a rumen pH effect on PUFA biohydrogenation. Russell et al. (40) have reported that changes in pH can differentially affect rumen bacteria. We speculate that the low pH in the rumen of cows fed the LF + NB diet may have influenced bacterial population(s) possessing specific enzymes for alternative biohydrogenation pathways. Bacteria capable of generating *trans*-10, *cis*-12–18:2 from linoleic acid have been reported (41) and formation of *trans*-10–18:1 has been suggested (4) to occur during further biohydrogenation of *trans*-10,*cis*-12–18:2 in the rumen. Nevertheless, in this study, both *cis*-9, *trans*-11–18:2 *and trans*-10, *cis*-12–18:2 were increased in duodenal CLA of cows fed the LF + NB diet. Thus, it appears that the low rumen pH influenced more than one biohydrogenation pathway.

As we reported previously (6), cows fed the LF + NB diet produced milk with the lowest fat percent but the reduction in milk fat was not significant ($P < 0.27$). In view of the association between *trans*-10, *cis*-12–18:2 and milk fat depression (42), the level of this isomer required to substantially reduce milk fat may be greater than was found in this study. Indeed, we observed milk fat depression (11) in lactating cows fed a diet resulting in considerably higher *trans*-10, *cis*-12 CLA in milk (0.5 g/d) compared with the level (0.14 g/d) determined in this experiment.

Except for the isomers with a *cis*-9 double bond, milk secretion of individual CLA isomers was less ($P < 0.03$) than duodenal flow, regardless of diet **(Fig. 1)**. This was also true for the individual tFA and suggests that, for most of the isomers, the amounts in milk were a reflection of the quantities of CLA and tFA present in the duodenum. These findings are consistent with studies (4,6,12 43,44) demonstrating that tFA and CLA are readily absorbed and transferred to milk fat. Nevertheless, in this study the total CLA in the duodenal flow were insufficient to account for the much higher amounts of milk CLA. For cows fed each diet, the amounts of *cis*-9, *trans*-11–18:2 and *trans*-7, *cis*-9–18:2 in milk were greater than their flows to the duodenum. Our data suggest that even if all of the *cis*-9 containing CLA available in the duodenal flow were transferred to milk, at least 93% of the *cis*-9,*trans*-11–18:2, and > 98% of *trans*-7, *cis*-9–18:2 were synthesized via desaturation of the appropriate *trans* monoenes. Conversion of dietary *trans*-11–18:1 to *cis*-9,*trans*-11–18:2 in human tissues was proposed by Parodi (45) based on the demonstration that Δ9-desaturase from rat liver microsomes can introduce a double bond at the 9 position of *trans*-11–18:1 to produce *cis*-9,*trans*-11–18:2 (46,47). The ability of microsomes isolated from bovine mammary tissue to convert stearoyl-CoA to oleic acid in the presence of an active Δ9-desaturase was reported earlier by Kinsella (48). More recently Griinari et al. (13) and Corl et al. (49), using abomasal infusion of sterculic oil to inhibit Δ9-desaturation, provided evidence that Δ9-desaturase is involved in the conversion of *trans*-11–18:1 to *cis*-9,*trans*-11–18:2 and can account for the higher than expected amounts of this CLA isomer in milk fat of lactating cows.

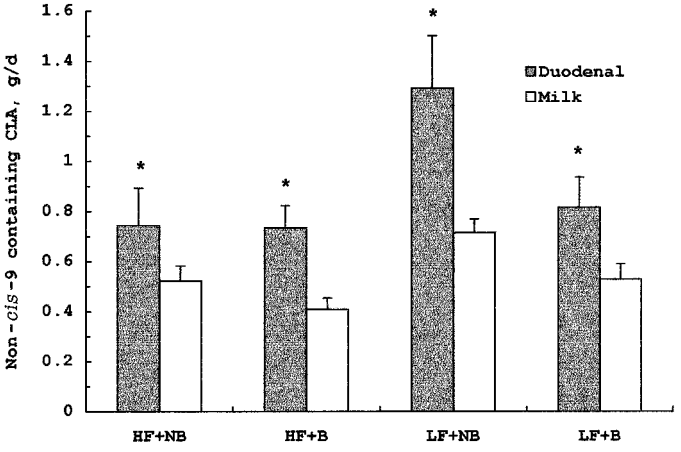

FIGURE 1 Total conjugated linoleic acid (CLA) isomers which do not contain a *cis*-9 double bond, in duodenal flow and milk fat of lactating dairy cows fed high (HF) or low (LF) forage diet with (B) or without (NB) buffer. Amounts of non-*cis*-9-containing CLA isomers in the duodenal flow were sufficient to account for those secreted in milk. Values are means ± SEM, $n = 4$. *$P < 0.03$ vs. milk fat.

We were interested in establishing whether the *trans*-7, *cis*-9 CLA could have been derived from the rumen. Indeed, the *trans*-7, *cis*-9 CLA was present in the duodenal lipid; however, regardless of diet the amounts of this isomer were minimal in relation to milk secretion. In contrast, *trans*-7, *cis*-9 CLA was the second most abundant isomer in milk fat and comprised 8–9% of the total CLA in cows fed each diet. Desaturation of *trans*-7–18:1 has been reported in microsomal preparations of rat liver (46) and by analogy to the formation of *cis*-9,*trans*-11–18:2, most of the *trans*-7, *cis*-9 CLA in milk may have been produced by the action of Δ9-desaturase on *trans*-7–18:1 in bovine tissue.

As has been suggested by others (29,49), the endogenous synthesis of CLA would depend on the supply of ruminally derived *trans*-18:1 fatty acid. In the case of the *cis*-9, *trans*-11–18:2, this does not seem to be a problem because the amounts of *trans*-11–18:1 presented to the duodenum were more than sufficient to account for the amounts of *cis*-9,*trans*-11 CLA observed in milk fat. If rumen biohydrogenation is the only means by which the *trans*-11 double bond is produced, then the sum of these two isomers in the duodenum must exceed the total of *cis*-9,*trans*-11–18:2 and *trans*-11–18:1 in the milk. This in fact was the case for cows fed each diet (**Fig. 2**) and confirmed that in addition to the duodenal *cis*-9,*trans*-11–18:2, sufficient *trans*-11–18:1 was present in the duodenum as a precursor for tissue synthesis of *cis*-9,*trans*-11 CLA via Δ9-desaturation. However, the availability of *trans*-7–18:1 was less clear, because the procedure used to quantify tFA isomers did not distinguish among the *trans*-6, *trans*-7, and *trans*-8–18:l isomers. Nevertheless, Katz and Keeney, (27) have demonstrated that the *trans*-7–18:1 isomer is produced in the rumen, and Parodi (30) has quantified this isomer in milk fat, using

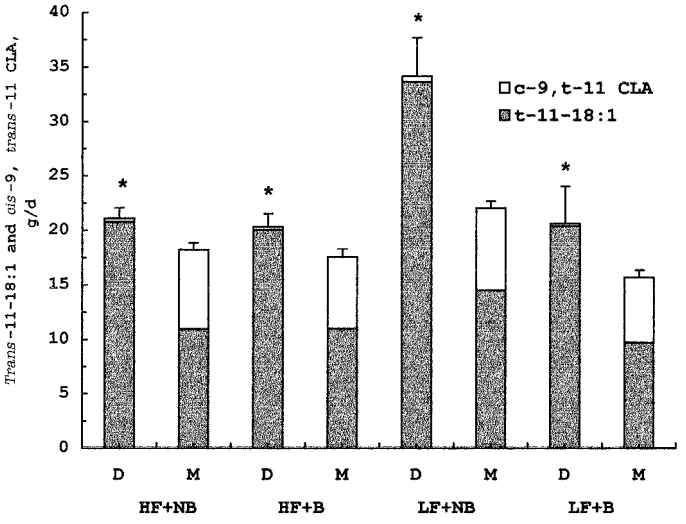

FIGURE 2 Total *trans*-11–18:1 and *cis*-9, *trans*-11 conjugated linoleic acid (CLA) isomers in duodenal flow (D) and milk fat (M) of lactating dairy cows fed high (HF) or low (LF) forage diet with (B) or without (NB) buffer. Sufficient *trans*-11-containing fatty acids were present in the duodenum to provide milk *trans*-11–18:1 and *cis*-9, *trans*-11 CLA. Values are means ± SEM, $n = 4$. *$P < 0.04$ vs. milk fat.

reductive ozonolysis. We contend that the increase in the *trans*-7, *cis*-9 CLA found in milk, compared to that in the duodenal lipid, is evidence for the postabsorptive use of *trans*-7–18:1 as a precursor for tissue synthesis of the *trans*-7, *cis*-9–18:2.

In summary the LF high concentrate diet, fed to lactating dairy cows, increased duodenal flow and milk secretion of CLA and tFA. The high ratios of tFA to CLA in the duodenal lipid of all cows suggested that minimal amounts of CLA, compared with tFA, escaped further biohydrogenation in the rumen. Regardless of the diet, the amounts of duodenal CLA were insufficient to account for the much higher quantities of CLA in milk. The results showed that the contribution of ruminally derived CLA to milk CLA content was minor compared with that synthesized endogenously, since at least 93% of the *cis*-9,*trans*-11–18:2 and almost the entire *trans*-7, *cis*-9 CLA were produced postruminally. Therefore, the principal source of milk CLA would be the postabsorptive synthesis via Δ9-desaturation of tFA produced during rumen biohydrogenation of dietary PUFA.

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