cis-9, *trans*-11 Conjugated Linoleic Acid Is Synthesized Directly from Vaccenic Acid in Lactating Dairy Cattle[1,2]

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ABSTRACT The utilization of ^{13}C-labeled vaccenic acid (VA) by lactating dairy cows to synthesize *cis*-9, *trans*-11 conjugated linoleic acid (CLA) was investigated. Primiparous ruminally cannulated Holstein cows ($n = 3$) were abomasally infused with 1.5 g of VA-1-^{13}C. Blood and milk samples were taken frequently before and after VA infusion. Milk and plasma lipid were extracted using chloroform:methanol. Plasma lipid was separated into triacylglycerol (TG), cholesterol ester (CE), phospholipid (PL), nonesterified fatty acid (NEFA), and mono- and diacylglycerol (MDG) fractions. Lipid was methylated, converted to dimethyl disulfide and Diels-Alder adducts, and analyzed by GC-MS. Increased enrichment of ^{13}C was determined using a 2-sample *t* test for each sample time compared with −24 h, with significance declared at $P < 0.05$. Enrichment in milk fat VA was detected at 4 (3.0%), 8 (8.3%), 12 (4.1%), 16 (2.2%), and 20 h (0.8%). Enrichment in VA was also detected in plasma TG, NEFA, PL, and MDG. Enrichment in milk fat *cis*-9, *trans*-11 CLA, the Δ9-desaturase product of VA, was detected at 4 (2.6%), 8 (6.6%), 12 (3.4%), 16 (1.7%), and 24 h (0.7%). Enrichment was not detected in *cis*-9, *trans*-11 CLA for any plasma lipid fraction. Modeling of the data showed the exponential decay in ^{13}C enrichment over time for both VA and *cis*-9, *trans*-11 CLA in milk fat. Conversion of dietary VA to *cis*-9, *trans*-11 CLA endogenously was confirmed with the mammary gland being the primary site of Δ9-desaturase activity; ~80% of milk fat *cis*-9, *trans*-11 CLA originated from VA. J. Nutr. 136: 570–575, 2006.

KEY WORDS: • *vaccenic acid* • cis-*9*, trans-*11 conjugated linoleic acid* • *desaturase* • *milk fat*

Ruminant products are an important source of conjugated linoleic acids (CLA)[4] in the human diet (1). Specific isomers of CLA affect various biological processes in humans and other animals. For example, the most abundant CLA isomer found in milk fat, *cis*-9, *trans*-11 (2), is potentially beneficial to human health (3). There are two sources for *cis*-9, *trans*-11 CLA synthesis in ruminant animals: *1*) the rumen via incomplete biohydrogenation of linoleic acid, and 2) desaturation of *trans*-11 18:1 (vaccenic acid; VA) by the Δ9-desaturase enzyme in animal tissues (4). Synthesis via the Δ9-desaturase enzyme was shown to be the primary source of *cis*-9, *trans*-11 CLA in milk fat (5). Additionally, the desaturation of VA to *cis*-9, *trans*-11 CLA was also shown to occur in humans (6) and rodents (7,8).

The majority of research examining the desaturation of VA to *cis*-9, *trans*-11 CLA has not used chemical tracers to establish conversion (5–8). In some instances (5,7,9), the Δ9-desaturase enzyme was chemically inhibited. Other investigations utilized quantification of the duodenal flow of VA and *cis*-9, *trans*-11 CLA to estimate the endogenous synthesis of *cis*-9, *trans*-11 CLA in milk (10,11). However, in one experiment (12) consisting of one human male subject, tracer methodology was employed to allow for a direct measurement of the conversion of VA to *cis*-9, *trans*-11 CLA in vivo.

Currently, there is limited information concerning the modification of individual dietary fatty acids in vivo. The objective of this experiment was to utilize ^{13}C-labeled VA to determine the endogenous synthesis of *cis*-9, *trans*-11 CLA from VA by the Δ9-desaturase enzyme in lactating dairy cows. The majority of *cis*-9, *trans*-11 CLA was hypothesized to be made by the Δ9-desaturase enzyme from VA, with the synthesis of *cis*-9, *trans*-11 CLA occurring in the mammary gland.

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[4] Abbreviations used: C, cholesterol; CE, cholesterol ester; CLA, conjugated linoleic acid; DMDS, dimethyl disulfide; E, enrichment; FAME, fatty acid methyl ester; MDG, mono- and diacylglycerols; MSD, mass selective detector; MTAD, 4-methyl-1,2,4-triazoline-3,5-dione; NEFA, nonesterified fatty acids; PL, phospholipid; TG, triacylglycerol; TTR, tracer:tracee ratio; VA, vaccenic acid.

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MATERIALS AND METHODS

Animals, treatment, and sampling. The University of Idaho Animal Care and Use Committee preapproved all of the procedures involving cows. Primiparous ruminally cannulated Holstein cows (n = 3; a mean of 371 ± 54 d in milk) were housed in tie-stalls and consumed their feed and water ad libitum (**Table 1**). Daily feed intake and milk production were measured. Each cow was administered a single bolus infusion of 1.5 g of vaccenic-1-^{13}C acid (Isotec) as a free fatty acid emulsified in 400 mg xanthan gum, 800 mg soy lecithin, and 60 mL water. Infusion lines were passed through the rumen cannula and secured in the abomasum with a rubber flange. The fatty acids were delivered as a bolus infusion over 10 min. The cows were milked using a portable bucket milking machine every 6 h for 24 h before the infusion, and every 4 h for 24 h postinfusion. At 24 h postinfusion, the cows were returned to the milking herd, and milk samples were taken in the parlor every 12 h for 60 h. Blood samples were taken via a jugular catheter at each milking and 0.5, 1, 1.5, 2, 3, and 6 h after the initiation of the infusion. Heparinized blood samples (10,000 U/L) were centrifuged for 20 min at 1000 × g at 4°C, and the plasma was collected. Plasma lipid classes were quantified using enzymatic kits (L-Type TG H, Free Cholesterol E, Cholesterol E, NEFA C, and Phospholipids B, Wako Chemicals) according to manufacturer's directions for microplate analysis. Plasma and milk samples were stored at −20°C.

Fatty acid analyses. Milk and plasma lipids were extracted using chloroform:methanol (2:1) (13). The extracted plasma lipid (1 mg) was fractionated into cholesterol esters (CE), triacylglycerols (TG), nonesterified fatty acids (NEFA), mono- and diacylglycerols (MDG), and phospholipid (PL) classes according to Hamilton and Comai (14). Milk, plasma TG, and plasma MDG lipids were methylated using base-catalyzed transesterification (15) with a reaction time of 10 min. Plasma CE and PL were methylated using the same method with a 24-h reaction time. Plasma NEFA were methylated in a 2-step procedure using methanolic-HCl and sodium methoxide according to Kramer et al. (16). The fatty acid methyl esters (FAME) (<1 mg) were converted to dimethyl disulfide (DMDS) derivatives (17) and 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) derivatives (18). The FAME were analyzed on a gas chromatograph (Hewlett-Packard 6890 Series with auto injector) fitted with a flame ionization detector and a 100 m × 0.25 mm, with 0.2 μm film capillary column coated with CP-Sil 88 (Chrompack). Initially, the oven temperature was 70°C (for 3 min); it was then increased to 175°C at a rate of 3°C/min and held for 3 min. The oven temperature was then increased to 185°C at a rate of 1°C/min and held for 20 min, increased to 215°C at a rate of 3°C/min, and then increased to 230°C at a rate of 10°C/min and held for 5 min. To quantify fatty acids, response correction factors were determined by the analysis of a butter oil standard with certified values (CRM 164; European Community Bureau of Reference, Brussels).

The DMDS and MTAD were analyzed by GC-MS [Agilent Technologies 6890N GC equipped with a 30 m × 0.25 mm with 0.2 μm film (5%-phenyl)-methylpolysiloxane HP-5ms capillary column and a 5973 inert series quadrupole mass selective detector (MSD) controlled by MSD ChemStation software (D.01.02.16) in the selective ion monitoring mode]. For the DMDS samples, the oven temperature was increased from 70 to 195°C at a rate of 20°C/min after injection of sample. The oven temperature was then increased to 225°C at a rate of 1°C/min and held for 5 min. Finally, the oven temperature was increased to 290°C at a rate of 10°C/min and held for 5 min. For the MTAD samples, the oven temperature was increased from 50 to 250°C at a rate of 20°C/min after injection of sample. The oven temperature was then increased to 325°C at a rate of 2°C/min followed by an increase to 340°C at a rate of 25°C/min.

Data analysis. For VA, the tracer:tracee ratio (TTR) was calculated from analysis of the DMDS. For *cis*-9, *trans*-11 CLA, the TTR was calculated from analysis of the MTAD. Both the DMDS and MTAD derivatives of FAME produce distinctive spectral fragments that are indicative of the double bond position when analyzed by MS. The TTR was calculated from the mass abundance of the ^{12}C and ^{13}C fragments (mass fragments 245 and 246 for VA; 322 and 323 for *cis*-9, *trans*-11 CLA) using the equation TTR = $^{13}C/^{12}C$. To account for the natural levels of ^{13}C, the mean TTR of samples taken before the infusion was subtracted from the TTR of all samples. Therefore, enrichment (E) of the fatty acid with ^{13}C at each sample period was calculated as (TTR – mean TTR $_{\text{before infusion}}$) × 100. The calculated E was adjusted for spectrum skewness using the correction factor 1/[1 + (0.011)(1)] (19).

For descriptive purposes, the calculated E and fatty acid concentration for VA and *cis*-9, *trans*-11 CLA for each sample postinfusion was compared with the E and fatty acid concentration of the sample at −24 h using a 2-sample t test (PROC TTEST, SAS version 9.1, SAS Institute) with significance declared at $P < 0.05$. All postinfusion E reported are greater ($P < 0.05$) than preinfusion E at −24 h unless otherwise stated. Additionally, plasma lipid class concentrations for each sample time for 24 h postinfusion were compared with the measurement obtained at −24 h using the 2-sample t test (PROC TTEST, SAS).

The decline in E over time for VA and *cis*-9, *trans*-11 CLA in milk fat from the observed maximum enrichment was modeled using an exponential decay function of the form:

$$E(t)=A\cdot e^{[-B\cdot(t-L)]},$$

where $E(t)$ is the predicted enrichment percentage at time t (h), A is the maximum percentage enrichment, B is the rate of decay, and L is the lag term set to 8 h. Parameter estimation was accomplished using the iterative Gauss-Newton nonlinear algorithm via PROC NLIN (SAS). Adequacy of fits was determined by the significance of the parameters, the magnitude of their correlation, and examination of the underlying residual structure. The area under each curve was calculated from 8 to 24 h. The ratio of the *cis*-9, *trans*-11 CLA to VA area was used to calculate the fraction of *cis*-9, *trans*-11 CLA originating from VA.

Additionally, the total grams of VA and *cis*-9, *trans*-11 CLA at each time point for 24 h pre- and postinfusion for each cow were calculated by converting fat yield (g) to total fatty acid yield (g) using 0.94 as a conversion factor. This conversion factor was calculated on the basis of the relative contribution of lipid classes in milk (20) and the fatty acid content based on the molecular weight of the lipid species using oleic acid to represent the average fatty acid. The calculated fatty acid content for each of the lipid classes was 73% for PL (based on a weighted mean of the relative contribution of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol), 95% for TG, 91% for DG, 79% for MG, 100% for FFA, and 79% for CE based on previously summarized data (20).

Total fatty acid yield was used to calculate the total grams of VA and *cis*-9, *trans*-11 CLA in milk fat. The grams of VA at each sample time were then separated into grams of ^{13}C-containing VA or grams of ^{12}C containing VA by multiplying the grams of VA by the TTR and dividing by (1 + VA TTR). The amount of ^{12}C VA (g) was

TABLE 1

Ingredients and chemical composition of the diet

Composition	g/kg of diet dry matter[1]
Ingredient	
Alfalfa silage	175
Barley silage	56
Cottonseed, whole	87
Alfalfa hay	209
Concentrate pellet[2]	472
Chemical analysis[3]	
Crude protein	181
Neutral detergent fiber	268
Acid detergent fiber	204
Ether extract	61

[1] Diet dry matter averaged 64.1%.

[2] Concentrate pellet contained 470 g/kg steam steam-rolled corn, 207 g/kg wheat midds, 144 g/kg finely ground corn, 52 g/kg soybean meal, 52 g/kg extruded soybean meal, 21 g/kg ground barley, 26 g/kg dried molasses, 2 g/kg tallow, 15 g/kg $CaCO_3$, 6 g/kg NaCl, and 6 g/kg MgO on a dry matter basis.

[3] The calculated net energy of lactation was 7.5 MJ/kg dry matter.

determined by difference. The same calculation was also used for *cis*-9, *trans*-11 CLA. For each cow, the grams ^{13}C-containing or grams ^{12}C-containing VA or *cis*-9, *trans*-11 CLA were summed for 24-h preinfusion and 24 h postinfusion. The pre- and postinfusion values for each variable were then averaged. The mean pre- and postinfusion grams of ^{13}C-containing or grams ^{12}C-containing VA values were used to predict values for each variable for a 24-h period by multiplying the ratio of postinfusion grams of ^{13}C VA to preinfusion grams of ^{13}C VA and multiplying by the postinfusion grams ^{13}C VA. The same calculation was used for ^{12}C values. These observed and predicted values were then used (**Table 2**) to compute the fraction of *cis*-9, *trans*-11 CLA originating from VA.

The contributions of fatty acids from plasma lipids were calculated as previously described for milk lipids using the measurements obtained in this study and based on previously summarized data (22). However, the fatty acid content of PL was calculated to be 72%, due to the different distribution of PL species (22).

RESULTS

Cows consumed (mean ± SEM) 19.1 ± 1.3 kg dry matter/d and produced 24.6 ± 1.6 kg milk/d with 3.8 ± 0.2% milk fat. None of these variables were altered throughout the duration of the experiment, including milk fatty acid composition (**Table 3**). The mean concentration of VA and *cis*-9, *trans*-11 CLA in milk fat before infusion at −24 h did not differ ($P > 0.05$) from the concentration at times of increased ^{13}C enrichment (data not shown). This indicates that the bolus abomasal infusion of 1.5 g ^{13}C-VA did not alter the steady-state fatty acid concentrations in the body fatty acid pools.

Compared with the −24 h values, no ^{13}C enrichment was detected for VA or *cis*-9, *trans*-11 CLA in milk lipid before abomasal infusion of vaccenic-1-^{13}C acid, as expected (**Fig. 1**). An initial ^{13}C enrichment increase for VA (3.5%) was detected at 4 h postinfusion. Enrichment reached a maximum at 8 h (8.3%). An increased enrichment compared with −24 h was also detected at 12 h (4.1%), 16 h (2.2%), and 20 h (0.8%). Increased enrichment was not detected after 20 h postinfusion. Additionally, an initial ^{13}C enrichment increase for *cis*-9, *trans*-11 CLA (2.6%) was detected 4 h postinfusion (Fig. 1). Enrichment reached an observed maximum at 8 h (6.6%). An increased enrichment compared with –24 h was also detected at 12 h (3.4%), 16 h (1.7%), and 24 h (0.7%).

Estimates of ^{13}C enrichment decreased over time for both VA and *cis*-9, *trans*-11 CLA from the time of observed maximum enrichment. Parameter estimates for the VA and *cis*-9, *trans*-11 CLA (**Table 4**) models both showed appropriate sign and magnitude to describe the decrease in ^{13}C enrichment over time. In each case, standard errors were small relative to the estimates and the asymptotic CI for the parameters did not encompass zero, indicating that each of the specified parameters was significant. Also, the magnitude of the correlations between parameters was small (<0.9), indicating lack of redundancy and that each parameter was necessary in the model. The predicted equation followed the data well and showed the expected exponential decay in the ^{13}C enrichment over time (**Fig. 2**). Residuals were randomly and uniformly distributed about zero with no discernable pattern. Using area under the curve, the amount of *cis*-9, *trans*-11 CLA arising from VA was estimated to be 83.4 ± 6.7% for 24 h postinfusion. The calculations used to determine the percentage of *cis*-9, *trans*-11 CLA originating from VA by mass output are shown in Table 2. The conversion of VA to *cis*-9, *trans*-11 CLA in milk estimated by mass determined that 83.1 ± 2.6% of milk *cis*-9, *trans*-11 CLA came from VA for measurements taken 24 h postinfusion.

Plasma lipid classes were quantified and their relative fatty acid content determined (**Table 5**). There were no concentration differences observed over time compared with concentrations at −24 h (data not shown). The majority of fatty acids in circulation were in PL (47.2%) and CE (47.0%); TG provided only 4.5% of the total fatty acids in circulation. The NEFA class contributed only 1.3% to the total fatty acid pool. The contribution to total plasma lipid was 2.0% for TG, 63.5% for CE, 31.7% for PL, 1.7% for NEFA, and 1.0% for free cholesterol.

Fatty acid analysis of individual lipid classes detected increased ^{13}C enrichment in VA in the TG, NEFA, and PL of plasma compared with –24 h using a 2-sample *t* test procedure (**Fig. 3**). In the TG fraction, ^{13}C enrichment was detected from 1 h (21.2%) until 4 h (5.7%) postinfusion with maximum enrichment occurring at 1.5 h (53.8%). In the PL fraction, increased enrichment was not detected until 2 h (0.5%) but was then maintained until 84 h (0.3%) postinfusion

TABLE 2

Calculations to determine the percentage of cis*-9,* trans*-11* CLA in milk originating from VA in Holstein cows[1]

Variable calculated	Value	Calculation	Equation
^{13}C VA infused, *g/cow*	1.5		
^{13}C VA absorbed, *g*	1.2	1.5·0.786[2]	(1)
^{13}C VA transferred to udder, *g*	0.4 ± 0.01	[(observed g ^{13}C VA post[2]) − (predicted g ^{13}C VA post)]+ [(observed g ^{13}C CLA post) − (predicted g ^{13}C CLA post)]	(2)
VA converted to CLA in udder, *%*	25.7 ± 0.76	100·[(observed g ^{13}C CLA post) − (predicted g ^{13}C CLA post)]/(g ^{13}C VA transferred to udder)	(3)
^{13}C VA transferred to adipose or oxidized, *g*	0.8 ± 0.01	(g ^{13}C VA absorbed) − (g ^{13}C VA transferred to udder)	(4)
Total VA pre,[3] *g*	12.0 ± 0.69	(g ^{13}C VA + g ^{12}C VA)/[1 − (% conversion of VA to CLA/100)]	(5)
Total VA post,[4] *g*	14.0 ± 0.63		(5)
VA converted to CLA pre, *g*	3.1 ± 0.26	(g VA) · (% conversion of VA to CLA/100)	(6)
VA converted to CLA post, *g*	3.6 ± 0.10		(6)
Total CLA pre, *g*	3.8 ± 0.09	(g ^{13}C CLA + g ^{12}C CLA)	(7)
Total CLA post, *g*	4.3 ± 0.25		(7)
CLA from VA pre, *%*	81.2 ± 5.11	[(g VA converted to CLA)/(g CLA)]·100	(8)
CLA from VA post, *%*	83.1 ± 2.59		(8)

1 Values are means ± SEM, $n = 3$.
2 Absorption efficiency from Romo et al. (21).
3 Total-pre represents measured total amount of VA or CLA output in milk for 24 h before abomasal infusion of ^{13}C VA.
4 Total-post represents measured total amount of VA or CLA output in milk for 24 h after abomasal infusion of ^{13}C VA.

TABLE 3

Fatty acid composition of milk fat[1]

Fatty acid	*g/100 g fatty acids*
4:0	3.3 ± 0.03
6:0	1.4 ± 0.02
8:0	0.65 ± 0.02
10:0	1.4 ± 0.04
12:0	1.5 ± 0.04
14:0	6.9 ± 0.15
14:1 *cis*-9	0.69 ± 0.03
15:0	0.74 ± 0.01
16:0	25.0 ± 0.13
16:1 *cis*-9	1.5 ± 0.03
17:0	0.64 ± 0.01
18:0	12.7 ± 0.19
18:1 *trans*-5 to -8	0.56 ± 0.01
18:1 *trans*-9	0.48 ± 0.004
18:1 *trans*-10	0.63 ± 0.01
18:1 *trans*-11	1.1 ± 0.02
18:1 *cis*-9	30.7 ± 0.25
18:2 *cis*-9, *cis*-12	3.3 ± 0.07
18:2 *cis*-9, *trans*-11	0.48 ± 0.01
18:3 *cis*-9, *cis*-12, *cis*-15	0.43 ± 0.02
20:0	0.04 ± 0.01
Others	5.8 ± 0.06

[1] Values are means ± SEM, $n = 3$. Values represent a mean of samples taken −24 h to 84 h relative to infusion of 1.5 g of vaccenic-1-^{13}C acid into the abomasum of lactating dairy cows.

with maximum enrichment occurring at 8 h (2.7%). The NEFA fraction was enriched from 1.5 h (22.9%) up to 3 h (11.9%) with an observed maximum enrichment detected at 1.5 h. Increased enrichment was detected only at 3 h (5.1%) postinfusion in the MDG fraction and no enrichment was detected in the CE fraction. Unlike the enrichment of VA in plasma lipid fractions, there was no increased enrichment of ^{13}C in *cis*-9, *trans*-11 CLA at any time in any plasma lipid fraction (data not shown).

DISCUSSION

Early reports on the desaturation of saturated fatty acids in vivo directly investigated the desaturation of deuterium-labeled saturated fatty acids fed to mice (23) and the in vitro desaturation of 1-^{14}C-stearyl-CoA and 1-^{14}C-palmityl-CoA by microsomal preparations (24). Recent studies shifted the focus to the use of unlabeled fatty acids and indirect measurements (e.g., measuring reciprocal changes without using tracer methodology)

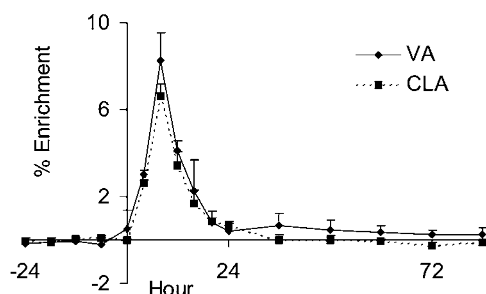

FIGURE 1 Enrichment of ^{13}C in VA and *cis*-9, *trans*-11 CLA in milk fat of lactating cows administered a bolus abomasal dose of 1.5 g vaccenic-1-^{13}C acid at time zero. Values are means ± SEM, $n = 3$.

TABLE 4

Estimated regression coefficients for the exponential models used to describe the decay in ^{13}C enrichment in vaccenic acid (VA) and cis-*9,* trans-*11 conjugated linoleic acid (CLA) in milk fat*[1]

Fatty acid	Variable	Parameter estimate	SE	95% Confidence interval Lower	Upper
VA	A	8.270	0.375	7.502	9.038
	B	0.174	0.016	0.142	0.206
CLA	A	6.589	0.193	6.193	6.984
	B	0.164	0.009	0.145	0.184

[1] Decay was calculated from 8 to 24 h after an abomasal dose of ^{13}C-VA in lactating cows ($n = 3$). Maximum percentage enrichment, A; rate of decay, B.

to document the activity of the Δ9-desaturase enzyme on VA. Using isotopically labeled fatty acids, the desaturation of VA to *cis*-9, *trans*-11 CLA was shown directly in vitro (25) and in vivo with only one human male subject (12). Indirectly, desaturation of VA to *cis*-9, *trans*-11 CLA was demonstrated in vivo in humans (6), mice (8), and cattle (5).

We demonstrated the direct conversion of VA to *cis*-9, *trans*-11 CLA in vivo using lactating dairy cattle. The abomasal infusion of a tracer dose of ^{13}C-labeled VA alleviated the need to chemically inhibit the Δ9-desaturase enzyme while allowing for a direct assessment of the Δ9-desaturase enzyme activity in the mammary gland of lactating dairy cattle, specifically on the conversion of VA to *cis*-9, *trans*-11 CLA. This approach enabled us to examine whole-animal metabolism of an individual fatty acid without potentially manipulating other fatty acids. Furthermore, the tracer dose of ^{13}C labeled VA allowed for an in vivo assay of the Δ9-desaturase enzyme activity.

Fatty acid substrates of the Δ9-desaturase enzyme in the mammary gland are contributed from the diet, mobilization of

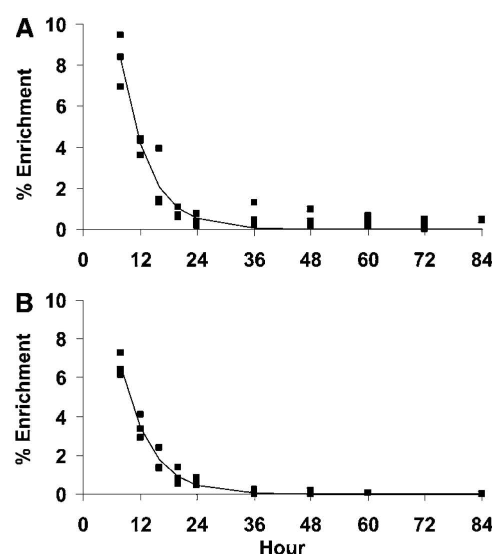

FIGURE 2 Relation between ^{13}C enrichment and time for vaccenic acid (*A*) and *cis*-9, *trans*-11 CLA (*B*) in milk fat of lactating cows ($n = 3$) administered a bolus dose of 1.5 g vaccenic-1-^{13}C acid at time zero. The chart represents the observed data points and predicted exponential regression model. *Panel A*: $E(t) = 8.270 \cdot e^{[-0.174 \cdot (t - 8)]}$; *Panel B*: $E(t) = 6.589 \cdot e^{[-0.164 \cdot (t - 8)]}$.

TABLE 5

Plasma lipid class quantification in lactating cows administered a bolus abomasal dose of 1.5 g vaccenic-1-^{13}C acid at time zero[1]

Plasma lipid	Plasma concentration	Fatty acid content in plasma	VA	CLA
	mmol/L	*mmol/L*	*g/100 g fatty acids*	
TG	0.28 ± 0.01	0.85 ± 0.03	0.7 ± 0.04	0.1 ± 0.01
PL	4.39 ± 0.08	8.82 ± 0.16	0.4 ± 0.01	0.1 ± 0.002
NEFA	0.24 ± 0.01	0.24 ± 0.01	0.4 ± 0.02	0.3 ± 0.02
CE	8.79 ± 0.20	8.78 ± 0.20	0.1 ± 0.004	0.1 ± 0.06
C	0.14 ± 0.01			

[1] Values are means ± SEM, n = 3. Samples were taken from −24 h to 84 h relative to the infusion of 1.5 g of vaccenic-1-^{13}C acid at 0 h into the abomasum of lactating dairy cows.

body fat, and synthesis in mammary tissue. In lactating dairy cattle, Griinari et al. (5) concluded that synthesis via the Δ9-desaturase enzyme is the primary source of *cis*-9, *trans*-11 CLA in the milk fat of cows fed a typical total mixed ration. By using the cyclopropene fatty acid in sterculic oil, they were able to block the activity of the Δ9-desaturase enzyme and determine that 64% of milk fat *cis*-9, *trans*-11 CLA was synthesized from VA in the mammary gland. Using similar methods, Corl et al. (26) estimated that 78% of *cis*-9, *trans*-11 CLA in milk fat came from desaturase activity on VA when cows were fed diets with or without partially hydrogenated vegetable oil. Furthermore,

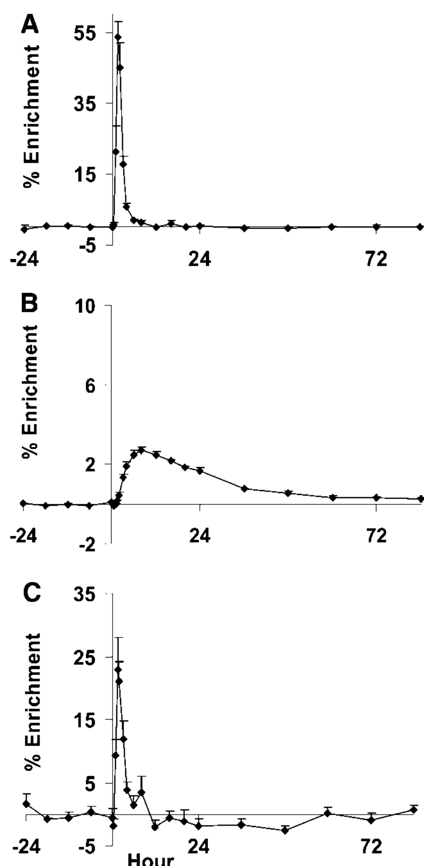

FIGURE 3 Enrichment of ^{13}C in VA in plasma TG (*A*), PL (B), and free fatty acids (*C*) of lactating cows administered a bolus abomasal dose of 1.5 g vaccenic-1-^{13}C acid at time zero. Values are means ± SEM, *n* = 3.

when pasture-fed lactating dairy cattle were treated with sterculic oil, 91% of the *cis*-9, *trans*-11 CLA in milk fat was projected to be from endogenous synthesis via the Δ9-desaturase enzyme (9). In another study with cows fed low- or high-forage diets with or without added buffer, 93% of *cis*-9, *trans*-11 CLA was estimated to originate from the desaturation of VA using the duodenal flow and milk fat contents of VA and *cis*-9, *trans*-11 CLA (10). As in the previously mentioned study (10), we also did not have to make any assumption about the extent of inhibition of the Δ9-desaturase enzyme, nor manipulate the activity of the enzyme, allowing for another approach to estimate the percentage of *cis*-9, *trans*-11 CLA originating from VA. The conversion of VA to *cis*-9, *trans*-11 CLA was calculated to be 83.1% based upon predicted output and 83.4% based upon area under the curve analysis under the experimental conditions with 3 cows. The production of 4.3 g/d of *cis*-9, *trans*-11 CLA in milk fat is most similar to that of Griinari et al. (5) where 4.9 g of *cis*-9, *trans*-11 CLA was produced each day when no oil was added to the diet. The other studies ranged from 6.9 g/d for buffer-supplemented cows (10) to 9.5 g/d for control pasture-fed cows (9). Nonetheless, these data do agree with the other estimates despite the variation of diet, animal, and overall production. This agreement on the percentage of VA converted to CLA across these conditions may indicate that the Δ9-desaturase enzyme is constant unless faced with a potent inhibitor. This constancy may contribute to its importance in the process of milk fat synthesis.

The conversion of dietary VA to *cis*-9, *trans*-11 CLA in tissues was also shown in rodents and humans. Carcass evaluation of growing mice consuming a diet high in VA showed that 11.4% of dietary VA was converted to *cis*-9, *trans*-11 CLA in the total carcass (8). Similar responses were seen in other studies in which rats or mice were fed VA (7,27,28). When nonlactating humans consumed diets high in *trans* fatty acids or VA, there was an increase in CLA in the serum (6,29). Additionally, reanalysis (12) of samples from a study originally published in 1978, showed a 30% enrichment of deuterium in *cis*-9, *trans*-11 CLA in the serum TG of one adult man who consumed deuterium-labeled VA. Unfortunately, there are no current published full-length articles evaluating the effect of the Δ9-desaturase enzyme in the mammary gland of lactating women on the conversion of VA to *cis*-9, *trans*-11 CLA. However, recent work from our laboratory published in abstract form (30) confirms the activity of the Δ9-desaturase enzyme in the mammary gland of lactating women on the conversion of VA to *cis*-9, *trans*-11 CLA.

Distribution of plasma lipid classes was similar to previously published data (22,31). However, low free cholesterol concentrations of ~1% were detected, compared with ~10% from previous data (22,31). The discrepancy may be attributed to analytical technique, stage of lactation, diet, or a variety of other factors. Limited data exist documenting the change in plasma lipids over the entire lactation. Conversely, there were no concentration changes detected over time when compared with −24 h measurements. This is in agreement with previous observations that circulating neutral lipids do not exhibit circadian patterns in lactating Holstein cows (31).

Dietary VA is rapidly incorporated into specific plasma lipid classes (Fig. 3). The short-term labeling of the VA fatty acid pool allowed for the tracking of this fatty acid into primarily TG, PL, and NEFA. Although the CE portion contains VA, there was no detection of transfer of the ^{13}C-VA throughout the short duration of the current experiment. Furthermore, by 6 h after the administration of the ^{13}C-VA, the PL fraction contained the majority of the plasma ^{13}C-VA. The sequestration of a substantial portion of VA into a lipid source

considered unavailable to the mammary gland is intriguing. Loor et al. (32) indicated that feeding unsaturated oils to lactating cows for 28 d increased plasma TG, PL, and CE while also increasing VA and *cis*-9, *trans*-11 CLA concentrations in each of these plasma fractions. Furthermore, it is suggested that at high arterial concentrations PL may provide fatty acids for milk fat synthesis (33). A long-term continuous infusion of ^{13}C-VA could provide further insight into the transfer of this fatty acid between plasma lipid pools.

The activity of the Δ9-desaturase enzyme in bovine tissues occurs primarily in mammary (34) and adipose tissue (35,36), with additional desaturation shown in liver, muscle, and intestinal mucosal microsomal preparations from Simmental cattle (37). However, no desaturase activity was detected in bovine liver from Angus and Braford cattle (35) or Charolais cattle in vitro (38). This variability in desaturase activity among tissues may be explained somewhat by dietary (37) and breed differences (39). In the current study, no ^{13}C *cis*-9, *trans*-11 CLA was detected in any plasma lipid fraction, indicating that the mammary gland was the major site for the conversion of VA to *cis*-9, *trans*-11 CLA in milk during the 24 h post-VA dose.

The abomasal infusion of VA labeled with a stable isotope (i.e., ^{13}C) enabled us to demonstrate an alternative robust method to account for the *cis*-9, *trans*-11 CLA produced from dietary VA and the transfer of VA and *cis*-9, *trans*-11 CLA from plasma to the mammary gland and into milk fat. These data confirm the magnitude of the contribution of dietary VA (~80%) to the synthesis of *cis*-9, *trans*-11 CLA in the whole animal under the specified dietary conditions. Ultimately, this information may be used to further elucidate current and future dietary manipulations that lead to increased concentrations of *cis*-9, *trans*-11 CLA and other fatty acids in ruminant products produced by the Δ9-desaturase enzyme.

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