Microbial biohydrogenation of oleic acid to *trans* isomers in vitro

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Abstract Ruminant products are significant sources of dietary *trans* fatty acids. *Trans* fatty acids, including various conjugated linoleic acid isomers, have been shown to act as metabolic modifiers of lipid metabolism. *Trans* fatty acids originate from biohydrogenation of dietary unsaturated fatty acids by gut microbes; however, the exact synthetic pathways are unclear. It was our goal to examine the biohydrogenation pathway for oleic acid, where oleic acid is hydrogenated directly to stearic acid. Our objective in this study was to trace the time course of appearance of ^{13}C in labeled oleic acid to determine if *trans* monoenes are formed from the ^{13}C-labeled oleic acid or if the ^{13}C appears only in stearic acid as described in reviews of earlier work. Enrichments were calculated from the mass abundance of ^{13}C in major fatty acid fragments and expressed as a percentage of total carbon isotopomers. Significant ^{13}C enrichment was found in stearic acid, oleic acid, *trans*-6, *trans*-7, and in all *trans* C18:1 in positions 9–16.■ We concluded that the biohydrogenation of oleic acid by mixed ruminal microbes involves the formation of several positional isomers of *trans* monoenes rather than only direct biohydrogenation to form stearic acid as previously described.— Mosley, E. E., G. L. Powell, M. B. Riley, and T. C. Jenkins. **Microbial biohydrogenation of oleic acid to *trans* isomers in vitro.** *J. Lipid Res.* 2002. **43:** 290–296.

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Meat and dairy products are significant sources of saturated fatty acids, *trans* fatty acids, and conjugated linoleic acid (CLA) for humans. In ruminants, *trans* fatty acids are formed during microbial biohydrogenation in the rumen, the largest stomach compartment of cattle. Microbial biohydrogenation is the process of converting unsaturated fatty acids to more saturated end products by gut microbes. Biohydrogenation is a unique biological process prevalent in the microbial ecosystem found within the rumen. In cows fed a typical forage diet, the major *trans* monoene isomer present in ruminal contents is *trans*-11 C18:1. Most of the remaining isomers have double bonds distributed equally among carbons 12–16 (1). The exact pathways for the synthesis of these positional isomers are not known. *Trans* C18:1 serve as precursors for the synthesis of saturated fatty acids in the rumen and of CLA at the tissue level. For example, *trans*-11 C18:1 is reduced in the rumen to form stearic acid or is desaturated by Δ^9-desaturase in the mammary tissue to produce *cis*-9, *trans*-11 C18:2, which is an abundant CLA isomer in meat and milk (2). This isomer has been shown to have antioxidant and anticarcinogenic properties (3).

Authoritative reviews on biohydrogenation (4) do not account for the formation of specific positional isomers except for *trans*-11, *cis*-15, and *trans*-15. It is accepted that linoleic and linolenic acids, typical fatty acids in ruminant diets, are converted to several monoene and diene intermediates containing *trans*-11 bonds during biohydrogenation. Oleic acid, also typical in ruminant diets, is usually described as being hydrogenated directly to stearic acid without the formation of *trans* intermediates (4). However, Ward et al. (5) observed that purified [1-^{14}C]oleic acid was rapidly converted by mixed ruminal microbes to stearic acid with a small amount (3%) of the radioactivity appearing in the *trans* monoenoic acid fraction. Furthermore, Selner and Schultz (6) observed a significant increase in the *trans* C18:1 fatty acid content of milk fat from 3% to 8% when oleic acid was fed to lactating dairy cows. These results support the possibility that *trans* C18:1 are synthesized from oleic acid by ruminal microbes.

Trans fatty acids, including various CLA isomers, are known to act as metabolic modifiers of lipid metabolism. When overweight and moderately obese humans were fed a mixture of CLA isomers, those receiving >3.4 g CLA/day had a significant reduction in body fat mass (7). Furthermore, in growing pigs the dietary intake of a mixture of CLA isomers including *trans*-10, *cis*-12 C18:2 results in decreased rates of body fat deposition (8). In hamsters, only diets containing a CLA isomer mix and not pure *cis*-9,

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trans-11 C18:2 result in lower weight gain (9). In lactating dairy cows, Griinari et al. (10) showed that dietary induced milk fat depression corresponds with increased *trans*-10 C18:1 in the milk fat. The production of *trans*-10 C18:1 has been attributed to the biohydrogenation of *trans*-10, *cis*-12 C18:2 (2). The *trans*-10, *cis*-12 C18:2 has been shown to be the primary isomer responsible for decreased milk fat synthesis (11). For example, post ruminal infusion of the *trans*-10, *cis*-12 C18:2 isomer resulted in reduced milk fat percentage and milk fat yield but infusion of the *cis*-9, *trans*-11 C18:2 isomer did not (11). The exact mechanism by which *trans* fatty acids alter fat metabolism is unknown. However, in mice *cis*-9, *trans*-11 C18:2 has been shown to have a potent effect on lipid transport and metabolism in vivo. This effect is attributed to the observation that the CLA is a high affinity ligand and activator for peroxisome proliferator-acivated receptor α (PPARα) (12).

In order to control the presence of *trans* fatty acids that act as metabolic inhibitors, the exact pathways for their synthesis must be elucidated. If production pathways are known, then ruminal biohydrogenation may be manipulated to control the formation of *trans* fatty acids. It was our goal to examine the biohydrogenation pathway for oleic acid and determine if the accepted pathway (oleic acid to stearic acid) was complete. To accomplish this we traced the time course of appearance of ^{13}C in labeled oleic acid to determine if *trans* monoenes are formed from the ^{13}C-labeled oleic acid or if the ^{13}C appears only in stearic acid as described in reviews of earlier work (4).

MATERIALS AND METHODS

Reagents

Reagent grade oleic acid (99% pure) and oleic-1-^{13}C acid (99% pure) were purchased from Aldrich Chemical Company (St. Louis, MO). All solvents were HPLC or gas chromatography (GC) grade. Dimethyl disulfide (DMDS) was purchased from Sigma-Aldrich Chemical Company. Silver nitrate crystal, anhydrous ethyl ether, iodine, and sodium thiosulfate were purchased from Fisher Scientific (Pittsburgh, PA).

Oleic-1-^{13}C acid purification

A total of approximately 160 mg of oleic-1-^{13}C acid was methylated according to Kramer et al. (13) and then separated into *cis* and *trans* monoene fractions using a modified procedure (see below) of Christie (14). The *cis* monoene fatty acid methyl esters (FAME) were converted to free acids by refluxing in 50 ml of 3N KOH in ethanol for 1 h and then titrating beyond the endpoint with 6N HCl. Thin layer chromatography was used to confirm the complete conversion of FAME to free fatty acids. Approximately 40 mg of the saponified oleic-1-^{13}C acid was transferred to a screw top glass culture tube and taken to dryness under nitrogen.

Microbial cultures

Conversion of oleic acid to *trans* monoenes was studied in cultures of mixed gut microbes taken from the stomach of cattle. Stomach contents were taken from the rumen of a Holstein cow with a surgically prepared rumen fistula and strained through two layers of cheesecloth. Cultures were maintained in 125 ml Erlenmeyer flasks containing 10 ml of the strained stomach contents mixed with 500 mg of ground hay, 40 ml of media, 300 µl

of added fat, and 2 ml of reducing solution according to Goering and Van Soest (15). The fat source for the labeled cultures was prepared by combining purified oleic-1-^{13}C acid (40 mg) with 60 mg of oleic acid in ethanol (60 mg/ml) for a final concentration of 100 mg/ml. The fat source for the unlabeled cultures consisted of oleic acid in ethanol (100 mg/ml). Cultures containing unlabeled or labeled oleic acid were run in triplicate at 39°C under anaerobic conditions. Samples (5 ml) were taken from each culture at 0, 12, 24, and 48 h and immediately frozen. The samples were freeze dried and then methylated according to Kramer et al. (13). When stored, all samples were stored in an organic solvent at −15°C.

### Solid phase extraction column separation

The FAME samples from each incubation time were taken to dryness under a stream of nitrogen gas and then dissolved in 0.4 ml of methylene chloride. The FAME were separated into saturated, *trans* monoene, *cis* monoene, and diene fractions using a modified procedure of Christie (14). The modified procedure is as follows: an Isolute® SCX-2 (International Sorbent Technology, Mid Glamorgan, UK) solid phase extraction column (500 mg, 10 ml reservoir) was wrapped to the level of the top of the absorbent bed with aluminum foil. The column was preconditioned by elution with 2 ml of acetonitrile. A solution of 20 mg of silver nitrate in 0.25 ml acetonitrile-water 10:1 (v/v) was allowed to flow through the solid phase extraction column. The column was flushed with acetonitrile (5 ml), acetone (5 ml), and methylene chloride (10 ml). The FAME sample in 0.4 ml methylene chloride was divided equally between two columns for better resolution and washed onto the column in methylene chloride (0.2 ml). Saturated fatty acids were eluted with methylene chloride (5 ml). The monoene fraction was separated into *trans* monoenes and *cis* monoenes by washing with 0.5% acetone in methylene chloride (5 ml) and 10% acetone in methylene chloride (5 ml), respectively. Dienes were eluted with acetone (5 ml). All fractions were eluted by gravity. Corresponding fractions from the two columns were combined and taken to dryness under a stream of nitrogen gas. The FAME in the saturated and diene fractions were dissolved in 100 µl of hexane and analyzed by gas chromatography-mass spectroscopy (GC-MS).

DMDS derivatization

DMDS adducts of the *trans* monoene and *cis* monoene fractions were prepared using a modified procedure of Yamamoto et al. (16). The modified procedure is as follows: the FAME fractions were treated with 0.35 ml of DMDS and 100 µl of iodine solution (6% iodine w/v in diethyl ether). The reaction mixtures were shaken in a 37°C water bath for 1 h and then diluted with diethyl ether-hexane (3 ml; 1:1, v/v). Iodine was removed by shaking with 10% sodium thiosulfate (200 µl). The organic phase was removed and the solvent was evaporated under a stream of nitrogen gas. The residue was dissolved in 100 µl of hexane and analyzed immediately by GC-MS. When stored, samples were stored no longer than 2 days in hexane at −15°C.

GC-MS

The FAME in the saturated and diene fractions were analyzed by gas chromatography with mass spectral detection (Varian 3400 gas chromatograph with Saturn II ion trap mass spectrometer, Varian Instruments Inc., Walnut Creek, CA) using a 30 m × 0.25 mm with 0.2 µm film Supelco 2380 column (Supelco, Inc., Bellefonte, PA). Column temperature was programmed from 140°C (after 3 min) to 220°C at 3°C/min and held for 5.5 min (total run time 35 min). The carrier gas was He (20 cm/s) with an injector split of 100:1. The DMDS derivatives in the *trans* and *cis* fractions were analyzed using the same GC-MS conditions
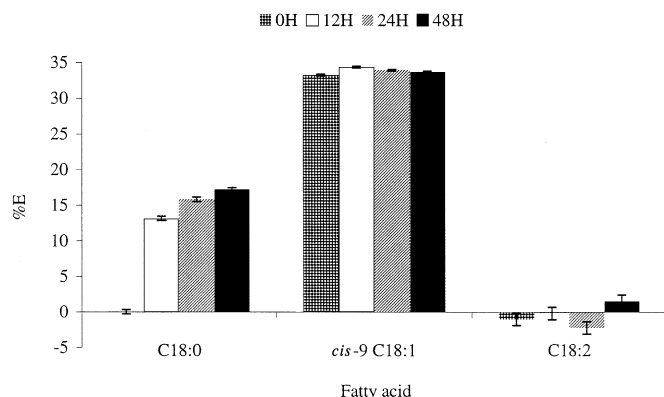
Fig. 1. The percentages of ^{13}C enrichment of oleic, stearic, and linoleic acids over time when a mixture (3:2) of oleic and oleic-1-^{13}C acids was added (30 mg per culture) to cultures of mixed ruminal microbes. Each bar is the mean of three replicates. Pooled standard errors are shown.

except that the column temperature was programmed from 160°C (after 3 min) to 190°C at 1.5°C/min and the temperature was held for 50 min (total run time 73 min).

Additional FAME samples from earlier in vitro cultures containing 0.5 mg of $C_{17:0}$ internal standard were analyzed on a gas chromatograph (Shimadzu GC-14A; Columbia, MD) equipped 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, Raritan, New Jersey). The injector and detector temperatures were both 250°C. The carrier gas was H_2 (33 cm/s) with an inlet pressure of 250 kPa. Each sample was run twice. First, the total fatty acid profile was determined. The column temperature was programmed from 70°C (after 4 min) to 175°C at 13°C/min and the temperature held for 27 min, then increased to 215°C at 4°C/min and held for 11 min. A second column temperature program from 160°C (held for 45 min) then increased to 215°C at 4°C/min and held for 11 min was used to separate the *cis* and *trans* octadecenoic acid isomers.

Calculations and statistics

The DMDS derivatives of FAME produce two distinctive spectral fragments that are indicative of the double bond position when analyzed by mass spectroscopy. The F fragment is the methyl thio adduct of the methyl end of the FAME. The G fragment is the methyl thio adduct of the carboxyl end of the FAME. The atom percent excess (APE) was calculated from the mass abundance of the G and G+1 fragments using the equation APE = (G+1)/[G+(G+1)]. In order to eliminate the natural levels of ^{13}C the average APE of unlabeled cultures was subtracted from the APE of labeled cultures. Therefore, enrichment (E) of the fatty acid with ^{13}C was calculated as (APE$_{labeled}$ − average APE$_{unlabeled}$) × 100. The percentage of each fatty acid originating from oleic acid biohydrogenation was calculated as [(average E)/(average E of oleic acid at time 0)] × 100.

An analysis of variance [PROC general linear model (GLM)] with main effect of time was evaluated. All results are expressed by their least square means (LSMEANS). The significance of the differences ($P < 0.05$) was assessed using SAS (SAS Institute, Inc., Cary, NC).

RESULTS

If oleic acid was biohydrogenated only directly to stearic acid, then only stearic acid would be enriched with ^{13}C. Instead, we found enrichment in stearic acid and many positional *trans* C18:1 fatty acids: *trans*-6, *trans*-7, *trans*-9, *trans*-10, *trans*-11, *trans*-12, *trans*-13, *trans*-14, *trans*-15, and *trans*-16.

As expected, ^{13}C enrichment (34%) was found in oleic acid at all times (**Fig. 1**). The enrichment of stearic acid (Fig. 1) increased over all incubation times from 0% at 0 h
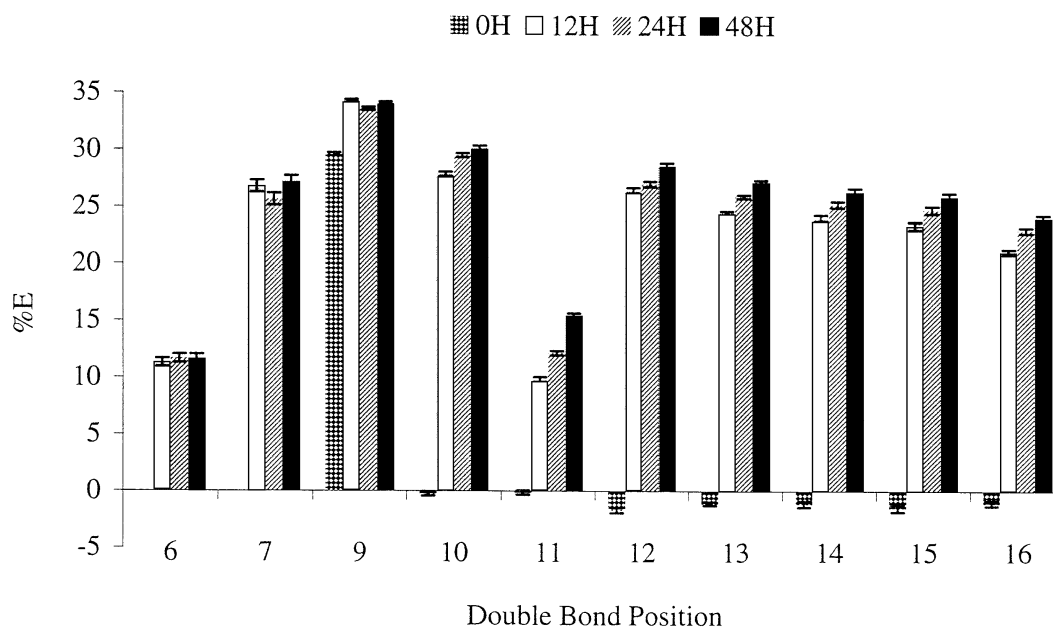
Fig. 2. The percentages of ^{13}C enrichment of *trans* C18:1 shown by double bond position and incubation time when a mixture (3:2) of oleic and oleic-1-^{13}C acids was added (30 mg per culture) to cultures of mixed ruminal microbes. Each bar is the mean of three replicates. Pooled standard errors are shown.

to a final enrichment of 17% at 48 h. *Trans*-6 and *trans*-7 C18:1 were not detected until 12 h, then their enrichments (11% and 26%, respectively) did not change ($P >$ 0.05) from 12 to 48 h (**Fig. 2**). *Trans*-9, *trans*-10, *trans*-11, *trans*-12, *trans*-13, *trans*-14, *trans*-15, and *trans*-16 C18:1 were detected at all times, but positive enrichment of all isomers, except *trans*-9, was not observed ($P < 0.05$) until 12 h (Fig. 2). Enrichments of the *trans*-10, *trans*-11, *trans*-13, *trans*-14, *trans*-15, and *trans*-16 isomers increased ($P <$ 0.05) from 12 to 24 h and from 24 to 48 h to final values of 30%, 15%, 27%, 26%, 26%, and 24% respectively. The enrichment values of *trans*-12 were similar ($P > 0.05$) from 12 to 24 h and increased ($P < 0.05$) from 24 to 48 h to a final value of 29%. The enrichment of *trans*-9 at 0 h (30%) was an artifact from the ^{13}C-labeled oleic acid (see Discussion). The final enrichment of *trans*-9 after 48 h was 34%. Also, because ruminal anaerobes cannot form fatty acids with more than one double bond by de novo synthesis, there was no enrichment ($P > 0.05$) found in linoleic acid at any incubation time (Fig. 1).

All fatty acids were analyzed for percentage originating from oleic acid (**Table 1**). The percent of stearic acid produced from oleic acid was 0 at 0 h and increased ($P <$ 0.05) over all incubation times to 52% at 48 h. *Trans*-6 and *trans*-7 C18:1 produced from oleic acid did not change ($P > 0.05$) from 12 to 48 h. *Trans*-9, *trans*-10, *trans*-11, *trans*-12, *trans*-13, *trans*-14, *trans*-15, and *trans*-16 C18:1 production from oleic acid increased ($P < 0.05$) from 0 to 48 h. As expected, linoleic acid was not produced ($P > 0.05$) from oleic acid at any incubation time.

In earlier in vitro cultures, oleic acid was the most abundant fatty acid at time 0 (**Fig. 3**), but its concentration decreased ($P < 0.05$) 60% (from 3.32 mg to 1.32 mg per 5 ml of culture) from 0 to 48 h. Although linoleic acid was present in much smaller concentrations, it still declined ($P < 0.05$) 69% (from 0.153 mg to 0.048 mg/5 ml) from 0
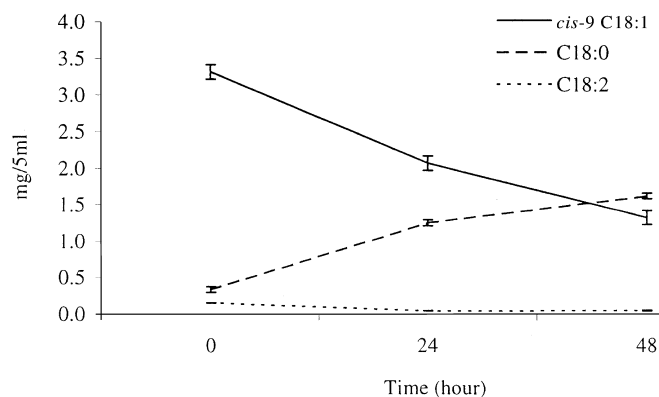

Fig. 3. Total (mg/5 ml of culture) of oleic, stearic, and linoleic acids from earlier experiments. Points are the mean of four replicates. Pooled standard errors are shown.

to 48 h. In contrast to oleic acid, the stearic acid concentration increased ($P < 0.05$) 3.8 times from 0 to 48 h. Although *trans*-10, *trans*-11, and *trans*-12 C18:1 were not detected at 0 h (**Fig. 4**), their concentrations increased ($P <$ 0.05) from 24 to 48 h (from 0.05 mg to 0.06 mg/5 ml, from 0.08 mg to 0.09 mg/5 ml, and from 0.02 mg to 0.03 mg/5 ml, respectively). The *trans*-9 C18:1 concentration also increased ($P < 0.05$) from 0 to 24 h (from 0.08 mg to 0.2 mg/5 ml) and slightly decreased ($P < 0.05$) from 24 to 48 h (from 0.2 mg to 0.18 mg/5 ml). The higher levels of *trans*-9 were due to *trans*-9 C18:1 contamination of the fat source used in the earlier in vitro cultures. *Trans*-6, *trans*-7, *trans*-13, *trans*-14, *trans*-15, and *trans*-16 C18:1 were not examined in earlier experiments for mg/5 ml of culture. However, their concentrations would appear to be similar to the concentrations of *trans*-10 and *trans*-12 based on peak area comparisons.

DISCUSSION

In our experiment, oleic acid was converted to stearic acid by stomach microbes, as depicted in current path-

TABLE 1. The percentage of fatty acids, including individual *trans* monoenes, derived from oleic acid when an oleic acid and oleic-1-^{13}C acid mix (3:2) were added (30 mg per culture)

Fatty Acid	Hour				SE
	0	12	24	48	
% from oleic acid					
C18:0 (stearic)	0.1	39.6	47.7	51.7	0.90
C18:1 isomers[a]					
cis-9 (oleic)	100.0	103.4	102.1	101.3	0.38
trans-6	ND	34.0	35.1	35.0	1.10
trans-7	ND	80.5	77.2	81.8	1.58
trans-9	89.1	103.2	101.0	102.4	0.42
trans-10	−0.7	83.7	88.7	90.6	0.58
trans-11	−0.4	29.6	36.6	46.5	0.57
trans-12	−5.1	79.5	81.2	86.0	0.69
trans-13	−3.3	73.5	77.8	81.7	0.48
trans-14	−3.5	72.2	75.8	79.2	0.81
trans-15	−4.5	70.2	74.4	77.8	0.97
trans-16	−3.2	63.3	69.0	72.3	0.74
C18:2 (linoleic)	−3.0	−0.6	−6.7	4.6	2.62

[a] Enrichment noted in *trans*-9 C18:1 at time 0 even after purification of fat source. Values are the mean of three replicates at each time. ND, not detected.
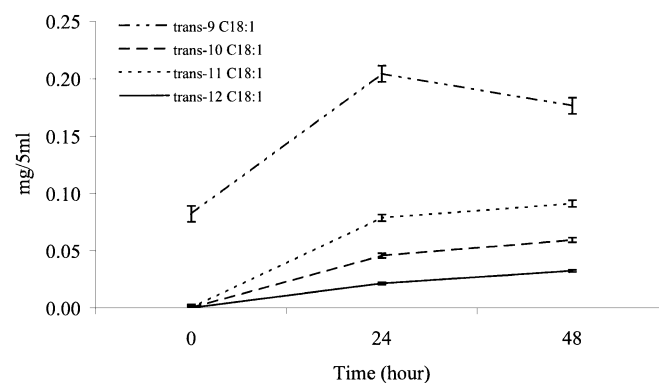

Fig. 4. Total (mg/5 ml of culture) of *trans*-9, *trans*-10, *trans*-11, and *trans*-12 C18:1 from earlier experiments. Points are the mean of four replicates. Pooled standard errors are shown.

ways. However, the results also showed that oleic acid was converted to a multitude of *trans* C18:1 positional isomers, contrary to currently accepted pathways for biohydrogenation of oleic acid, involving the direct conversion to stearic acid. It is unknown whether the *trans* C18:1 that are formed from oleic acid are intermediates or if they are end products of biohydrogenation. Nonetheless, the formation of these *trans* C18:1 from oleic acid is interesting and potentially important.

Trans monoenes having double bonds from carbons 6–16 originated from the biohydrogenation of oleic acid. The percentage of *trans* monoenes that originated from the biohydrogenation of oleic acid was <56% for the *trans*-6 and *trans*-11 C18:1 isomers. For all other positional isomers, the percentage that originated from oleic acid was >70%. Unlabeled C18:2 and C18:3 also were available in the hay substrate for biohydrogenation to *trans* fatty acids. Oleic acid was the most abundant fatty acid present in the cultures, and if C18:2 or C18:3 were present in greater concentrations, they could have contributed more to the formation of *trans* fatty acids.

In earlier experiments, oleic-1-^{13}C acid was found to be contaminated with ^{13}C-labeled *trans*-9 C18:1 (2.8%) and stearic acid (1.6%), which made it difficult to determine with certainty that all *trans* C18:1 isomers came only from the biohydrogenation of oleic acid. The oleic-1-^{13}C acid was purified using silver ion chromatography (see Materials and Methods) to remove the ^{13}C-labeled contaminants. Despite the high purity of oleic-1-^{13}C acid, there was still significant label (30%) found in *trans*-9 C18:1 at 0 h, suggesting that the label was derived from the labeled oleic acid. The *trans*-9 and *trans*-10 C18:1 peaks appeared as small shoulders on a much larger oleic acid peak at 0 h. Analysis of the mass spectra of the *trans*-10 C18:1 isomer included G and G+1 fragments (217 and 218, respectively) that matched the pattern of fragmentation of the oleic acid. The enrichment of these fragments could be attributed only to the oleic acid, because *trans*-10 C18:1 has a completely different set of G and G+1 fragments (231 and 232, respectively). However, the mass spectral fragments of geometric isomers, such as *cis*- and *trans*-9 C18:1, are exactly the same. Thus it cannot be excluded that the enrichment found in the *trans*-9 mass spectra was an artifact of the ^{13}C-labeled oleic acid because of the incomplete separation of the *cis*-9 and *trans*-9 C18:1.

In order to test the hypothesis of this study that gut microbes hydrogenate oleic acid to *trans* intermediates, it was essential to use mixed microbes in the cultures instead of a single species. The identification of a specific microbial species that can perform all of the steps of biohydrogenation would be difficult. Many different strains of pure bacterial cultures have been tested for biohydrogenation activity. However, fatty acid hydrogenation by many bacterial strains was negligible or incomplete (4). Ruminal bacterial are symbiotic in that they exchange intermediates of biohydrogenation between populations. Therefore, ruminal bacteria are grouped according to which substrate is used during the process of biohydrogenation (17). Furthermore, single bacterial species are not recognized for having the ability to carry out all steps of biohydrogenation. Kemp and Lander (17) noted that there must be a balance of bacterial groups for complete hydrogenation of unsaturated fatty acids to occur and for the accumulation of all possible intermediates. Ruminal bacteria have been allocated to two groups, A and B (17). Group A are those described as able to hydrogenate linoleic acid and α-linolenic acid to *trans*-11-octadecenoic acid but are unable to hydrogenate octadecenoic acids. Group B bacteria hydrogenate oleic, *trans*-11 C18:1, and linoleic acids to stearic acid. Thus it is necessary, when testing biohydrogenation pathways, to use mixed ruminal microbes to observe the production of all possible products.

Only a limited number of pure strains of ruminal bacteria have been shown to use oleic acid as a substrate. When used, oleic acid may be hydrated, hydrogenated, or isomerized (5, 18–22). *Fusocillus babrahamensis* P2/2, Fusocillus T344, and R8/5 Gram-negative rod were the only three bacterial strains of those studied that converted oleic acid to stearic acid. *F. babrahamensis* P2/2 also converted oleic acid to hydroxystearic acid (19–21). While Fusocillus T344 also converted oleic acid to *trans*-11 C18:1 (19, 20), *Butyrivibrio fibrisolvens* could not modify oleic acid in any way (18). However, when purified oleic acid was added to cultures of mixed ruminal microbes, it was converted to stearic acid and traces of *trans* C18:1 (5). Hudson et al. (22) showed that ruminal bacterial strains of *Selenomonas ruminatium* and *Enterococcus faecalis* also hydrated oleic acid to 10-hydroxystearic acid.

Previous studies have shown an increase in the concentration of *trans* C18:1 in milk fat when diets high in oleic acid were fed to lactating dairy cows. Selner and Schultz (6) fed oleic acid and observed an increase in the *trans* C18:1 concentration in milk fat (from 3% to 8%). Similarly, Jenkins (23) fed high oleic canola oil (78% oleic acid) and observed a significant increase in the *trans* C18:1 concentration in milk fat (from 1.72% to 4.22%). One possibility for the increase in production of *trans* C18:1 in milk fat could be that the isomers are formed during the biohydrogenation of oleic acid in the rumen. Another possibility could be that the oleic acid interfered with the biohydrogenation of linoleic acid resulting in an accumulation of *trans* C18:1.

There are several possible processes for the production of *trans* C18:1 isomers from oleic acid. One could be that the rumen bacteria possess a multitude of *cis/trans* isomerases. It is accepted that there is an isomerase that converts the *cis*-12 bond of linoleic and linolenic acids to a *trans*-11 bond (4). Furthermore, Mortimer and Niehaus (24) incubated a soluble enzyme preparation from a non-ruminal bacteria, *Pseudomonas* strain NRRL 3266, with oleic acid. They found that the enzyme preparation transformed oleic acid to *trans*-10 C18:1, but only at pH 5.

Another possibility could be that the *trans* C18:1 isomers are formed as a result of simple chemical double bond migration (2). Ward et al. (5) suggested that the accumulation of *trans* monoenes from the biohydrogenation of ^{14}C-labeled linoleic acid was due to double bond migration. This conclusion was based on the observation

that the chemical hydrogenation of linoleic acid with a metal catalyst resulted in migration of the double bond and formation of *trans* acids. However, enzymatic and metal catalyzed hydrogenation are not parallel processes (2). If this process does occur without catalysis, it must be slow, as we were able to store our ^{13}C-labeled oleic acid after purification without experiencing migration of the double bond.

Meat and dairy products are sources of saturated fatty acids, *trans* fatty acids, and conjugated linoleic acid (CLA). *Trans* C18:1 from ruminal biohydrogenation serve as precursors for the synthesis of saturated fatty acids in the rumen and of CLA at the tissue level. For example, *trans*-11 C18:1 is reduced in the rumen to form stearic acid or is desaturated by Δ^9-desaturase in the mammary tissue, with *cis*-9, *trans*-11 C18:2 as the product, which is an abundant CLA isomer in meat and milk (2). This isomer has been shown to have antioxidant and anticarcinogenic properties (3). Additionally, recent studies have shown that CLA is linked to decreased body fat deposition in animals and humans (7–9). The *trans*-10, *cis*-12 C18:2 has been shown to be the primary isomer responsible for decreased milk fat synthesis (11). Furthermore, the concentration of *trans*-10 C18:1 in milk fat is directly correlated with milk fat depression (10). It has been suggested that the production of *trans*-10 C18:1 could be attributed to the biohydrogenation of *trans*-10, *cis*-12 C18:2 (2). However, we have shown that *trans*-10 C18:1 is also derived from oleic acid.

We examined the conversion of oleic acid under standard in vitro conditions (15). Under different conditions, the results may vary. For instance, the pH must be maintained within a range (6–7) for normal microbial growth and metabolism (25). The type of feed, or substrate, consumed affects the rumen pH. Thus, the diet will affect the concentration of various rumen microbes. This shift in the bacterial population leads to changes in fermentation products. Furthermore, at pH 5, *Pseudomonas* strain NRRL 3266 isomerized oleic acid to *trans*-10-octadecenoic acid, but at pH 7, it was hydrated to 10-hydroxystearic acid (24). Hudson et al. (26) also noted that more hydration of oleic acid occurred at lower pH.

The type of fat used as a substrate may also cause the results to vary. The concentration, saturation, and esterification of fatty acid substrates affect the extent of biohydrogenation (27). The presence of 0.1% (v/v) linoleic acid inhibited growth and resulted in no hydrated products by *Enterococcus faecalis* (26). The stage of bacterial growth must also be considered. *E. faecalis* hydrated oleic acid only after growth had stopped and the carbon source had been depleted (26).

The biohydrogenation pathway of oleic acid has not been thoroughly explored in review articles (4). Our study demonstrates the ability of mixed ruminal microbes to convert oleic acid to a multitude of *trans* positional isomers. In the culture conditions used, most *trans* isomers were produced primarily from the biohydrogenation of oleic acid. This is an important step in predicting the type and amount of *trans* positional isomers. With further studies, it may be possible to control the production of *trans* fatty acids that have metabolic and physiological benefits.█

REFERENCES

1. Bickerstaffe, D., D. E. Noakes, and E. F. Annison. 1972. Quantitative aspects of fatty acid biohydrogenation, absorption and transfer into milk fat in the lactating goat, with special reference to the *cis*- and *trans*-isomers of octadecenoate and linoleate. *Biochem. J.* **130:** 607–617.
2. Griinari, J. M., and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. *In* Advances in Conjugated Linoleic Acid Research. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, editors. AOCS Press, Champaign, IL. 180–200.
3. McDonald, H. B. 2000. Conjugated linoleic acid and disease preventions: A review of current knowledge. *J. Am. Coll. Nutr.* **19:** 111S–118S.
4. Harfoot, C. G., and G. P. Hazelwood. 1988. Lipid metabolism in the rumen. *In* The Rumen Microbial Ecosystem. P. N. Hobson, editor. Elsevier Science Publishing, New York. 285–322.
5. Ward, P. F. V., T. W. Scott, and R. M. C. Dawson. 1964. The hydrogenation of unsaturated fatty acids in the ovine digestive tract. *Biochem. J.* **92:** 60–68.
6. Selner, D. R., and L. H. Schultz. 1980. Effects of feeding oleic acid or hydrogenated vegetable oils to lactating cows. *J. Dairy Sci.* **63:** 1235–1241.
7. Blankson, H., J. A. Stakkestad, H. Fagertun, E. Thom, J. Wadstein, and O. Gudmundsen. 2000. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J. Nutr.* **130:** 2943–2948.
8. Ostrowska, E., M. Muralitharan, R. F. Cross, D. E. Bauman, and F. R. Dunshea. 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. *J. Nutr.* **129:** 2037–2042.
9. Gavino, V. C., G. Gavino, M. Leblanc, and B. Tuchweber. 2000. An isomeric mixture of conjugated linoleic acids but not pure *cis*-9, *trans*-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. *J. Nutr.* **130:** 27–29.
10. Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. V. Nurmela. 1998. *Trans*-octadecenoic acids and milk fat depression in lactating dairy cows. *J. Dairy Sci.* **81:** 1251–1261.
11. Baumgard, L. H., B. J. Corl, D. A. Dwyer, A. Saebo, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* **278:** R179–R184.
12. Moya-Camarena, S. Y., J. P. Vanden Heuvel, S. G. Blanchard, L. A. Leesnitzer, and M. A. Belury. 1999. Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARα. *J. Lipid Res.* **40:** 1426–1433.
13. Kramer, J. K. G., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. *Lipids.* **32:** 1219–1228.
14. Christie, W. W. 1989. Silver ion chromatography using solid-phase extraction columns packed with bonded-sulfonic acid phase. *J. Lipid Res.* **30:** 1471–1473.
15. Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.
16. Yamamoto, K., A. Shibahara, T. Nakayama, and G. Kajimoto. 1991. Determination of double-bond positions in methylene-interrupted dienoic fatty acids by GC-MS as their dimethyl disulfide adducts. *Chem. Phys. Lipids.* **60:** 39–50.

17. Kemp, P., and D. J. Lander. 1984. Hydrogenation *in vitro* of α-linolenic acid to stearic acid by mixed cultures of pure strains of rumen bacteria. *J. Gen. Microbiol.* **130:** 527–533.
18. Kepler, C. R., K. P. Hirons, J. J. McNeill, and S. B. Tove. 1966. Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrosolvens. J. Biol.Chem.* **241:** 1350–1354.
19. Kemp, P., R. W. White, and D. J. Lander. 1975. The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including a new species. *J. Gen. Microbiol.* **90:** 100–114.
20. Hazlewood, G. P., P. Kemp, D. Lander, and R. M. C. Dawson. 1976. C$_{18}$ unsaturated fatty acid hydrogenation patterns of some rumen bacteria and their ability to hydrolyse exogenous phospholipid. *Br. J. Nutr.* **35:** 293–297.
21. White, R. W., P. Kemp, and R. M. C. Dawson. 1970. Isolation of a rumen bacterium that hydrogenates oleic acid as well as linoleic acid and linolenic acid. *Biochem. J.* **116:** 767–768.
22. Hudson, J. A., C. A. M. MacKenzie, and K. N. Joblin. 1995. Conversion of oleic acid to 10-hyroxystearic acid by two species of ruminal bacteria. *Appl. Microbiol. Biotechnol.* **44:** 1–6.
23. Jenkins, T. C. 2000. Feeding oleamide to lactating Jersey cows 1. Effects on lactation performance and milk fatty acid composition. *J. Dairy Sci.* **83:** 332–337.
24. Mortimer, C. E., and W. G. Niehaus. 1972. Enzymatic isomerization of oleic acid to *trans*-Δ^{10}-octadecenoic acid. *Biochem. Biophys. Res. Commun.* **49:** 1650–1656.
25. VanSoest, P. J. 1994. Nutritional Ecology of the Ruminant, 2nd edition, Cornell University Press, Ithaca, NY. 253–255.
26. Hudson, J. A., C. A. M. MacKenzie, and K. N. Joblin. 1996. Factors affecting the formation of 10-hydroxystearic acid from oleic acid by a ruminal strain of Enterococcus faecalis. *Appl. Microbiol. Biotechnol.* **45:** 404–407.
27. Noble, R. C., J. H. Moore, and C. G. Harfoot. 1974. Observations on the pattern on biohydrogenation of esterified and unesterified linoleic acid in the rumen. *Br. J. Nutr.* **31:** 99–108.