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 13C in labeled oleic acid to determine if trans monoenoic acids formed from the 13C-labeled oleic acid or if the 13C appears only in stearic acid as described in reviews of earlier work. Enrichments were calculated from the mass abundance of 13C in major fatty acid fragments and expressed as a percentage of total carbon isotopomers. Significant 13C 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 monoenoic acids 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.

Supplementary key words biohydrogenation • oleic acid • trans monoenoic acids • ruminal microbes

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. Biroydrogenation is a unique biological process prevalent in the microbial ecosystem found within the rumen. In cows fed a typical forage diet, the major trans monoenoic 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 Δ⁹-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 [1-13C]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 >5.4 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,
trans-11 C18:2 result in lower weight gain (9). In lactating dairy cows, Grinari 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-activated 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 13C in labeled oleic acid to determine if trans monoenes are formed from the 13C-labeled oleic acid or if the 13C 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-1-13C 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-1-13C acid purification

A total of approximately 160 mg of oleic-1-13C acid was methylated according to Kramer et al. (15) and then separated into cis and trans monoenes 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-1-13C 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-1-13C 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 0.5% acetone in 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 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.
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 H₂ (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 ¹³C the average APE of unlabeled cultures was subtracted from the APE of labeled cultures. Therefore, enrichment (E) of the fatty acid with ¹³C was calculated as \( (APE_{labeled} - average \ APE_{unlabeled}) \times 100 \). The percentage of each fatty acid originating from oleic acid biohydrogenation was calculated as \( [(average \ E)/(average \ E \ of \ oleic \ acid \ at \ time \ 0)] \times 100 \).

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 \( (P \leq 0.05) \) 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 ¹³C. 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, ¹³C 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
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 13C-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 (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 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 0.05 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-

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

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

* 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.
ways. However, the results also showed that oleic acid was converted to a multitude of \textit{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 \textit{trans} C18:1 that are formed from oleic acid are intermediates or if they are end products of biohydrogenation. Nonetheless, the formation of these \textit{trans} C18:1 from oleic acid is interesting and potentially important.

\textit{Trans} monoenes having double bonds from carbons 6–16 originated from the biohydrogenation of oleic acid. The percentage of \textit{trans} monoenes that originated from the biohydrogenation of oleic acid was <56\% for the \textit{trans}-6 and \textit{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 \textit{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 \textit{trans} fatty acids.

In earlier experiments, oleic-1-\textsuperscript{13}C acid was found to be contaminated with \textsuperscript{13}C-labeled \textit{trans} \textit{C18:1} (2.8\%) and stearic acid (1.6\%), which made it difficult to determine with certainty that all \textit{trans} C18:1 isomers came only from the biohydrogenation of oleic acid. The oleic-1-\textsuperscript{13}C acid was purified using silver ion chromatography (see Materials and Methods) to remove the \textsuperscript{13}C-labeled contaminants. Despite the high purity of oleic-1-\textsuperscript{13}C acid, there was still significant label (30\%) found in \textsuperscript{13}C-labeled contaminants. The enrichment of these fragments could be attributed only to the \textit{oleic acid}, because \textsuperscript{13}C-labeled isomers were unlabeled and \textit{trans} isomers at 0 h, suggesting that the label was derived from the labeled oleic acid. The \textit{trans} \textit{C18:1} peaks appeared as small shoulders on a much larger oleic acid peak at 0 h.

Analysis of the mass spectra of the \textit{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 \textit{oleic acid}, because \textsuperscript{13}C-labeled \textit{trans} \textit{C18:1} has a completely different set of G and G+1 fragments (231 and 232, respectively). However, the mass spectral fragment of geometric isomers, such as \textit{cis}- and \textit{trans} \textit{C18:1}, are exactly the same. Thus it cannot be excluded that the enrichment found in the \textit{trans} mass spectra was an artifact of the \textsuperscript{13}C-labeled oleic acid because of the incomplete separation of the \textit{cis} and \textit{trans} \textit{C18:1}.

In order to test the hypothesis of this study that gut microbes hydrogenate oleic acid to \textit{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 \textit{cis}-18:3 to \textit{trans}-11-octadecenoic acid but are unable to hydrogenate octadecenoic acids. Group B bacteria hydrogenate oleic, \textit{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). \textit{Fusocillus babrahemensis} P2/2, \textit{Fusocillus} T344, and R8/5 Gram-negative rod were the only three bacterial strains of those studied that converted oleic acid to stearic acid. \textit{F. babrahemensis} P2/2 also converted oleic acid to hydroxystearic acid (19–21). While \textit{Fusocillus} T344 also converted oleic acid to \textit{trans}-11 C18:1 (19, 20), \textit{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 \textit{trans} C18:1 (5). Hudson et al. (22) showed that ruminal bacterial strains of \textit{Selenomonas ruminatum} and \textit{Enterococcus faecalis} also hydrated oleic acid to 10-hydroxystearic acid.

Previous studies have shown an increase in the concentration of \textit{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 \textit{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 \textit{trans} C18:1 concentration in milk fat (from 1.72\% to 4.22\%). One possibility for the increase in production of \textit{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 \textit{trans} C18:1.

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

Another possibility could be that the \textit{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 \textit{trans} monoenes from the biohydrogenation of \textsuperscript{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 13C-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 Δ9-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.}

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