Cis-9, Trans-11 Conjugated Linoleic Acid Is Synthesized from Vaccenic Acid in Lactating Women

Erin E. Mosley, Michelle K. McGuire, Janet E. Williams, and Mark A. McGuire

Abstract

We studied the incorporation of the trans-11 vaccenic-1-13C acid (13C-VA) into milk and endogenous synthesis of cis-9, trans-11 conjugated linoleic acid (CLA) in lactating women. Subjects (n = 4) were 247 ± 30 d postpartum, weighed 70.8 ± 3.7 kg, breast-fed at least 6 times/d and consumed self-selected diets. After an overnight fast, they consumed the 13C-VA (2.5 mg/kg body wt). Milk samples were obtained by complete breast expression at 0, 2, 4, 8, 12, 18, 24, and 48 h post-13C-VA ingestion. Lipid was extracted using chloroform:methanol. Fatty acids were methylated and converted to dimethyl disulfide and Diels-Alder derivatives before analysis by gas chromatography mass spectrometry. The mean 13C-enrichment of milk VA was 3.1% at 8 h and reached maximal enrichment of 7.6% at 18 h. The 13C enrichment of milk cis-9, trans-11 CLA reached a maximum of 0.4% at 18 h, confirming its conversion of VA to the Δ9-desaturase enzyme product. In the subjects examined, a portion (≤10%) of the cis-9, trans-11 CLA present in milk was endogenously synthesized from VA. J. Nutr. 136: 2297–2301, 2006.

Introduction

Dairy products are a key source of certain fatty acids, particularly conjugated linoleic acids (CLA)1 for the human consumer (1). The most abundant CLA isomer in milk fat is the cis-9, trans-11 form (2). Ip et al. (3) showed that cis-9, trans-11 CLA possesses anticarcinogenic properties in rodents, and others have demonstrated this effect in human tumor cells (4). The consumption of cis-9, trans-11 CLA-enriched foods has been investigated as a way to increase the concentration of cis-9, trans-11 CLA in the blood and milk of humans (5,6). However, one caveat with CLA work has indirectly determined that VA can be converted to cis-9, trans-11 CLA, although these data suggest that the conversion in humans may be less than what occurs in cows. Most research has focused on improving the incorporation of cis-9, trans-11 CLA to the consumer through endogenous desaturation. Previous work has directly determined that VA can be converted to cis-9, trans-11 CLA, although these data suggest that the conversion in humans may be less than what occurs in cows. Most research examining the desaturation of VA to cis-9, trans-11 CLA in animals and humans has not used chemical tracers to establish conversion (8,10–12). In some instances (8,10,14), the activity of the Δ9-desaturase enzyme has been chemically inhibited. Only in one human experiment (15), consisting of one male subject, was chemical tracer methodology employed. This methodology is ideal because it circumvents the need for chemical inhibition of the enzyme and allows for a direct measurement of the conversion of VA to cis-9, trans-11 CLA in vivo.

In the United States, new labeling laws require the identification of the content of total trans fatty acids per serving. Because of the potential negative effects of trans fatty acids, the Institute of Medicine (13) indicates that the intake of trans fatty acids should be “as low as possible.” Therefore, understanding the metabolism of trans fatty acid isomers may yield insight into preventing human disease. Some trans fatty acids may afford health benefits. For example, VA is unique in that it may provide cis-9, trans-11 CLA to the consumer through endogenous desaturation.

1 Supported in part by the Idaho Beef Council, the Idaho Agricultural Experiment station, NIH-BRIN, a National Research Initiative Competitive Grant 2003-35206-13869 from the USDA Cooperative State Research, Education, and Extension Service, and NIH and the National Center for Research Resources Center of Biomedical Research Excellence (COBRE), Grant P20 RR15887.
2 Recipient of the University of Idaho Presidential Doctoral Research Fellowship.
3 Abbreviations used: CE, cholesterol ester; CLA, conjugated linoleic acid; DMDS, dimethyl disulfide; E, enrichment; NEFA, nonesterified fatty acids; PL, phospholipid; TG, triglycerides; TTR, tracer to tracee ratio; VA, vaccenic acid.
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Materials and Methods

Subjects, treatment, sampling, and analysis. The University of Idaho Human Assurances Committee and Washington State University Institutional Review Board approved this study. Written informed consent was obtained from all participants. Subjects (n = 4) were 247 ± 30 d (means ± SEM) postpartum, weighed 70.8 ± 3.7 kg, and were breastfeeding at least 6 times/d. All women were consuming self-selected diets. After an overnight fast, subjects consumed the 13C-VA (2.5 mg/kg body weight mixed with 100 g of applesauce) at 0 (pre) and 2, 4, 8, 18, 24, and 48 h post-VA ingestion. Blood was allowed to clot for 20 min at room temperature, centrifuged at 2000 × g for 15 min at 4°C, and the serum was collected and stored at −20°C. Serum lipid classes were quantified using enzymatic kits (L-Type TG H, Free Cholesterol E, Cholesterol E, NEFA cholesterol, and Phospholipids B from Wako Chemicals) according to the manufacturer’s directions for microplate analysis.

Milk samples (20 mL) were obtained by complete breast expression at 0, 2, 4, 8, 12, 18, 24, and 48 h post-VA ingestion using an electric breast pump (Model SMR-B-RI Ameda-Egnell). Samples were immediately frozen and stored at −20°C. Milk and serum lipids were analyzed as previously described (9). Dietary intake was estimated from written dietary records on 1 weekday and 1 weekend day 1 wk prior to the initiation of the study. Data from the dietary records were analyzed using a computer-based dietary assessment program (Genesis R&D, version 7.33, ESHA Research) to provide a general description of nutrient intakes.

Data analyses. For VA, the tracer enrichment ratio (TTR) was calculated from analysis of the dimethyl disulfide derivatives (DMDS). For cis-9, trans-11 CLA, the TTR was calculated from analysis of the methyl-1,2,4-triazoline-3,5-dione derivatives (MTAD). Both the DMDS and MTAD derivatives of fatty acid methyl ester 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 13C and 12C fragments (mass fragments 245 and 246 for VA; 322 and 323 for cis-9, trans-11 CLA) using the equation TTR = 13C/12C. To account for the natural levels of 13C, the mean TTR of samples taken before the infusion was subtracted from the TTR of all samples. Enrichment of the fatty acid with 13C at each sample period was calculated as (TTR − TTR 0h) × 100. The calculated enrichment was adjusted for spectrum skewness (16). Curves were fit to the enrichment values for VA and cis-9, trans-11 CLA in milk for subjects 1, 2, and 4. Area under each curve was calculated, and the ratio of cis-9, trans-11 CLA to VA was used to determine the percentage of cis-9, trans-11 CLA originating from VA (9). Descriptive statistical analyses were performed (PROC UNIVARIATE; SAS, version 9.1; SAS Institute). Statistical analyses of differences among intakes were performed (PROC UNIVARIATE; SAS, version 9.1; SAS Institute). Statistical analyses of differences among enrichment patterns were not performed because of the minimal number of observations and wide variation observed. Data are presented as means ± SEM (n = 4).

Results

Analysis of 2-d dietary records indicated that the women consumed 9106 ± 239 g of food with 2746 ± 302 kJ/d from fat. Intakes of protein, carbohydrate, and fat were 86 ± 8, 296 ± 35, and 73 ± 9 g/d, respectively. Overall milk fat content was 3.6 ± 0.3%. Individual milk fat percentages were 4.9 ± 0.4, 4.7 ± 0.6, 3.1 ± 0.4, and 1.7 ± 0.2% for subjects 1, 2, 3, and 4, respectively. The milk fatty acid profile (Table 1) was similar to previously published data (17) with cis-9 18:1 (oleic acid) representing the most abundant fatty acid followed by 16:0 and cis-9, cis-12 18:2. The weight percentages of VA and cis-9, trans-11 CLA in milk fat at 0 h were similar to the weight percentages at other sampling times irrespective of 13C-VA ingestion (Table 2). This indicates that the consumption of 2.5 mg vaccenic-13C acid/kg body wt as an oral bolus dose did not alter the steady-state fatty acid concentrations in the body fatty acid pools.

### Table 1

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>g/100 g fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>6:0</td>
<td>0.10 ± 0.003</td>
</tr>
<tr>
<td>8:0</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>10:0</td>
<td>1.25 ± 0.03</td>
</tr>
<tr>
<td>18:0</td>
<td>6.12 ± 0.27</td>
</tr>
<tr>
<td>cis-9 14:1</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>15:0</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>16:0</td>
<td>19.14 ± 0.31</td>
</tr>
<tr>
<td>cis-9 16:1</td>
<td>1.41 ± 0.14</td>
</tr>
<tr>
<td>17:0</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>18:0</td>
<td>6.69 ± 0.21</td>
</tr>
<tr>
<td>trans-7 to -10, 12, and -16 18:1</td>
<td>3.00 ± 0.15</td>
</tr>
<tr>
<td>trans-11 18:1</td>
<td>1.05 ± 0.06</td>
</tr>
<tr>
<td>cis-11 to -15 18:1</td>
<td>3.34 ± 0.18</td>
</tr>
<tr>
<td>cis-9 18:1</td>
<td>31.36 ± 0.44</td>
</tr>
<tr>
<td>trans-182 (total)</td>
<td>1.00 ± 0.11</td>
</tr>
<tr>
<td>cis-9, cis-12 18:2</td>
<td>15.15 ± 0.63</td>
</tr>
<tr>
<td>cis-9, trans-11 18:2</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>cis-9, cis-12, cis-15 18:3</td>
<td>1.19 ± 0.05</td>
</tr>
<tr>
<td>20:0</td>
<td>0.12 ± 0.003</td>
</tr>
<tr>
<td>22:0</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>20:4 (n-6)</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>22:5 (n-3)</td>
<td>0.19 ± 0.004</td>
</tr>
<tr>
<td>22:6 (n-3)</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Others</td>
<td>2.03 ± 0.03</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 32 (4 women sampled 8 times from 0 to 48 h).

Enrichment of milk lipids with 13C. No 13C enrichment was detected for VA (Fig. 1A) or cis-9, trans-11 CLA in milk lipid at 0 h before 13C-VA (Fig. 1B) ingestion. In milk fat, the 13C enrichment of VA was detected only in subjects 1, 2, and 4. A similar pattern for enrichment was observed for cis-9, trans-11 CLA in milk fat. The maximum 13C enrichment of VA in milk fat for all subjects occurred between 12 (7.5 ± 4.0%) and 18 h (7.6 ± 3.7%). The 13C enrichment of cis-9, trans-11 CLA in milk fat reached a maximum at 18 h (0.4 ± 0.2%). Using area under the curve analysis, we estimated that 7.4, 4.7, and 8.6% of the cis-9, trans-11 CLA originated from VA for subjects 1, 2, and 4, respectively.

Enrichment of serum lipids with 13C. As observed with milk fat, subject 3 had minimal incorporation of 13C-VA into the serum lipid fractions. However, analysis of data from all subjects detected enrichment of 13C-VA in serum triglycerides (TG), cholesterol esters (CE), phospholipids (PL), and nonessential fatty acids (NEFA). In the TG fraction, 13C enrichments measured at 2, 4, 8, 24, and 48 h postdosing were 2.9 ± 0.9, 6.4 ± 1.6, 11.7 ± 3.7, 1.9 ± 0.4, and 0.3 ± 0.1%, respectively. Enrichments of 13C in VA in the CE fraction measured at 2, 4, 8, 24, and 48 h postdose were 3.0 ± 0.9, 4.0 ± 1.2, 7.6 ± 3.7, 2.0 ± 0.2, and 1.0 ± 0.3%, respectively. The PL fraction 13C enrichments of VA at 2, 4, 8, 24, and 48 h were 0.2 ± 0.2, 0.6 ± 0.3, 4.9 ± 2.0, 3.2 ± 1.1, and 1.0 ± 0.3%, respectively. In the NEFA fraction, enrichments of VA measured at 2, 4, 8, 24, and 48 h were 0.9 ± 1.3, 2.2 ± 0.7, 7.2 ± 2.6, 0.5 ± 0.6, and 0.3 ± 0.6%, respectively.

Additionally, 13C enrichments of cis-9, trans-11 CLA in the TG, CE, PL, and NEFA of serum were detected. In the TG
fraction, enrichments of cis-9, trans-11 CLA measured at 2, 4, 8, 24, and 48 h were 0.2 ± 0.2, 0.4 ± 0.4, 1.7 ± 1.0, 0.7 ± 0.6, and 0.2 ± 0.4%, respectively. For the CE fraction, enrichments of cis-9, trans-11 CLA measured at 2, 4, 8, 24, and 48 h were 0.5 ± 0.5, 0.6 ± 0.3, 1.6 ± 0.6, 1.1 ± 0.5, and 0.4 ± 0.4%, respectively. Enrichments measured in the PL fraction at 2, 4, 8, 24, and 48 h were 0.3 ± 0.2, 0.2 ± 0.2, 0.2 ± 0.2, 0.5 ± 0.3, and 0.5 ± 0.2%, respectively. Enrichments of 13C in the NEFA fraction measured at 2, 4, 8, 24, and 48 h were 1.1 ± 0.6, 0.0 ± 0.2, 1.4 ± 0.9, 0.5 ± 0.4, and 1.3 ± 0.9%, respectively. Again, as for milk fat, subject 3 incorporated minimal 13C in cis-9, trans-11 CLA in any serum lipid fractions.

Finally, PL, CE, and TG fractions contributed to the majority of serum lipids, with PL representing the most abundant lipid fraction, followed by CE, and then TG (Table 3). Additionally, the TG and PL fractions contained the largest mass of VA.

**Discussion**

Milk fat secretion is regulated in part by the melting point of fatty acids within the milk fat droplet (18). Incorporating short chain saturated and long chain unsaturated fatty acids may facilitate the mammary gland's ability to maintain adequate lipid droplet liquidity (19). Furthermore, analysis of human milk lipids suggests that lauric, oleic, linoleic, and arachidonic acid concentrations are strongly correlated with the melting point of milk lipid (20). However, the action of the Δ9-desaturase enzyme and its impact on the maintenance of liquidity of lipid through synthesis of monounsaturated fatty acids, such as oleic acid, was deemed absent in the human mammary gland (19). The role of the Δ9-desaturase enzyme in the synthesis of milk lipids is well defined in lactating dairy cattle (21,22) but poorly defined in humans. Two isoforms of the Δ9-desaturase enzyme gene have been identified in human tissues, with expression predominantly in the liver, pancreas, and brain (23). However, the existence of this enzyme in the human mammary gland is debatable due to lack of published data.

Desaturation of fatty acids in vivo in humans using stable isotope labeling has been documented. Desaturation of stearic acid to oleic acid was detected in only 1 of 5 men consuming 2H-labeled myristic and 13C-labeled palmitic and stearic acids (24). Whereas the desaturation of 2H-labeled palmitic (3.9%, n = 5) and stearic acids (9.2%, n = 7) in adult men was detected, substantial subject variability was attributed to differences in dietary fatty acids (25). However, when 2 adult males consumed 2H-labeled palmitic and stearic acids in the form of triglycerides, no desaturation was detected (26). Using stearic (n = 4; 3 females and 1 male) and palmitic (n = 3; 1 female and 2 males) acids uniformly labeled with 13C, desaturation of stearic acid was detected at 14%, whereas palmitic acid desaturation was <2% (27). Furthermore, conversion of stearic to palmitic acid was 2%, and conversion of palmitic to stearic acid was 6%. These conversion processes were detected in chylomicrons and VLDL, indicating that conversion probably occurred in the intestine and liver (27). In all studies where desaturation was detected, the desaturation of stearic acid occurred to a greater extent than that of palmitic acid in vivo in humans. Similar to these studies, our data are quite variable (Fig. 1). However, 3 of the 4 subjects appeared to desaturate VA to cis-9, trans-11 CLA, confirming the activity of the Δ9-desaturase enzyme on VA in human tissue. This variation may be attributed to differences in diet, physiological state, or genetic potential. For example, milk

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**TABLE 2** VA and cis-9, trans-11 CLA in milk fat at each sample time from women who consumed vaccenic-13C acid

<table>
<thead>
<tr>
<th>Time postingestion, h</th>
<th>g/100 g fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatty acid</td>
</tr>
<tr>
<td></td>
<td>VA</td>
</tr>
<tr>
<td></td>
<td>cis-9, trans-11 CLA</td>
</tr>
</tbody>
</table>

1 Values are means, n = 4.

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**TABLE 3** Serum lipid concentrations in lactating women who consumed vaccenic-13C acid

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Lipid concentration (mmol/L)</th>
<th>Lipid fatty acid concentration (mmol/L)</th>
<th>VA (mol/100 mol fatty acids)</th>
<th>CLA (mol/100 mol fatty acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>1.40 ± 0.20</td>
<td>4.21 ± 0.59</td>
<td>0.56 ± 0.05</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>PL</td>
<td>3.04 ± 0.23</td>
<td>6.08 ± 0.45</td>
<td>0.40 ± 0.03</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.22 ± 0.02</td>
<td>0.22 ± 0.02</td>
<td>0.15 ± 0.04</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td>CE</td>
<td>4.53 ± 0.42</td>
<td>4.53 ± 0.42</td>
<td>0.11 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.08 ± 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 32 (4 women sampled 8 times from 0 to 48 h).
samples from subject 3 contained the greatest weight percentage of cis-9, trans-11 CLA but only the second highest amount of VA; very limited $^{13}$C enrichment was detected in either of these fatty acids. There was no abnormal dietary intake, subject weight, or milk or blood variables in this subject. The only notable difference was that her VA weight percentage of PL fatty acids was 0.60% compared with 0.30, 0.31, and 0.44% for subjects 1, 2, and 4, respectively. As PL is the largest contributor to total fatty acids in serum, this larger VA pool would have diluted the $^{13}$C label, resulting in lower enrichment values. Considering the wide variation among subjects and the minimal detection of $^{13}$C label in cis-9, trans-11 CLA in serum lipids and milk, we are uncertain whether the majority of the desaturation of VA to form cis-9, trans-11 CLA occurs in the mammary gland or other tissues. Nonetheless, the presence of $^{13}$C-labeled cis-9, trans-11 CLA in serum lipids and milk suggests $\Delta^9$-desaturase enzyme activity in the human body. To more accurately identify the contribution of the $\Delta^9$-desaturase activity in mammary and nonmammary tissues to the endogenously synthesized cis-9, trans-11 CLA that is incorporated into milk fat, more women need to be examined.

The desaturation of VA to cis-9, trans-11 CLA has only been shown directly using stably labeled chemical tracers in vivo with one human male subject (15) and in lactating dairy cattle (9), and indirectly using unlabeled fats in vivo in humans (10), mice (12), and cattle (8). When nonlactating healthy humans ($n = 40$) consumed diets high in trans fatty acids, there was a 30% increase of total CLA in the serum (28). Furthermore, VA was consumed diets high in trans fatty acids, there was a 30% increase of total CLA in the serum (28). Furthermore, VA was converted to cis-9, trans-11 CLA in humans as demonstrated indirectly from the measurement of cis-9, trans-11 11 CLA in the serum of nonlactating humans consuming a VA-rich diet (10). However, there was considerable individual variation, with a mean conversion rate of only 19%. Additionally, reanalysis (15) of samples from a study originally published in 1978 showed a 30% enrichment of $^2$H in cis-9, trans-11 CLA in the serum of one human adult male who consumed $^2$H-labeled VA. In each of the previously mentioned human studies, the liver would likely be the main site of desaturation (23,29). In contrast, we studied conversion of VA to cis-9, trans-11 CLA in both mammary and nonmammary sites and estimated that up to 10% of cis-9, trans-11 CLA in milk was from the desaturation of VA. This value is substantially lower than the ~80% estimated to originate from the desaturation of VA in lactating dairy cattle (9).

The fatty acids VA and cis-9, trans-11 CLA have been studied extensively for their potentially beneficial properties in preventing cancer and other diseases. For example, Ip et al. (3) showed that CLA can inhibit tumor formation and growth in rodents, whereas more recent work demonstrates the anticarcinogenic effect of cis-9, trans-11 CLA in a variety of human tumor cells (4). Furthermore, specific isomers of CLA affect various metabolic activities in humans and other animals (1,30,31). The anticarcinogenic activity of cis-9, trans-11 CLA may be linked to the desaturation of VA to form cis-9, trans-11 CLA as demonstrated by MCF-7 and SW480 cancer cells in vitro (32). In vivo evidence also supports the necessity of VA conversion to cis-9, trans-11 CLA for maximal anticarcinogenic effects. For example, when rats were fed increasing dosages of VA with a limited addition of dietary cis-9, trans-11 CLA, the incidence of chemically induced mammary carcinomas decreased as cis-9, trans-11 CLA accumulated in the mammary fat pad (11). Similarly, when VA was fed with and without cyclopentenoic fatty acids (inhibitors of the $\Delta^9$-desaturase enzyme) from sterulic oil, the addition of sterulic oil reversed the anticarcinogenic effects that occurred when only VA was fed to rats (33). Our data, showing the incorporation of VA into various serum lipid classes within 2 to 8 h post-VA ingestion, indicates that it is rapidly utilized in the body. The rapid incorporation into serum TG, CE, and PL ensures that VA is available to a variety of tissues, considering these lipid pools account for nearly 99% of total serum fatty acids (Table 3).

In conclusion, this experiment in lactating women confirms that $^{13}$C-labeled fatty acids may be used as a tool to measure the activity of the $\Delta^9$-desaturase enzyme in vivo. Currently, there is a push to increase both VA and cis-9, trans-11 CLA in products consumed by humans to provide the cis-9, trans-11 CLA directly and indirectly via desaturation of VA. However, the minimal enrichment found in cis-9, trans-11 CLA in human serum casts doubt on the total contribution of cis-9, trans-11 CLA from the desaturation of VA in nonmammary tissues. Furthermore, the wide variation observed among subjects represents a challenge to investigators seeking to determine an accurate estimate of conversion. Future studies including many subjects will ultimately be necessary to further elucidate our understanding of the regulation of the $\Delta^9$-desaturase enzyme activity in humans. In addition, future studies should be designed to investigate possible factors, such as maternal diet and body composition, that contribute to the differences in $\Delta^9$-desaturase enzyme activity among women.

**Literature Cited**


32. Miller A, McGrath E, Stanton C, Devery R. Vaccenic acid (t11–18:1) is converted to c9,t11-CLA in MCF-7 and SW480 cancer cells. Lipids. 2003;38:623–32.