Bioconversion of vaccenic acid to conjugated linoleic acid in humans

Anu M Turpeinen, Marja Mutanen, Antti Aro, Irma Salminen, Samar Basu, Donald L Palmquist, and J Mikko Grönari

ABSTRACT
Background: Vaccenic acid (11-trans octadecenoic acid; VA), a major trans fatty acid in the fat of ruminants, is produced in the rumen and converted in tissues to rumenic acid (9-cis, 11-trans octadecenoic acid; RA), an isomer of conjugated linoleic acid, by Δ9-desaturase. There are indications that this conversion also occurs in humans.

Objective: The aim of this controlled intervention was to study the conversion of VA to RA in humans after consumption of diets with increasing amounts of VA.

Design: Thirty healthy subjects consumed a baseline diet rich in oleic acid for 2 wk. The subjects were then divided into 3 groups (n = 10 per group) and provided a diet containing 1.5, 3.0, or 4.5 g VA/d for 9 d. All diets contained equal amounts of macronutrients and differed only in their fatty acid compositions. The fats were mixed into conventional foods, and nearly all food was provided during the study.

Results: The proportion of VA in serum total fatty acids increased 94%, 307%, and 620% above baseline with the 1.5-, 3.0-, and 4.5-g VA/d diets, respectively. This was associated with a linear increase in the proportion of RA. The conversion rate was 19% on average, 94%, 307%, and 620% above baseline with the 1.5-, 3.0-, and 4.5-g VA/d diets, respectively. This was associated with a linear increase in the proportion of RA. The conversion rate was 19% on average, and differed only in their fatty acid compositions. The fats were mixed into conventional foods, and nearly all food was provided during the study.

Conclusions: The results quantify the desaturation of VA to RA in humans. Conversion is likely to contribute significantly to the amount of RA available to the body, and dietary intakes of VA should thus be taken into account when predicting RA status. Am J Clin Nutr 2002;76:504–10.

KEY WORDS Vaccenic acid, rumenic acid, conjugated linoleic acid, trans fatty acids, endogenous synthesis, Δ9-desaturase, isoprostanes

INTRODUCTION
Conjugated linoleic acid (CLA) is a collective term for isomers of linoleic acid with conjugated double bonds in several positions and conformations. CLA has received considerable interest during the past decade, because it has been shown to promote various beneficial health-related effects in animals, including anticarcinogenic and antiatherogenic effects and effects on body composition and fat metabolism (see 1 for a review).

Ruminant fat is the main dietary source of CLA (2). Consumption of milk fat correlates with CLA concentrations in plasma (3), adipose tissue (4), and human milk (5). The daily intake of CLA in Western populations has been estimated to be 50–300 mg/d. The current intake, however, is lower than intakes that have had beneficial health effects in animal studies.

Vaccenic acid (11-trans-octadecenoic acid; VA) is a major trans fatty acid in milk fat, constituting ≈1.7% (range: 0.4–4%) of the total fatty acid content (6). VA is also an intermediate in the biohydrogenation of polyunsaturated 18-carbon fatty acids to stearic acid in the rumen, and is the major precursor of CLA in milk fat (7). Desaturation of VA to rumenic acid (9-cis, 11-trans octadecenoic acid; RA) in tissues is catalyzed by Δ9-desaturase (EC 1.14.99.5) (8, 9). Bioconversion of VA to RA was shown and quantified in a recent study in mice (10). A study in humans noted that the CLA concentration in serum total lipids increases with consumption of a diet high in trans oleic acid (18:1), but the role of Δ9-desaturase was not confirmed in this context (11). Conversion of VA to RA in humans could substantially increase the amount of CLA available to the body.

Our aim was to confirm and quantify the conversion of VA to RA in humans. We conducted a controlled dietary intervention at 3 VA intakes in healthy humans. This design allowed us to estimate the extent to which dietary VA is converted to RA. Recent studies showed increases in the urinary excretion of lipid oxidation products after dietary CLA supplementation (12, 13). To examine whether endogenously formed CLA has a similar effect, we analyzed the urinary excretion of an indicator of arachidonic acid oxidation, 8-isoprostaglandin F2α (8-iso-PGF2α).

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2 Supported by the National Dairy Council (United States), the Dairy Research and Development Corporation (Australia), and the Dairy Farmers of Canada. The high-oleic acid oil was provided by the Raisio Group, Raisio, Finland.

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SYNTHESIS OF CONJUGATED LINOLEIC ACID IN HUMANS

TABLE 1

Characteristics of subjects before the study

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 22)</th>
<th>Men (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>24.3 ± 7.1</td>
<td>28.6 ± 6.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.5 ± 8.5</td>
<td>79.3 ± 7.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168 ± 7.1</td>
<td>184 ± 4.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 ± 2.8</td>
<td>23.5 ± 2.2</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>4.5 ± 0.8</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Serum triacylglycerol (mmol/L)</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.4</td>
</tr>
</tbody>
</table>

1 t ± SD.
2 Significantly different from women, P < 0.001.

SUBJECTS AND METHODS

Subjects

Thirty healthy subjects (22 women and 8 men) were recruited to the study. The subjects were screened for serum total cholesterol, triacylglycerols, blood pressure, and urinary glucose and protein. They completed a questionnaire about their dietary habits, alcohol consumption, and other lifestyle factors. The mean age of the subjects was 26 y and they were of normal weight [body mass index (BMI; in kg/m²) 19.0–25.5], were normocholesterolemic (serum total cholesterol 3.2–5.4 mmol/L), and were normotensive [blood pressure: < 140 (systolic) and < 90 mm Hg (diastolic)]. None of the subjects smoked, and 8 women used oral contraceptives. The baseline diet contained 30% of energy as fat, 15% as protein, and 55% as carbohydrates. A weekly rotating menu was used. On weekdays the subjects ate lunch at the Division of Nutrition, where they received the food to be consumed the rest of the day and the next morning. On Fridays they were given the food to be consumed over the weekend. Each participant’s food was weighed according to their energy level. Energy level was determined before the study began by using calculated surface area (14), age, and self-reported physical activity. Body weight in light clothing was recorded biweekly, and energy intake was adjusted to avoid weight changes.

In addition to the food supplied, which provided 90% of the daily energy intake, the subjects were allowed to choose a limited amount of fat- and cholesterol-free food items daily to provide the remaining 10% of daily energy intake. The participants kept diaries in which they recorded the foods they selected, the amount of free-choice foods eaten, any signs of illness, all medications used, and any deviation from their diets.

 Duplicate portions of the diets were collected for 1 wk and homogenized. Before homogenization, 5 g butyl hydroxytoluene/kg (Merck, Germany) was added to prevent oxidation. The homogenized samples were freeze-dried and analyzed for total fat and nitrogen contents at the Agricultural Research Center of Finland, Jokioinen, and for fatty acid composition at the National Public Health Institute, Helsinki. The analyzed values were combined with the values calculated from the free-choice foods.

Blood and urine sampling

Fasting venous blood samples were collected with minimal stasis between 0730 and 1000 at the end of the baseline period (day 0) and on 4 mornings (days 2, 4, 6, and 9) during the experimental period. The blood samples for each subject were taken at the same time of the morning throughout the study.

Two 24-h urine samples were collected at the end of the baseline and experimental periods with the use of a Japanese aliquot cup (KK Izumi Seisakusyo Co, Ltd, Japan). The urine samples were divided into aliquots and stored at −70°C. Urinary isoprostanes were analyzed from pooled individual samples from each period.

Serum total lipids and VLDLs

Serum total lipids were extracted and methylated as described by Folch et al (15) and Stoffel et al (16). Methylation was done with 1% H₃SO₄ in dry methanol. In our preliminary studies we found that 1% provides sufficient methylation but does not cause isomerization of CLA to a significant extent. The percentile distribution of methylated fatty acids was determined by gas chromatography (GC; model 6890 with the use of workstation software A.06.01; Hewlett-Packard, Palo Alto, CA) with a 60-m Supelco SP 2380 column (Supelco, Inc, Bellefonte, PA) and hydrogen as carrier gas. Fatty acid peaks from 14:0 to 22:6 were identified in a temperature-programmed run. Interassay variation was 0.5–2% for GC peaks >1% and 1.5–5% for peaks <1%. For RA, variation was 3%. The column does not separate 10-trans 18:1 and 11-trans 18:1 (VA) isomers. However, the baseline diet contained negligible amounts of both 10-trans and VA and there were no 10-trans isomers in the experimental fat. Thus, the 10–11-trans peak is referred to as VA.

VLDL was isolated from fresh serum samples by ultracentrifugation at a density of < 1.019 g/L (100 000 rpm, 3 h, and 5°C) according to the method of Havel et al (17). A Beckman model L preparative centrifuge (Beckman Instruments, Inc, Palo Alto, CA) with a type TFT 45.6 rotor (Kontron Instruments, Zürich) was used, and the solution densities were adjusted with potassium bromide. The lipoprotein fraction was removed with
the use of a standard tube-slicing technique (Spinco Tube Slicer; Beckman; 18) and stored at 4°C. VLDL-triglycerol fatty acids were separated by thin-layer chromatography (19). Fatty acids were methylated and determined by GC as described for serum total fatty acids. VLDL triacylglycerols were further analyzed with Ag⁺ HPLC to separate 7-trans, 9-cis, and 9-cis, 11-trans CLA isomers (20).

**Urinary isoprostanes**

8-iso-PGF₂α, a major isoprostane and an indicator of nonenzymatic arachidonic acid oxidation, was analyzed from unextracted urine samples with the use of radioimmunoassay (21). In brief, an antibody raised in rabbits by immunization with 8-iso-PGF₂α was used. The cross-reactivities of the antibody with 8-iso-15-keto-13,14-dihydro-PGF₂α; 8-iso-PGF₂β; PGF₂α; 15-keto-PGF₂α; 15-keto-13,14-dihydro-PGF₂α; thromboxane B₂; 11-β-PGF₂α; 9-β-PGF₂α; and 8-iso-PGF₂β were 1.7%, 9.8%, 1.1%, 0.01%, 0.01%, 0.1%, 0.03%, 1.8%, and 0.6%, respectively. The detection limit of the assay was ≈ 23 pmol/L. Intraassay mean values for the low and high spiked standards in the human plasma are 95.6% and 101%, respectively. Intraassay CVs of the low and high standards in the human plasma were 14.5% and 12.2%, respectively. The urinary concentrations of 8-iso-PGF₂α were adjusted for creatinine values, which were measured with the use of a commercial kit (IL Test; Monarch Instrument, Amherst, NH).

**Statistical analysis**

Fatty acid data were analyzed as repeated measures according to the following model:

\[ Y_{ijkl} = m + D_i + G_j + BC_{k,j} + p_{k,i} + T_l + DT_{il} + E_{ijkl} \]  

where m is the overall mean, \( D_i \) is the fixed effect of the \( i \)th treatment (\( i = 1, 2, 3 \)), \( G_j \) is the fixed effect of the \( j \)th sex (\( j = 1, 2 \)), \( B \) is a regression coefficient, \( C_{k,j} \) is the covariate measurement on person \( k \) of the \( j \)th treatment group, \( p_{k,i} \) is the random effect of the \( k \)th person within the \( i \)th treatment group (\( k = 1, \ldots, k \)), \( T_l \) is the fixed effect of the \( l \)th day of measurement (\( l = 1, 2, 3, 4 \)), \( DT_{il} \) is the treatment × day interaction, and \( E_{ijkl} \) is the random residual error.

The MIXED procedure of SAS (version 8.1; SAS Institute, Cary, NC) was used to estimate the parameters of the mixed model. The covariate value was the measurement from day 0 of the study. A first-order autoregressive structure was used to model the error variance–covariance matrix. The IML procedure of SAS was used to generate linear, quadratic, and cubic orthogonal polynomial coefficients for the unequally spaced days of measurements and their interactions with treatment. Two-factor repeated-measures analysis of variance was used to analyze the isoprostane data. Correlations were calculated by using Pearson’s correlation analysis. Differences with a \( P \) value < 0.05 were considered significant.

**Estimation of the rate of bioconversion**

The slope of linear regression of increases in RA versus VA plus RA in serum total lipids on day \( x \) – day 0 [ie, \( \Delta RA \) versus (\( \Delta VA + \Delta RA \))] represents the rate of conversion and was used as an estimate of average conversion over the range of VA intakes studied.

**RESULTS**

All subjects successfully completed the study. No significant changes in body weight were observed during the study. Double-portion analysis of the baseline and experimental diets showed that the intake of macronutrients was not significantly different during the 2 periods, whereas significant differences in fatty acid composition were observed (Table 2): the baseline diet was low in VA (< 0.2 g) and the intakes of VA in the 3 experimental diets were as planned. The CLA intake from the baseline diet was also low and remained unchanged throughout the study.

Changes in serum total fatty acid composition during the intervention are presented in Table 3 and for VA and RA in Figure 1. In all 3 groups, the proportion of VA increased until day 4, and the response was linear (\( P < 0.001 \)). RA also increased and reached a plateau after day 2 in all groups, but an increase was again seen on day 9; the dose response was linear (\( P < 0.0001 \)).

VLDL triacylglycerols (data not shown), measured on days 0, 4, and 9 in the group who consumed 3.0 g VA/d, showed a pattern for VA and RA that was similar to the pattern seen in serum total fatty acids. Although the proportion of VA had reached a plateau by day 4 and thereafter decreased slightly, RA still showed an upward trend.

Use of the slope of the linear regression \( \Delta RA \) versus \( \Delta VA + \Delta RA \) as an estimate of conversion gave an average conversion rate of 19% (Figure 2). This approach is valid because the dietary supply of CLA was small and constant (Table 2); therefore, any increase in plasma RA could occur only by desaturation of dietary VA. Significant interindividual differences were observed in the conversion rate: 2- to 3-fold differences in total increases in plasma RA were seen in all groups. In the 1.5-g group, one subject was a nonresponder (ie, no significant change in RA was seen, although serum VA increased). Four subjects in the 4.5-g group were identified as low responders (ie, those with an average increase in VA, but an increase in RA > 25% below the average) (Figure 2).

No significant differences in serum fatty acids, except in VA and RA, were observed between the 3 groups (Table 3). Quadratic effects of sample day were seen for several fatty acids. However, none of the trends were large or influenced the data importantly.

**Table 2**

Mean daily intake of nutrients from 10-MJ baseline and experimental diets according to duplicate-portion analysis and the calculated contribution of freely selected items

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Baseline diet</th>
<th>1.5 g/d</th>
<th>3 g/d</th>
<th>4.5 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% of energy)</td>
<td>14.8</td>
<td>14.4</td>
<td>14.4</td>
<td>14.4</td>
</tr>
<tr>
<td>Carbohydrates (% of energy)</td>
<td>54.4</td>
<td>54.8</td>
<td>54.8</td>
<td>54.8</td>
</tr>
<tr>
<td>Total fat (% of energy)</td>
<td>29.9</td>
<td>29.9</td>
<td>30.0</td>
<td>30.1</td>
</tr>
<tr>
<td>Alcohol (% of energy)</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>&lt; 0.2</td>
<td>1.7</td>
<td>3.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Saturated (g)</td>
<td>16.7</td>
<td>18.8</td>
<td>18.3</td>
<td>18.0</td>
</tr>
<tr>
<td>Oleic acid (g)</td>
<td>41.0</td>
<td>39.4</td>
<td>40.0</td>
<td>42.2</td>
</tr>
<tr>
<td>Vaccumic acid (g)</td>
<td>38.9</td>
<td>36.1</td>
<td>36.0</td>
<td>35.4</td>
</tr>
<tr>
<td>Polyunsaturated (g)</td>
<td>&lt; 0.2</td>
<td>1.7</td>
<td>3.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Conjugated linoleic acid (g)</td>
<td>8.1</td>
<td>7.2</td>
<td>7.2</td>
<td>7.0</td>
</tr>
</tbody>
</table>
| 1Calculated as the difference between total energy intake and energy intake from protein, fat, and alcohol.
No changes were seen in the concentration of the 7-trans, 9-cis CLA isomer (data not shown), which coelutes with RA on GC. The 7-trans, 9-cis CLA isomer represented only a minimal proportion (≈5%) of the RA peak throughout the study.

The urinary excretion of 8-iso-PGF$_{2\alpha}$ increased significantly from baseline ($P < 0.001$) in all 3 groups (0.294 to 0.468, 0.243 to 0.457, and 0.339 to 0.560 nmol/mmol creatinine in the 1.5-, 3.0-, and 4.5-g groups, respectively), representing 59%, 88%, and 65%
increases, respectively. No significant differences between groups (P = 0.147) and no correlation between serum RA and urinary 8-iso-PGF$_2$α was found.

**DISCUSSION**

We showed that VA was desaturated to RA in humans and that a linear increase in serum RA was associated with increases in serum VA.

The existence of Δ⁹-desaturase, an enzyme that desaturates saturated fatty acids to monounsaturated fatty acids (eg, palmitic to palmitoleic acid and stearic acid to 18:1) was first identified in rats > 25 y ago (22) and thereafter in mice, bovines, and chickens (23). It was later shown that the same enzyme is also responsible for the conversion of VA to RA (8, 9). To quantify Δ⁹-desaturase activity and the conversion of VA to RA, pure VA was fed to mice in a recent study (10). Eleven percent of dietary VA was converted to RA, as assessed by carcass analysis. Of the VA absorbed and stored, 51% was converted. Also, in lactating dairy cows, 64% of the CLA in milk fat was estimated to be of endogenous origin (7).

A similar pathway for desaturation of VA was suggested to occur in humans, whereas evidence for this has been somewhat inconsistent. No conversion was detected in 2 subjects fed a single dose of deuterium-labeled VA (7–8 g as triacylglycerol) and followed for 48 h (24). However, a recent reanalysis of the data of one subject led the authors to conclude that VA was converted to RA (25). A controlled human intervention study showed a significant accumulation of CLA (from 0.32% to 0.43% of plasma fatty acids), despite an 87% reduction in CLA intake, in serum fatty acids of 40 healthy subjects who consumed a diet rich in VA (3 g/d) for 5 wk after consumption of a diet high in dairy fat (11). In a group consuming a high–stearic acid diet after the diet high in dairy fat, serum CLA decreased from 0.34% to 0.17%.

Consistent with the results of our preliminary study in 3 volunteers (data not shown), serum VA and RA reached a plateau after 2–4 d in the present study. However, we observed an increase in serum RA again on the last day (day 9) of the study in all groups. An increase in VLDL triacylglycerols was also seen, indicating that the increase was not due to enrichment of RA in serum pools with slower turnover rates, such as phospholipids or cholesterol esters. The reason for this increase in unknown, but we cannot exclude the possibility of a “weekend effect” because the last sample was taken on a Monday morning. Although the subjects were given instructions on when to eat the desserts and bakery products containing VA, eating habits during the weekend may have been slightly different from those adopted on weekdays.

Although slight changes in serum saturated fatty acids and in long-chain PUFs were seen during the study, significant differences between the 3 groups were observed only in VA and RA. Because the proportion of 7-trans, 9-cis CLA separated from RA by Ag⁺ HPLC remained unchanged, it was concluded that appreciable amounts of 7-trans 18:1 were not present in the diet to be desaturated to 7-trans, 9-cis CLA and did not contribute to changes in the RA peak.

The conversion rate over the range of VA intakes studied was 19%. Interindividual differences were prominent, ranging from one nonresponder in the 1.5-g group to a conversion rate >30% in another subject. The finding of 4 low responders in the 4.5-g group suggests that the enzyme became saturated in some individuals. However, because ruminant fat contains 2–3 times more VA than RA (26, 27), even low rates of conversion contribute significantly to body CLA status. The present data suggest that the total contribution of ruminant fat to body CLA status is likely to be on average 1.5 times the CLA content because of endogenous synthesis from VA.
The distribution of 9-desaturase activity in human tissues is unknown. Liver is the principal tissue for fatty acid synthesis in humans and presumably also has the highest 9-desaturase activity. It has been postulated that CLA might also be synthesized from linoleic acid through anaerobic microbial activity in the large bowel (28). However, a diet rich in linoleic acid (safflower oil) did not increase plasma CLA in humans, suggesting that it is unlikely that synthesis in the bowel would have a significant contribution to body CLA status (29).

Dietary factors may significantly affect desaturase activity. In animals, dietary PUFAs suppress 9-desaturase activity (30), whereas cholesterol (31), carbohydrates (32), and a fat-free diet (33) are known to increase the activity. In mice, increasing dietary PUFAs from 4% to 10% decreased the desaturation of VA to RA by 30% (10). In the present study, the baseline and experimental diets were designed to provide similar intakes of cholesterol, and the intake of PUFAs was limited to 3% of energy (7–8 g/d).

Early studies suggest that antioxidant activity could explain the anticarcinogenic (34, 35) and antiatherogenic (36) effects of CLA, but several, although not all (37), recent studies have shown the susceptibility of CLA to in vitro oxidation to be comparable with or higher than that of linoleic acid (38–41). The anticarcinogenic effects and cytotoxicity of CLA in cancer cell lines may be partly due to increased lipid oxidation (42, 43). Isoprostanes are formed in the body from PUFAs via nonenzymatic pathways, in contrast with eicosanoids, which are formed via enzymatic pathways. Isoprostanes are seen as in vivo indicators of lipid oxidation because increased synthesis was reported in a variety of clinical conditions associated with oxidative stress (44). Two recent studies in humans showed increased excretion of isoprostanes after CLA feeding (12, 13). Urinary 8-iso-PGF2α (a product of the nonenzymatic peroxidation of arachidonic acid) and 15-keto-dihydro-PGF2α (a product of the enzymatic peroxidation of arachidonid acid) both increased after daily CLA supplementation (4.2 g/d, containing equal amounts of the 9-cis, 11-trans, and 10-trans, 12-cis CLA isomers) of healthy subjects for 3 mo (12). Enhancement in urinary isoprostanes was also seen in middle-aged men with abdominal obesity after only 1 mo of consuming mixed isomers of CLA (4.2 g/d) (13). Isoprostane concentrations returned to baseline 2 wk after the cessation of CLA intake. In agreement with the results of Basu et al (12, 13), we observed an increase in the urinary excretion of 8-iso-PGF2α in all groups with intake of mixed isomers of CLA, although not as pronounced as in the aforementioned studies. This suggests that the effects of exogenously (dietary) and endogenously formed CLA on isoprostane production are not different.

The intake of CLA in Western populations is too low to provide the beneficial effects observed in animal studies. However, data from the present study warrant a reevaluation of the amount of CLA available to the body and indicate that the dietary intake of VA should also be taken into account when predicting CLA status. Conversion of VA to RA by 9-desaturase probably contributes significantly to CLA status.

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