trans Palmitoleic acid arises endogenously from dietary vaccenic acid1–3

Anke Jaudszus, Ronny Kramer, Maria Pfeuffer, Alexander Roth, Gerhard Jahreis, and Katrin Kuhnt

ABSTRACT
Background: trans Palmitoleic acid (t-16:1n-7, or 16:1n9 in the δ nomenclature usually applied to trans fatty acids and used herein) arouses great scientific interest because it has been suggested to serve as a biomarker for lower risks of type 2 diabetes and coronary artery disease. Objective: Although 16:1n9 has been assumed to derive from dietary sources, we examined the hypothesis that 16:1n9 might also be endogenously produced from its metabolic precursor vaccenic acid (t-18:1n-7 or 18:1n11). Design: We reevaluated fatty acid data obtained from one human intervention study and one cellular model in both of which 18:1n11 was supplemented. Both studies have already been published, but to our knowledge, 16:1n9 has not yet been considered. This reanalysis of the datasets was reasonable because a new methodology for identifying 16:1 cis and trans isomers allowed us to address the subject presented in this article. Results: Data showed that the systemic or intracellular increase in 16:1n9 was strongly correlated with the increase in 18:1n11 after the dietary intake or cellular uptake of 18:1n11. The conversion rate in humans was, on average, 17%. Conclusion: Our findings suggest that endogenous 16:1n9 is not, as has been assumed, exclusively diet derived but may also be produced by the partial β oxidation of dietary 18:1n11. Am J Clin Nutr 2014;99:431–5.

INTRODUCTION

trans Fatty acids (tFAs)4 are the subject of an ongoing discussion on both suggested positive and negative associations with metabolic and cardiovascular risk factors (1). 16:1n9, which is one of several 16:1 isomers in dairy fat (2), has been linked to beneficial metabolic effects of dairy consumption. Mozaffarian et al (3, 4) reported that high plasma phospholipid 16:1n9 was cross-sectionally associated with favorable plasma triglycerides and lower fasting insulin and prospectively associated with a substantially lower incidence of type 2 diabetes in the elderly. These authors assumed that circulating 16:1n9 derives principally from dietary sources and, in contrast to its cis isomer 16:1c9, is not endogenously synthesized (3). Observations described in the current work strongly indicated that 16:1n9 is also produced endogenously by chain shortening of 18:1n11. These results were based on and are complementary to one human intervention study (5–7) and one in vitro study (8), which have been previously published in their entirety.

SUBJECTS AND METHODS
Human study
A placebo-controlled, double-blind, randomized, human-intervention study in a parallel design was conducted in young, healthy men and women, with the original aim to evaluate the endogenous δ-9-desaturation of orally administered 18:1n11 compared with 18:1n12. A group size of 10 subjects was estimated to provide >95% power at α = 0.05 (PASS 6.0; NCSS) to detect a difference of the primary outcome defined in the original study [ie, an increase in serum c9,t11–conjugated linoleic acid (CLA) (5)]. In brief, after a 2-wk baseline period without ruminant fat intake for all participants (n = 24), subjects of the isomeric mixture group (n = 12; 6 men and 6 women) consumed 2.9 g 18:1n11/d and 2.9 g 18:1n12/d, together with small amounts of the corresponding cis isomers. This isomeric mixture or an oleic acid–rich control oil [control group n = 12 (6 men and 6 women)] was incorporated into a chocolate spread (Table 1) that was consumed daily along with an otherwise ruminant-fat–free diet over a period of 6 wk. Participants were instructed to substitute the ruminant staples with, eg, soy milk, vegetable margarine and spreads, and meat from nonruminant species in their habitual diets. Blood samples were obtained at the end of the 2-wk baseline and 6-wk intervention period. To ensure comparability, all participants received a standardized ruminant-fat–free diet over the last 7 d of study periods that was based on previously determined energy requirements. Detailed information on the study design, characterization of participants, and outcomes has

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2 Supported by a grant from the German Research Council (JA 893). The standard for cistrans 16:1 fatty acid methyl esters was a kind gift from Ali-Reza Fardin Kia and Pierluigi Delmonte.
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4 Abbreviations used: CLA, conjugated linoleic acid; FAME, fatty acid methyl ester; GC, gas chromatographic; PBMC, peripheral blood mononuclear cell; PPAR, peroxisome proliferator-activated receptor; tFA, trans fatty acid.

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be previously shown (5–7). The study was approved by the Ethics Committee of the Friedrich Schiller University of Jena and took place at the Department of Nutritional Physiology in Jena.

**In vitro study**

All materials, conditions, and procedures of purifying and culturing human peripheral blood mononuclear cells (PBMCs) for the purpose of a gas chromatographic (GC) analysis of the cellular fatty acid distribution after incubation with 18:1t11 have been described in detail elsewhere (8).

**Fatty acid analysis**

Total lipids were extracted from washed cell pellets and transesterified by incubating samples with 0.5N methanolic sodium hydroxide at 100°C for 10 min followed by methanolic boron trifluoride (10% wt/wt; Supelco) treatment at 100°C for 2 min. Lipid extracts from serum samples were transesterified with sodium methylate and 1,1,3,3-tetramethylguanidine, according to Kuhnt et al (5). Subsequently, fatty acid methyl esters (FAMEs) were extracted with n-hexane. Analysis of the fatty acid distribution including FAs was performed by combining the following 2 GC methods: total FAMEs from 4 to 26 were determined with the help of a fused-silica capillary column with medium polarity (DB-225 MS: 60-m × 0.25-mm inside diameter with a 0.25-μm film thickness; Agilent Technologies). Second, a fused-silica capillary column with high polarity (CP-silselect; 200-m × 0.25-mm inside diameter with a 0.25-μm film thickness; Varian) was used to separate hexadecenoic and octadecenoic acid methylesters with cis and trans configurations according to Degen et al (9). The peak area integration was accomplished by using GC-solution software (version 2.3; Shimadzu) compared with previously measured reference standards [BR2, BR4, ME93, and Menhadan (Larodan/CPS-Chemie); 463 and 674 (Nu-Chek-Prep); CLA standard (Sigma-Aldrich)]. The standard for cis/trans 16:1 FAMEs was obtained from Ali-Reza Fardin Kia and Pierluigi Delmonte (10). The conversion rate of 18:1r11 to 16:1r9 was calculated as follows.

\[
\text{Conversion rate} = \frac{\Delta 16:1r9}{\Delta 16:1r9 + \Delta 18:1r11} \times 100
\]

whereby \( \Delta 16:1r9 = \text{intervention 16:1r9} - \text{baseline 16:1r9} \) (2) and \( \Delta 18:1r11 = \text{intervention 18:1r11} - \text{baseline 18:1r11} \) (3).

**Statistics**

Differences in percentages of serum fatty acids were evaluated by using a linear mixed model with the fixed factors treatment (isomeric mixture and control) and time (baseline and intervention) and the interaction of these 2 factors. The normality and homoscedasticity of studentized residuals were judged by visual inspection of the quantile-quantile plot and predicted compared with a residual plot. A random intercept that was specific for each subject was included to control for interindividual differences. A Tukey-Kramer test was conducted as a post hoc test, and \( P \) values were adjusted for multiple comparisons. For the evaluation of data on cellular fatty acid profiles, the factor treatment (without and with 18:1r11) was entered into the model without the random intercept. \( P \) values for the association between 16:1 isomers and 18:1 precursors were calculated by using Pearson’s correlation. The significance of difference was set at \( P < 0.05 \). All calculations were carried out with SAS 9.3 software (PROC MIXED; SAS).

**RESULTS**

The ingestion of the 18:1r11-enriched spread over a period of 6 wk was reflected by 8-fold elevated serum concentrations of 18:1r11 compared with both baseline (\( P < 0.001 \)) and control group after intervention (\( P < 0.001 \); Figure 1A). This increase was accompanied by a significant increase in 16:1r9 (5-fold; \( P < 0.001 \) for both baseline and control group after intervention; Figure 1A). Because the diet and the spread consumed were free of 16:1r9 (Table 1), and a strong correlation was observed between the 2 fatty acids (\( R^2 = 0.808 \)), it was most likely that 16:1r9 arose from 18:1r11 as a result of endogenous chain shortening by 2 carbon atoms. The other isomers contained within the spread likewise showed up in serum of participants who consumed the isomeric mixture diet (Figure 1B). As seen

**TABLE 1**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Pure spread</th>
<th>Spread with control oil</th>
<th>Spread with isomeric mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \sum 16:1r9-11 )</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16:1r9</td>
<td>( \leq 0.1 )</td>
<td>( \leq 0.1 )</td>
<td>0</td>
</tr>
<tr>
<td>( \sum 16:1c10-12 )</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18:1r11</td>
<td>( \leq 0.1 )</td>
<td>( \leq 0.1 )</td>
<td>( \leq 0.1 )</td>
</tr>
<tr>
<td>18:1c12</td>
<td>( \leq 0.1 )</td>
<td>( \leq 0.1 )</td>
<td>( \leq 0.1 )</td>
</tr>
<tr>
<td>( \sum 18:1c13-16 )</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18:1c9</td>
<td>28.7</td>
<td>28.3</td>
<td>24.2</td>
</tr>
<tr>
<td>18:1c11</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>18:1c12</td>
<td>( \leq 0.1 )</td>
<td>( \leq 0.1 )</td>
<td>1.1</td>
</tr>
<tr>
<td>( \sum 18:1c13-15 )</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
for 18:1t11, the respective 16:1 isomers increased too (Figure 1B, Table 2), and a similarly strong association was shown between 16:1t10 and 18:1t12 ($R^2 = 0.901$) as well as 16:1c10 and 18:1c12 ($R^2 = 0.797$). Because base concentrations of 18:1c11 and 16:1c9 were comparatively high, no additional intervention effect was observed (Table 2). The conversion rate of 18:1t11 to 16:1t9, which was estimated according to the conversion rate of 18:1t11 to $c9,t11$-CLA (5), ranged from 10% to 30% and was, on average, 17%. Because both $c9,t11$-CLA (initial readout) and 16:1t9 (current readout) increased after the ingestion of 18:1t11, we further wanted to know whether and how strong respective conversion rates were associated. There was a weak and nonsignificant positive association between the conversion rate of 18:1t11 to 16:1t9 and the conversion rate of 18:1t11 to $c9,t11$-CLA ($R^2 = 0.261$, with one participant who showed no conversion of 18:1t11 to $c9,t11$-CLA not considered; data not shown).

Base concentrations of 18:1t11 in native human PBMCs were low (0.12 ± 0.02% of total FAMEs) (Figure 2). The percentage of 18:1t11 increased within the cellular lipid fraction over the incubation period of 24 h with 11 $\mu$mol 18:1t11/L and, finally, accounted for 17.1 ± 3.7% of total FAMEs ($P = 0.006$ compared with the DMSO control). 16:1t9 was detectable only in marginal amounts in native PBMCs (0.01 ± 0.01%) but increased 25-fold to 0.27 ± 0.04% of total FAMEs after incubation with 11 $\mu$mol 18:1t11/L ($P < 0.001$; Figure 2). These data further supported the hypothesis of an endogenous partial $\beta$ oxidation of the 18 to 16 corpus. Neither 14:1 nor 12:1 isomers increased as additional putative metabolites of $\beta$ oxidation (data not shown).

**FIGURE 1.** 16:1t9 increases in serum after ingestion of 18:1t11. A: Participants were allocated to either iso mix ($n = 12$) or ctrl ($n = 12$) groups. Baseline data represent serum fatty acid data after a 2-wk adaptation period (baseline) throughout which a ruminant-fat–free but otherwise unsupplemented diet was consumed. The subsequent intervention period lasted 6 wk, during which participants consumed the iso mix or ctrl supplement. Before blood withdrawals, a standardized diet was consumed for 7 d. Data are expressed as boxplots showing medians, 0.25 and 0.75 quartiles, and whiskers with lowest and highest values of $n = 12$ group. B: Representative partial chromatograms of 18:1 and 16:1 isomers (column: CP-select, 200 m; Varian) of one participant in the iso mix in comparison with one participant in the ctrl group after the intervention period, ctrl, control; FAME, fatty acid methyl ester; iso mix, isomeric mixture; std, standard for trans (red) and cis (blue) 16:1 fatty acid methyl esters as a reference.

**DISCUSSION**

18:1t11 is the dominant isomer in ruminant tFAs but can also be formed during the partial hydrogenation of vegetable oils. Therefore, it may not be surprising that the concentration of 16:1t9 in plasma phospholipids was significantly associated with the intake of margarine and cookies as well as dairy products in the Multi-Ethnic Study of Atherosclerosis cohort (4).

Fatty acid $\beta$ oxidation is a universal property of peroxisomes in most, if not all, organisms (11). The synthesis of DHA (22:6n–3) involves one cycle of $\beta$ oxidation in peroxisomes. There has been indirect evidence of a retroconversion of DHA to EPA (20:5n–3) in human red blood cells (12) and of docosapentaenoic acid (22:5n–3) to EPA in animal tissues (13). Experiments with labeled DHA showed that retroconversion via chain shortening was increased with older age (14). But partial $\beta$
oxidation seems to be a more widespread phenomenon and also affects fatty acids with shorter chain lengths. For instance, palmitoleic acid (16:1\textsubscript{c}7; alternatively named 16:1\textsubscript{n}29) is formed from endogenously synthesized oleic acid (18:1\textsubscript{c}9\textsuperscript{o}r 18:1\textsubscript{n}29) (15). This observation confirmed previous data on the considerable retroconversion by the chain shortening of both labeled oleic to palmitoleic acid and stearic (18:0) to palmitic (16:0) acid in human hepatocellular carcinoma cells (16). In these experiments, this process was markedly stimulated by adding the peroxisome proliferator-activated receptor (PPAR) \(g\) ligand troglitazone (16). Because isomers of CLAs including \(c9, t11\)-CLA are well-known PPAR ligands, this process offers an explanation of why corresponding 16:2-conjugated dienes appeared in rat tissues after the consumption of \(c9, t11\)-CLA enriched milk fat (17) and in human colorectal adenocarcinoma cells incubated with alpine milk fatty acids (9).

As described previously, our in vitro data indicated that 18:1\textsubscript{t}11 also acts, at least in part, in a PPAR\(g\) ligand-like fashion in PBMCs (8). In our human study, the intake of \(t\)FAs stimulated the gene expression of PPAR\(g\) and (in men only) the peroxisomal bifunctional enzyme in monocytes (7), the latter of which catalyzes the second step of peroxisomal \(b\) oxidation.

### TABLE 2

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Human-intervention study iso mix group</th>
<th>In vitro study treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Intervention</td>
<td>DMSO control</td>
</tr>
<tr>
<td>16:1\textsubscript{t}9</td>
<td>0.04 ± 0</td>
<td>0.13 ± 0.01***</td>
</tr>
<tr>
<td>16:1\textsubscript{t}10</td>
<td>0.03 ± 0</td>
<td>0.12 ± 0.01***</td>
</tr>
<tr>
<td>16:1\textsubscript{t}11</td>
<td>2.47 ± 0.26</td>
<td>2.25 ± 0.19</td>
</tr>
<tr>
<td>16:1\textsubscript{c}7</td>
<td>0.01 ± 0</td>
<td>0.05 ± 0.01***</td>
</tr>
<tr>
<td>16:1\textsubscript{c}11</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>18:1\textsubscript{t}11</td>
<td>0.06 ± 0</td>
<td>0.48 ± 0.03***</td>
</tr>
<tr>
<td>18:1\textsubscript{t}12</td>
<td>0.07 ± 0.01</td>
<td>0.84 ± 0.04***</td>
</tr>
<tr>
<td>18:1\textsubscript{t}13</td>
<td>0.06 ± 0</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>18:1\textsubscript{c}7</td>
<td>2.18 ± 0.06</td>
<td>2.47 ± 0.08</td>
</tr>
<tr>
<td>18:1\textsubscript{c}11</td>
<td>0.04 ± 0</td>
<td>0.47 ± 0.03***</td>
</tr>
<tr>
<td>18:1\textsubscript{c}13</td>
<td>0.04 ± 0</td>
<td>0.05 ± 0.03</td>
</tr>
</tbody>
</table>

All values (% FAMEs) are means ± SEMs. Data from the control group of the human study were fed into the model but are not shown. ***Between baseline and intervention or between DMSO control and 18:1\textsubscript{t}11, \(P < 0.001\); **between DMSO control and 18:1\textsubscript{t}11 treatment, \(P < 0.01\). Differences between groups were determined by using a linear mixed model followed by Tukey-Kramer post hoc test. FAME, fatty acid methyl ester; iso mix, isomeric mixture.

### FIGURE 2

Mean (±SEM) 16:1\textsubscript{t}9 increases in peripheral blood mononuclear cells after incubation with 18:1\textsubscript{t}11 (\(n = 4\)). Human peripheral blood mononuclear cells were incubated without (DMSO-ctrl) or with 11 \(\mu\)mol 18:1\textsubscript{t}11/L for 24 h. Subsequently, fatty acid profiles of cells were determined by using GC-FID.

A: Different lowercase letters indicate a statistically significant difference (\(P < 0.01\)). B: Partial chromatogram of 16:1 isomers (column: CP-select, 200 m; Varian) in reference to a cow-milk sample and to the std. ctrl; control; FAME, fatty acid methyl ester; GC-FID, gas chromatography with flame ionization detection; iso mix, isomeric mixture; std, standard for \textit{trans} (red) and \textit{cis} (blue) 16:1 fatty acid methyl esters as a reference.
hepatocellular carcinoma cells, 18 fatty acids, such as 18:1r11, induced messenger-RNA expression of acyl–coenzyme A oxidase-1, which is the first enzyme of the peroxisomal β-oxidation system (18). Moreover, rFA-enriched partially hydrogenated vegetable oil compared with respective native vegetable oils enhanced β-oxidation in rat liver peroxisomes (19).

However, our finding of 16:1r9 production on 18:1r11 supply was merely circumstantial evidence and provided no final proof of such a conversion. Nevertheless, under the defined experimental conditions, there was no other likely explanation for this phenomenon. Of note, there was also an increase of 16:1r10 (likely derived from 18:1r12) and 16:1c10 (likely derived from 18:1c12) in serum. Prospectively, studies that use 18:1r11 labeled with stable isotopes will doubtlessly prove whether 16:1r9 is produced via chain shortening as it was previously shown for 16:1c7 production from 18:1c9 (16). Such studies may also reveal whether 18:1r11 or its metabolite c9,r11-CLA induced a general stimulation of peroxisomal β-oxidation.

In conclusion, our data strongly indicate that circulating 16:1r9, which is considered a biomarker for lower risk of diabetes (3, 4) and coronary artery disease (20), arises endogenously from 18:1r11, and the human body is able to provide this fatty acid itself after the consumption of whole-fat dairy products.

The authors’ responsibilities were as follows—AJ and KK: designed and conducted the research; KK: generated the random allocation sequence for the human-intervention study, enrolled participants, and assigned participant participants to interventions; RK: analyzed data; AR: performed the statistical analysis; AJ and MP: wrote the manuscript; and GJ: supervised the work. None of the authors declared a conflict of interest.

REFERENCES