trans Palmitoleic acid arises endogenously from dietary vaccenic acid\textsuperscript{1–3}

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ABSTRACT

Background: trans Palmitoleic acid (t-16:1\textsubscript{n–7}, or 16:1\textsubscript{9} in the \textdelta no-
menculation usually applied to trans fatty acids and used herein) arouses great scientific interest because it has been suggested to serve as a bio-
marker for lower risks of type 2 diabetes and coronary artery disease.

Objective: Although 16:1\textsubscript{9} has been assumed to derive from di-
etary sources, we examined the hypothesis that 16:1\textsubscript{9} might also be
endogenously produced from its metabolic precursor vaccenic acid (t-18:1\textsubscript{n–7} or 18:1\textsubscript{11}).

Design: We reevaluated fatty acid data obtained from one human
intervention study and one cellular model in both of which 18:1\textsubscript{11} was supplemented. Both studies have already been published, but to
our knowledge, 16:1\textsubscript{9} has not yet been considered. This reanalysis
of the datasets was reasonable because a new methodology for iden-
tifying 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
16:1\textsubscript{9} was strongly correlated with the increase in 18:1\textsubscript{11} after the
dietary intake or cellular uptake of 18:1\textsubscript{11}. The conversion rate in
humans was, on average, 17%.

Conclusion: Our findings suggest that endogenous 16:1\textsubscript{9} is not, as
has been assumed, exclusively diet derived but may also be produced by
the partial \textbeta oxidation of dietary 18:1\textsubscript{11}. Am J Clin Nutr

INTRODUCTION

trans Fatty acids (tFAs)\textsuperscript{4} are the subject of an ongoing dis-
cussion on both suggested positive and negative associations
with metabolic and cardiovascular risk factors.\textsuperscript{1} 16:1\textsubscript{9}, which
is one of several 16:1 isomers in dairy fat,\textsuperscript{2} has been linked to benefi-
cial metabolic effects of dairy consumption. Mozaffarian et al (3, 4) reported that high plasma phospholipid 16:1\textsubscript{9} was
cross-sectionally associated with favorable plasma triglycerides
and lower fasting insulin and prospectively associated with
favorable plasma triglycerides in healthy men and women, with the original aim to evaluate the
dependent \textdelta-9-desaturation of orally administered 18:1\textsubscript{11} compared with 18:1\textsubscript{12}. A group size of 10 subjects was estimated
to provide >95% power at \textalpha = 0.05 (PASS 6.0; NCSS) to detect
a difference of the primary outcome defined in the original study
[i.e., an increase in serum 9,11–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 18:1\textsubscript{11}/d and
2.9 g 18:1\textsubscript{12}/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, e.g., soy milk,
vegetable margarine and spreads, and meat from nonruminant spe-
cies 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 pre-
viously determined energy requirements. Detailed information on
the study design, characterization of participants, and outcomes has

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standard for cis\textit{trans} 16:1 fatty acid methyl esters was a kind gift from
Ali-Reza Fardin Kia and Pierluigi Delmonte.

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\textsuperscript{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; rFA, trans fatty acid.

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been 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 18:1\textsubscript{11} 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 -hexane. Analysis of the fatty acid distribution including tFAs 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-select: 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 Menhaden (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 18:1\textsubscript{11} to 16:1\textsubscript{9} was calculated as follows

\[
\text{Conversion rate} = \frac{\Delta16:1r9}{\Delta18:1r11} \times 100
\]

whereby

\[
\Delta16:1r9 = \text{intervention 16:1r9} - \text{baseline 16:1r9}
\]

and

\[
\Delta18:1r11 = \text{intervention 18:1r11} - \text{baseline 18:1r11}
\]

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 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 F values were adjusted for multiple comparisons. For the evaluation of data on cellular fatty acid profiles, the factor treatment (without and with 18:1\textsubscript{11}) was entered into the model without the random intercept. F 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 18:1\textsubscript{11}-enriched spread over a period of 6 wk was reflected by 8-fold elevated serum concentrations of 18:1\textsubscript{11} 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 16:1\textsubscript{9} (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 16:1\textsubscript{9} (Table 1), and a strong correlation was observed between the 2 fatty acids (R\textsuperscript{2} = 0.808), it was most likely that 16:1\textsubscript{9} arose from 18:1\textsubscript{11} 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
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 μmol 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 μmol 18:1t11/L ($P < 0.001$; Figure 2). These data further supported the hypothesis of an endogenous partial β-oxidation of the 18 to 16 corpus. Neither 14:1 nor 12:1 isomers increased as additional putative metabolites of β-oxidation (data not shown).

**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 β-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 β-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 β-oxidation is not the only mechanism that can accommodate the demand for EPA in ruminants, as increased β-oxidation was also observed in ruminant-fed cows (15). The contribution of β-oxidation to the formation of EPA in ruminants remains to be elucidated, but peroxisomal β-oxidation appears to be a major route for EPA formation in these animals. The presence of β-oxidation in ruminants suggests that EPA could be a natural or intrinsic part of their tissue composition.
oxidation seems to be a more widespread phenomenon and also affects fatty acids with shorter chain lengths. For instance, palmitoleic acid (16:1\text{c}_7; alternatively named 16:1n_{29}) is formed from endogenously synthesized oleic acid (18:1\text{c}_9or 18:1n_{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) \text{g} ligand troglitazone (16). Because isomers of CLAs including \text{c}_9,\text{t}_{11}-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 \text{c}_9,\text{t}_{11}-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\text{t}_{11} also acts, at least in part, in a PPAR \text{g} ligand-like fashion in PBMCs (8). In our human study, the intake of \text{t}_{11}FAs stimulated the gene expression of PPAR \text{g} and (in men only) the peroxisomal bifunctional enzyme in monocytes (7), the latter of which catalyzes the second step of peroxisomal \text{b} oxidation. In human

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Baseline</th>
<th>Intervention</th>
<th>DMSO control</th>
<th>18:1r11</th>
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</thead>
<tbody>
<tr>
<td>16:1\text{r}_9</td>
<td>0.04 ± 0</td>
<td>0.13 ± 0.01***</td>
<td>0.01 ± 0.01</td>
<td>0.27 ± 0.04***</td>
</tr>
<tr>
<td>16:1r10</td>
<td>0.03 ± 0</td>
<td>0.12 ± 0.01***</td>
<td>0.02 ± 0.02</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>16:1r11</td>
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<td>0.02 ± 0.01</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>16:1r9</td>
<td>2.47 ± 0.26</td>
<td>2.25 ± 0.19</td>
<td>0.31 ± 0.02</td>
<td>0.44 ± 0.22</td>
</tr>
<tr>
<td>16:1r10</td>
<td>0.01 ± 0</td>
<td>0.05 ± 0.01***</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>16:1r11</td>
<td>0.07 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.03 ± 0.03</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>18:1r11</td>
<td>0.06 ± 0</td>
<td>0.48 ± 0.03***</td>
<td>0.12 ± 0.02</td>
<td>17.07 ± 3.68**</td>
</tr>
<tr>
<td>18:1r12</td>
<td>0.07 ± 0.01</td>
<td>0.84 ± 0.04***</td>
<td>0.08 ± 0.05</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>18:1r13</td>
<td>0.06 ± 0</td>
<td>0.07 ± 0.01</td>
<td>0.14 ± 0.02</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td>18:1r11</td>
<td>2.18 ± 0.06</td>
<td>2.47 ± 0.08</td>
<td>2.32 ± 0.05</td>
<td>2.48 ± 0.12</td>
</tr>
<tr>
<td>18:1r12</td>
<td>0.04 ± 0</td>
<td>0.47 ± 0.03***</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>18:1r13</td>
<td>0.04 ± 0</td>
<td>0.05 ± 0.03</td>
<td>0.07 ± 0.03</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 18:1r11, \text{P} < 0.001; **between DMSO control and 18:1r11 treatment, \text{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.

**TABLE 2**

**Increase in 16:1 isomers after ingestion or incubation with 18:1 precursors**

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**FIGURE 2.** Mean (±SEM) 16:1r9 increases in peripheral blood mononuclear cells after incubation with 18:1r11 (n = 4). Human peripheral blood mononuclear cells were incubated without (DMSO-ctrl) or with 11 \text{mmol} 18:1r11/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 (\text{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 trans (red) and cis (blue) 16:1 fatty acid methyl esters as a reference.
hepatocellular carcinoma cells, 18 fatty acids, such as 18:1\(\text{cis-9}\), induced messenger-RNA expression of acyl–coenzyme A oxidase-1, which is the first enzyme of the peroxisomal \(\beta\)-oxidation system (18). Moreover, \(\alpha\)FA-enriched partially hydrogenated vegetable oil compared with respective native vegetable oils enhanced \(\beta\)-oxidation in rat peroxisomes (19).

However, our finding of 16:1\(\text{cis-9}\) production on 18:1\(\text{cis-11}\) 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 16:1\(\text{cis-10}\) (likely derived from 18:1\(\text{cis-12}\)) and 16:1\(\text{cis-10}\) (likely derived from 18:1\(\text{cis-12}\)) in serum. Prospectively, studies that use 18:1\(\text{cis-11}\) labeled with stable isotopes will doubtlessly prove whether 16:1\(\text{cis-9}\) is produced via chain shortening as it was previously shown for 16:1\(\text{cis-7}\) production from 18:1\(\text{cis-9}\) (16). Such studies may also reveal whether 18:1\(\text{cis-11}\) or its metabolite \(9\)-\(\alpha\)11-CLA induced a general stimulation of peroxisomal \(\beta\)-oxidation.

In conclusion, our data strongly indicate that circulating 16:1\(\text{cis-9}\), which is considered a biomarker for lower risk of diabetes (3, 4) and coronary artery disease (20), arises endogenously from 18:1\(\text{cis-11}\), 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 participants to interventions; RK: analyzed data; AR: performed the statistical analysis; AJ and MP: wrote the manuscript; and GF: supervised the work. None of the authors declared a conflict of interest.

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