Vaccenic acid and trans fatty acid isomers from partially hydrogenated oil both adversely affect LDL cholesterol: a double-blind, randomized controlled trial1–3

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ABSTRACT

Background: Adverse effects of industrially produced trans fatty acids (iTFAs) on the risk of coronary artery disease are well documented in the scientific literature; however, effects of naturally occurring trans fatty acids (TFAs) from ruminant animals (rTFA), such as vaccenic acid (VA) and cis-9,trans-11 conjugated linoleic acid (c9,t11-CLA), are less clear. Although animal and cell studies suggest that VA and c9,t11-CLA may be hypocholesterolemic and antiatherogenic, epidemiologic data comparing rTFAs and iTFAs are inconsistent, and human intervention studies have been limited, underpowered, and not well controlled.

Objective: We determined the effects of VA, c9,t11-CLA, and iTFA, in the context of highly controlled diets (24 d each), on lipoprotein risk factors compared with a control diet.

Results: We conducted a double-blind, randomized, crossover feeding trial in 106 healthy adults [mean ± SD age: 47 ± 10.8 y; body mass index (in kg/m²): 28.5 ± 4.0; low-density lipoprotein (LDL) cholesterol: 3.24 ± 0.63 mmol/L]. Diets were designed to have stearic acid replaced with the following TFA isomers (percentage of energy): 0.1% mixed isomers of TFA (control), ~3% VA, ~3% iTFA, or 1% c9,t11-CLA. Total dietary fat (34% of energy) and other macronutrients were matched. Total cholesterol (TC), LDL cholesterol, triacylglycerol, lipoprotein(a), and apolipoprotein AI, and apolipoprotein B were higher after VA than after iTFA; high-density lipoprotein (HDL) cholesterol and apolipoprotein AI also were higher after VA. Compared with control, VA and iTFA both increased TC, LDL cholesterol, ratio of TC to HDL cholesterol, and apolipoprotein B (2–6% change; P < 0.05); VA also increased HDL cholesterol, apolipoprotein AI, apolipoprotein B, and lipoprotein(a) (2–6% change; P < 0.05), whereas iTFA did not. c9, t11-CLA lowered triacylglycerol (P ≤ 0.01) and had no effect on other lipoprotein risk factors.

Conclusions: With respect to risk of cardiovascular disease, these results are consistent with current nutrition labeling guidelines, with the requirement of VA, but not c9,t11-CLA, to be listed under TFA on the Nutrition Facts Panel. This trial was registered at clinicaltrials.gov as NCT00942656. Am J Clin Nutr 2015;102:1339–46.

Keywords: cardiovascular disease risk, industrial trans fatty acids, partially hydrogenated vegetable oil, ruminant trans fatty acids, vaccenic acid

INTRODUCTION

The effects of industrially produced trans fatty acids (iTFAs) on risk factors for chronic diseases, such as cardiovascular disease (CVD), are well documented. In contrast, the health effects of naturally occurring trans fatty acids (TFAs) found in ruminant fat, or ruminant trans fatty acids (rTFAs), are less clear. Although these 2 dietary sources of TFA (iTFA and rTFA) share common stereoisomers, the proportion of the stereoisomers differs, with vaccenic acid (VA, 18:1t11) and elaidic acid (18:1t9) being the predominant stereoisomers in rTFA and iTFA, respectively (1). Conjugated linoleic acid (CLA) also is present in rTFA, with cis-9,trans-11 CLA (c9,t11-CLA) being the most predominant isomer. Animal and cell culture studies demonstrate that the effects of rTFA may differ from those of iTFA, and they suggest that VA and c9,t11-CLA may be hypocholesterolemic and antiatherogenic (2). Some epidemiologic studies report a direct association of estimated iTFA intake with risk of coronary artery disease (CAD) and an inverse or no association with rTFA intake (3–6); however, other studies have

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2 Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

3 Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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7 Abbreviations used: apoAI, apolipoprotein AI; apoB, apolipoprotein B; CAD, coronary artery disease; CLA, conjugated linoleic acid; CVD, cardiovascular disease; c9,t11-CLA, cis-9,trans-11 conjugated linoleic acid; IDL, intermediate-density lipoprotein; iTFA, industrially produced trans fatty acid; rTFA, ruminant trans fatty acid; TC, total cholesterol; TFA, trans fatty acid; VA, vaccenic acid.

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reported that intake of TFA isomers, regardless of source, is
associated with CAD risk (7).

Dietary interventions in humans are limited (8–11), and results
from previous studies are difficult to compare and interpret be-
cause of differences in study design, lack of a control group (9),
or inadequate statistical power (10). Most previous human
studies were not highly controlled dietary interventions but
rather free-living studies (8, 9) or controlled-feeding studies in
which saturated and unsaturated fatty acids were not matched
across diets (10). Previous studies have used dairy products
enriched with rTFA, as the result of dietary manipulation of
dairy cows (12). In the process of modifying milk fat to increase
rTFA, other fatty acids also are altered (i.e., decreases in hy-
percholesterolemic SFAs and increases in neutral and hypo-
cholesterolemic fatty acids). Thus, these products are useful for
measuring the effect of rTFA as a group, but they do not allow
for the specific comparisons of individual rTFA isomers.

Concomitant with the decreasing use of iTFA in the food
supply, the proportion of dietary rTFA is increasing in relation to
total TFA intake. Based on intake data from the US Food and
Drug Administration, and assuming that intake of naturally
occurring TFAs has remained stable over the past decade at 1.2 g/
person per day, the decrease in TFA from industrial sources from
4.6 to 1.3 g/person per day corresponds to an increase in the propor-
tion of TFA from natural sources from 21% to 48% (13). An
understanding of the similarities or differences of the effects
of TFA isomers from rTFA compared with those from iTFA on
CVD risk factors is important because these effects could have an
impact on food labeling or other regulatory processes.

Because of the limited number of dietary intervention studies,
as well as the limitations of existing studies, we conducted
a double-blind, randomized, crossover, controlled-feeding study
to compare the effects of iTFA and purified VA and c9,t11-CLA
on risk factors for CVD. By using purified VA, we overcame
confounding effects of changes in other fatty acids that occur
when natural products are enriched.

METHODS

Study design

The study was registered with clinicaltrials.gov as NCT00942656,
and details of study design have been described previously (14).
Briefly, we used a double-blind, randomized, crossover design. There
were four 24-d diet periods in which participants received a completely
controlled diet designed to have stearic acid replaced with one of the
following TFA isomer preparations: 0.1% energy from mixed TFA
isomers (control), 3.3% of energy from VA, 3.3% of energy from
mixed isomers of TFA from partially hydrogenated vegetable oil
(iTFA), or 0.9% of energy from c9,t11-CLA. The added energy from
TFA replaced energy from stearic acid so that all diets contained
34% of energy from fat.

Controlled-feeding intervention

To achieve the desired fatty acid profile of each diet, we
produced test fats (provided by the Nestlé Research Center) by
blending different oils and fats. These test fats were in-
orporated into a variety of foods, including baked goods,
sauces, spreads, and mashed potatoes, which were identical
across all treatments with the exception of the test fat. All
other foods in the diet that did not include the test fats were
identical across all treatments. To achieve the required VA
concentration in the VA diet, we synthesized VA tri-
vaccenate and randomized it with other fats and oils. To
eliminate the possible effects of randomized fatty acids in
this test fat, we also randomized the 3 other fat and oil
blends. The detailed methods of production of the experi-
mental fats, as well as their fatty acid composition, have
been reported previously (14).

Details of the planned diets and feeding protocol have been
published previously (14). Briefly, participants came to the
Beltsville Human Nutrition Research Center to consume both
breakfast and dinner, Monday through Friday, under the super-
vision of a dietitian, investigator, or study personnel. Lunch and
weekend meals were packed for offsite consumption. Thus, all
food to be consumed during the intervention periods was provided
to the participants. Diets were designed such that macronutrient
composition was similar across treatment diets. Investigators,
participants, and other study personnel were blinded to the
composition of the test fats, and fats were color-coded throughout
the duration of the intervention and analyses of samples.

Participants were assigned to a treatment sequence by study
investigators and fed at an energy intake necessary to maintain
body weight. Body weight was measured, and patterns of weight
loss or weight gain were monitored. When adjustments in
energy intake were necessary to maintain body weight, portion
size was adjusted such that the nutrient content of the diet was
the same for all participants regardless of energy intake. Thus,
the absolute amount of TFA and other nutrients varied, but the
relative proportion (percentage of energy) was comparable for
all participants.

Chemical composition of the diets

During each of the 4 diet periods, diet composites were created
by collecting each of the 7 d in the menu rotation at different
calorie levels as previously described (14, 15).

Subject recruitment and selection

Adult men and women, between 25 and 65 y of age with a BMI
(in kg/m²) between 20 and 38, were recruited from the Baltimore-
Washington area to come to the Beltsville Human Nutrition
Research Center for screening. Participants were enrolled by
study investigators and were nonsmokers, free of active CVD
diabetes, and not taking medications or supplements that
alter lipid metabolism. In addition, participants were required
to have total plasma cholesterol <7.24 mmol/L, fasting tri-
acylglycerol <3.39 mmol/L, blood pressure <160/100 mm Hg
(controlled with certain medications), and fasting glucose
<7 mmol/L. Inclusion and exclusion criteria were established to
recruit generally healthy individuals across a wide age range but
potentially at risk of CVD. Recruitment began in 2008, and
study participants completed the intervention in 2009. Of the
115 participants who were enrolled in the study, 95 completed the
intervention. Detailed exclusion criteria and recruitment statistics
have been published previously (14). Study procedures were in
accordance with the Declaration of Helsinki. The protocol was
approved by the MedStar Health Institutional Review Board, and all participants provided written informed consent.

Blood collections

Fasting blood samples (12-h fast) were collected by venipuncture on 2 d, separated by at least 24 h, before the start of the intervention (baseline) and at the end of each of the 4 diet periods. Serum and plasma were collected from blood and stored at −80°C until completion of the study for analyses (14).

Laboratory analyses

Lipids and lipoproteins

Plasma was analyzed for concentrations of total cholesterol (TC) (16) and triacylglycerol (17), as well as for HDL cholesterol, measured after precipitation of apolipoprotein B (apoB)–containing lipoproteins in plasma (Corning Express 550 automated analyzer; Ciba-Corning Diagnostics Corp.) (18). Lipid measurements were standardized through the CDC–National Heart, Lung, and Blood Institute Lipid Standardization Program. LDL cholesterol was calculated by using the Friedewald equation (19). Lipoprotein(a) was measured in triplicate (CV <10%) by sandwich-style ELISA. The lipoprotein(a) antiserum was purified by precipitation (International Immunology Corporation). All controls were validated by outside measurement (Northwest Lipid Laboratory). Plasma apoB and apolipoprotein A-I (apoAI) concentrations were measured with an immunoturbidimetric assay (20, 21). Reagents, standards, and reference plasma controls, with and without elevated lipids, are included in the immunoturbidimetric assay kit (Bacton Assay Systems, Inc.). Calibrators and reference controls were assigned concentration values with the use of International Federation of Clinical Chemistry standard reference materials SP1 for apoAI and SP3-07 for apoB. Plasma apolipoprotein AII was measured in triplicate (CV <10%) by sandwich-style ELISA. The apolipoprotein AII antiserum was purified by precipitation (International Immunology Corporation). All controls were validated by outside measurement (Northwest Lipid Laboratory).

VLDL, intermediate-density lipoprotein (IDL), and LDL subfractions were analyzed by ion mobility, which uniquely allows for direct particle quantification in defined lipoprotein size intervals after a procedure to remove nonlipoprotein proteins (22). Interassay variation was reduced by inclusion of 2 in-house controls in each preparatory process and duplicate analysis (CV <15%). Lipoprotein subfractions were defined according to previous nomenclature (23) as HDL small (equivalent to HDL3 +2a), HDL large (equivalent to HDL2b), LDL large (LDL I), LDL medium (LDL II), LDL small (LDL III), LDL very small (LDL IV), IDL large (IDL 1), and IDL small (IDL 2) (22). On the basis of peak LDL diameter, individuals were classified into 1 of 3 LDL size phenotypes: A (predominance of large LDL particles), B (predominance of small, dense LDL particles), or intermediate (type A/B).

Markers of inflammation and adhesion molecules

Fibrinogen and factor VII were measured in sodium citrate plasma by automated coagulation/light-scattering assay (Instrumentation Laboratory Company) performed on a Coulter ACL 1000 coagulation analyzer (Beckman Coulter Inc.). C-reactive protein, IL-6, and E-selectin were measured in serum by sandwich ELISA (R&D Systems) performed on an automated ELISA system (Dynex Technologies Inc.).

Glucose, insulin, and insulin resistance

Glucose was measured in sodium fluoride/EDTA plasma with an automated enzymatic/colorimetric assay (Dimension Clinical Chemistry System; Siemens Health Care Diagnostics Inc.). Insulin was measured in serum by ELISA (Millipore Corp.) performed on an automated ELISA system (Dynex Technologies Inc.). HOMA-IR was calculated by multiplying fasting serum concentrations of glucose (mg/dL) and insulin (mg/dL) and dividing by 405 (24).

Statistical analyses

Participants were stratified by sex and LDL cholesterol from screening and were randomly assigned by a study investigator to a treatment sequence consisting of each of the 4 treatment diets (14). Data for statistical analyses were included for participants who completed at least one of the 4 diet periods. Values from the 2 samples collected at each study time point were averaged and used for statistical analyses. Data were reviewed for outliers before statistical analyses. All statistical analyses were performed with SAS (Version 9.2; SAS Institute) by using a mixed model (PROC MIXED) to determine whether effects were statistically significant (P ≤ 0.05). Pretreatment baseline values of the dependent variables were used to adjust posttreatment values. Other independent variables that were tested included characteristics of the individual (BMI, sex), design variables (period and sequence in which the diets were administered), and their interactive effects. Contrast statements were used to make the following comparisons: iTFA and control, VA and control, iTFA and VA, and c9,t11-CLA and control. Sample size was calculated based on previous lipid studies conducted at the Beltsville Human Nutrition Research Center. On the basis of these studies, we predicted a mean ± SD change in LDL cholesterol (primary outcome) of 0.116 ± 0.349 mmol/L when comparing the control and iTFA diets. Sample size (n = 120) was calculated based on detection with 95% probability and 90% power while accounting for a~15% dropout rate, which has been observed in previous studies of similar design.

RESULTS

Of the 116 participants who were randomized in the study, 106 completed at least one of the 4 treatments. The baseline characteristics of the 106 study participants whose data are included in statistical analyses are presented in Table 1. The reasons for dropping out of the study protocol have been reported previously (14). Body mass of participants was stable across the intervention, with mean ± SEM body weight at the beginning and end of the intervention of 80.5 ± 1.3 and 79.2 ± 1.4 kg, respectively. Mean ± SEM energy intake during the intervention was 2700 ± 360 and 2220 ± 310 kcal/d for men and women, respectively.

Chemical analyses of the diets (Table 2) confirmed that actual composition was similar to the designed composition. Total saturated, monounsaturated, and polyunsaturated fatty acid content of the consumed diets was within 1% of energy of the planned diets. The amount of VA in the VA diet was 0.6% of energy higher than the total TFA in the iTFA diet (3.86% of energy compared with 3.26% of energy, respectively).
TABLE 1
Baseline characteristics of 106 study participants

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
</tr>
<tr>
<td>Female</td>
<td>59</td>
</tr>
<tr>
<td>Age, y</td>
<td>47 ± 10.82</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>80.5 ± 13.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.5 ± 4.0</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.00 ± 0.74</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.24 ± 0.63</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.24 ± 0.30</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.12 ± 0.50</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>127.6 ± 13.6</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76.1 ± 9.2</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.17 ± 0.5</td>
</tr>
</tbody>
</table>

DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerol.

Mean ± SD (all such values).

When the VA diet and the iTFA diet were compared, consumption of the VA diet led to higher TC (P < 0.001), LDL cholesterol (P < 0.05), triacylglycerol (P < 0.05), apoB (P < 0.01), and lipoprotein(a) (P < 0.01), as well as higher HDL cholesterol (P < 0.01) and apoAI (P < 0.05). In comparison with the control diet, consumption of the iTFA diet increased TC (P < 0.05), LDL cholesterol (P < 0.01), apoB (P < 0.05), and the ratio of TC:HDL cholesterol (P < 0.05) (Table 3). The VA diet increased TC (P < 0.0001), LDL cholesterol (P < 0.0001), lipoprotein(a) (P < 0.01), apoB (P < 0.0001), and the ratio of TC:HDL cholesterol (P < 0.0001) compared with control. In addition, VA increased HDL cholesterol (P < 0.05) and apoAI (P < 0.0001), whereas the iTFA diet did not.

Dietary effects on lipoprotein subfractions (Table 4) were generally consistent with those for the standard lipid and lipoprotein measures. When the VA and iTFA diets were compared, consumption of VA increased large HDL (P < 0.01), small VLDL (P < 0.01), IDL (P < 0.001), and large LDL (P < 0.01), medium LDL (P < 0.01), small LDL in men (P < 0.05) but not in women, as well as very small LDL (P < 0.05). Compared with control, VA increased intermediate VLDL (P < 0.05) and small VLDL (P < 0.001), IDL (P < 0.001), and large and medium LDL (P < 0.01), and small LDL in men (P < 0.001) but not in women. Consumption of the iTFA diet decreased large HDL compared with the control diet. c9,t11-CLA did not change lipoprotein subfractions compared with the control diet. Changes in LDL peak diameter were not different across the treatments. Most of the study participants were classified as LDL phenotype A (84% A, 6% B, 10% A/B) at baseline. There was no overall change in LDL phenotype due to diet (data not shown). Fibrinogen was lower after consumption of the VA diet compared with the control (P < 0.0001) and iTFA diets (P < 0.001; Suplemental Table 1). Markers of inflammation and adhesion molecules were not different across treatments. In addition, there

TABLE 2
Chemical analysis of treatment diets (percentage of energy)

<table>
<thead>
<tr>
<th></th>
<th>Control1</th>
<th>iTFA</th>
<th>VA</th>
<th>c9,t11-CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>17.0 ± 0.13</td>
<td>17.2 ± 0.07</td>
<td>17.0 ± 0.12</td>
<td>17.1 ± 0.12</td>
</tr>
<tr>
<td>Fat</td>
<td>33.3 ± 0.31</td>
<td>33.0 ± 0.19</td>
<td>33.4 ± 0.23</td>
<td>33.3 ± 0.17</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>49.7 ± 0.25</td>
<td>49.8 ± 0.15</td>
<td>49.5 ± 0.20</td>
<td>49.6 ± 0.15</td>
</tr>
<tr>
<td>SFAs</td>
<td>15.9 ± 0.04</td>
<td>12.5 ± 0.05</td>
<td>12.4 ± 0.02</td>
<td>14.9 ± 0.04</td>
</tr>
<tr>
<td>Lauric</td>
<td>0.43 ± 0.006</td>
<td>0.42 ± 0.007</td>
<td>0.40 ± 0.003</td>
<td>0.43 ± 0.005</td>
</tr>
<tr>
<td>Myristic</td>
<td>0.05 ± 0.005</td>
<td>0.05 ± 0.003</td>
<td>0.05 ± 0.002</td>
<td>0.05 ± 0.004</td>
</tr>
<tr>
<td>Palmitic</td>
<td>5.74 ± 0.055</td>
<td>5.90 ± 0.104</td>
<td>5.93 ± 0.025</td>
<td>5.84 ± 0.063</td>
</tr>
<tr>
<td>Stearic</td>
<td>9.24 ± 0.105</td>
<td>5.68 ± 0.115</td>
<td>5.61 ± 0.058</td>
<td>8.18 ± 0.126</td>
</tr>
<tr>
<td>Other</td>
<td>0.44 ± 0.002</td>
<td>0.44 ± 0.002</td>
<td>0.38 ± 0.002</td>
<td>0.44 ± 0.003</td>
</tr>
<tr>
<td>MUFAs</td>
<td>9.85 ± 0.070</td>
<td>9.77 ± 0.089</td>
<td>9.74 ± 0.047</td>
<td>9.87 ± 0.048</td>
</tr>
<tr>
<td>Oleic</td>
<td>9.17 ± 0.138</td>
<td>8.92 ± 0.176</td>
<td>8.94 ± 0.090</td>
<td>9.18 ± 0.092</td>
</tr>
<tr>
<td>Other</td>
<td>0.68 ± 0.002</td>
<td>0.86 ± 0.002</td>
<td>0.81 ± 0.003</td>
<td>0.69 ± 0.004</td>
</tr>
<tr>
<td>PUFAs</td>
<td>5.67 ± 0.015</td>
<td>5.89 ± 0.026</td>
<td>5.77 ± 0.031</td>
<td>5.81 ± 0.017</td>
</tr>
<tr>
<td>Linoleic</td>
<td>5.10 ± 0.037</td>
<td>5.30 ± 0.062</td>
<td>5.20 ± 0.080</td>
<td>5.23 ± 0.041</td>
</tr>
<tr>
<td>α-Linolenic</td>
<td>0.45 ± 0.005</td>
<td>0.46 ± 0.012</td>
<td>0.45 ± 0.009</td>
<td>0.46 ± 0.005</td>
</tr>
<tr>
<td>Other</td>
<td>0.13 ± 0.002</td>
<td>0.13 ± 0.004</td>
<td>0.13 ± 0.003</td>
<td>0.12 ± 0.004</td>
</tr>
<tr>
<td>Trans fatty acids</td>
<td>0.28 ± 0.002</td>
<td>3.26 ± 0.014</td>
<td>3.93 ± 0.012</td>
<td>0.32 ± 0.009</td>
</tr>
<tr>
<td>Palmitelaidic</td>
<td>0.02 ± 0.000</td>
<td>0.02 ± 0.001</td>
<td>0.02 ± 0.001</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td>Elaidic</td>
<td>0.21 ± 0.007</td>
<td>2.87 ± 0.050</td>
<td>0.00 ± 0.000</td>
<td>0.24 ± 0.032</td>
</tr>
<tr>
<td>trans-Vaccenic</td>
<td>0.02 ± 0.001</td>
<td>0.30 ± 0.005</td>
<td>3.86 ± 0.044</td>
<td>0.02 ± 0.003</td>
</tr>
<tr>
<td>Other</td>
<td>0.03 ± 0.000</td>
<td>0.06 ± 0.000</td>
<td>0.05 ± 0.001</td>
<td>0.04 ± 0.001</td>
</tr>
<tr>
<td>Conjugated linoleic acids</td>
<td>0.04 ± 0.002</td>
<td>0.06 ± 0.002</td>
<td>0.04 ± 0.002</td>
<td>0.04 ± 0.001</td>
</tr>
<tr>
<td>Linoleic</td>
<td>0.02 ± 0.001</td>
<td>0.04 ± 0.001</td>
<td>0.02 ± 0.001</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td>Conjugated linoleic</td>
<td>0.02 ± 0.002</td>
<td>0.02 ± 0.003</td>
<td>0.03 ± 0.003</td>
<td>0.82 ± 0.034</td>
</tr>
</tbody>
</table>

1Values are means ± SEMs, n = 8 samples for each treatment diet. Chemical composition of diets from chemical analyses of weekly composites of food collected throughout the study intervention period. c9,t11-CLA, cis-9,trans-11 conjugated linoleic acid; iTFA, industrially produced trans fatty acid; VA, vaccenic acid.

Control is the control diet from which energy from stearic acid was replaced with energy from iTFA, VA, or c9,t11-CLA.
were no differences in glucose, insulin, or HOMA-IR across treatments (Supplemental Table 1).

**DISCUSSION**

**Atherogenic lipoproteins**

To our knowledge, the present study is the first adequately powered human study to compare the effects of a highly controlled diet containing purified VA with that of one containing iTFA, as well as a control diet, on markers of CVD risk. The increase in LDL cholesterol after consumption of the VA diet is consistent with a previous study in men that reported an increase in LDL cholesterol after a diet high in rTFA-enriched butter (3.7% of energy from rTFA) compared with a low TFA control diet (0.8% energy) and moderate rTFA diet (1.5% of energy) (10). The lipoprotein subfraction data from the present study confirm the adverse effects of VA on atherogenic lipoproteins, with increases across subfractions compared with the iTFA and control diets. Our data demonstrate that VA and partially hydrogenated vegetable oil both adversely affect atherogenic lipoproteins, with higher concentrations of LDL cholesterol, apoB, triacylglycerol, and lipoprotein(a) after the VA diet.

**TABLE 4**

<table>
<thead>
<tr>
<th></th>
<th>Control²</th>
<th>iTFA</th>
<th>VA</th>
<th>c9,11-CLA</th>
<th>VA vs. iTFA</th>
<th>VA vs. control</th>
<th>iTFA vs. control</th>
<th>CLA vs. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mmol/L</td>
<td>4.86 ± 0.04</td>
<td>4.95 ± 0.04</td>
<td>5.08 ± 0.04</td>
<td>4.80 ± 0.04</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
<td>0.0234</td>
<td>0.1182</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.94 ± 0.04</td>
<td>3.04 ± 0.04</td>
<td>3.12 ± 0.04</td>
<td>2.93 ± 0.04</td>
<td>0.0114</td>
<td>&lt;0.0001</td>
<td>0.0028</td>
<td>0.6054</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.40 ± 0.02</td>
<td>1.40 ± 0.02</td>
<td>1.43 ± 0.02</td>
<td>1.39 ± 0.02</td>
<td>0.0026</td>
<td>0.0110</td>
<td>0.6315</td>
<td>0.2927</td>
</tr>
<tr>
<td>TC:HDL cholesterol</td>
<td>3.64 ± 0.04</td>
<td>3.71 ± 0.04</td>
<td>3.75 ± 0.04</td>
<td>3.61 ± 0.04</td>
<td>0.2932</td>
<td>0.0005</td>
<td>0.0126</td>
<td>0.3675</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.13 ± 0.03</td>
<td>1.11 ± 0.03</td>
<td>1.16 ± 0.03</td>
<td>1.06 ± 0.03*</td>
<td>0.0029</td>
<td>0.1488</td>
<td>0.4518</td>
<td>0.0026</td>
</tr>
<tr>
<td>Apo(a), nmol/L</td>
<td>0.95 ± 0.02</td>
<td>0.95 ± 0.02</td>
<td>0.95 ± 0.02</td>
<td>0.95 ± 0.02</td>
<td>0.0042</td>
<td>0.0016</td>
<td>0.7562</td>
<td>0.9138</td>
</tr>
<tr>
<td>ApoAI, g/L</td>
<td>1.30 ± 0.01</td>
<td>1.31 ± 0.01</td>
<td>1.35 ± 0.01*</td>
<td>1.30 ± 0.01</td>
<td>0.0043</td>
<td>&lt;0.0001</td>
<td>0.1097</td>
<td>0.5505</td>
</tr>
<tr>
<td>ApoAII, g/L</td>
<td>0.29 ± 0.004</td>
<td>0.28 ± 0.004</td>
<td>0.29 ± 0.004</td>
<td>0.28 ± 0.004</td>
<td>0.3163</td>
<td>0.7013</td>
<td>0.5320</td>
<td>0.1726</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>0.85 ± 0.009</td>
<td>0.87 ± 0.009*</td>
<td>0.89 ± 0.009*</td>
<td>0.84 ± 0.009</td>
<td>0.0025</td>
<td>&lt;0.0001</td>
<td>0.0173</td>
<td>0.1231</td>
</tr>
</tbody>
</table>

¹n = 106. All statistical analyses were performed with SAS (version 9.2; SAS Institute) by using a mixed-model analysis (PROC MIXED) to determine whether effects were significant (P ≤ 0.05). Contrast statements were used to make the following comparisons: iTFA and control, VA and control, iTFA and VA, and c9,11-CLA and control. ²Significant difference compared with control as defined by P ≤ 0.05; ³significant difference between iTFA and VA as defined by P ≤ 0.05. ApoAI, apolipoprotein AI; ApoAII, apolipoprotein AII; ApoB, apolipoprotein B; CLA, conjugated linoleic acid; c9,11-CLA, cis-9, trans-11 conjugated linoleic acid; iTFA, industrially produced trans fatty acid; Lp(a), lipoprotein(a); TC, total cholesterol; TG, triacylglycerol; VA, vaccenic acid. ²Control is the control diet from which energy from stearic acid was replaced with energy from iTFA, VA, or c9,11-CLA. ³Mean ± SEM (all such values).
Investigate the specific effects of highly purified VA in humans.

Strengths and limitations

The use of VA, within the context of a highly controlled diet, was a strength in the current study because it allowed us to investigate the specific effects of highly purified VA in humans, which until now has been unknown. In previous studies, which used dairy products (regular or naturally enriched with rTFA), ascertaining the specific effects of VA is difficult, because multiple fatty acids are modified from this enrichment. An additional strength of our study was the use of stearic acid as the control. The replacement of stearic acid for VA, iTFA, and c9,t11-CLA allowed all diets to contain a similar amount of fat and provided a comparison to a diet with a fatty acid that has neutral effects on LDL cholesterol and HDL cholesterol.

It is important to note that the amount of VA used in the VA diet (3.93% of energy) and the amount of iTFA used in the iTFA diet (3.26% of energy) were higher than current intakes in the United States, estimated to be ~0.35% of energy (~6–8 times the estimated intake) (1, 26) and ~0.56% of energy (13), respectively. However, subgroups in the population may have such intakes, particularly of iTFA. Although the VA and iTFA diets were designed to have matched energy from TFA, the actual chemical composition of the diets differed in TFA amount by 0.67% of energy. It is unclear whether this small difference in TFA amount between the VA and iTFA diets had any impact.

Both natural and industrial food sources contain multiple TFA isomers. trans-18:1 is the predominant TFA in both sources, but the isomeric distribution of trans-18:1 differs. Although VA is the predominant TFA in ruminant fat (43–60% of total 18:1 TFA isomers), produced during biohydrogenation in ruminant animals, it also is produced during the hydrogenation of plant oils and consequently present in partially hydrogenated vegetable oil (~15% of total 18:1 TFA isomers) (43). The use of synthesized VA in a randomized triglyceride as part of a highly controlled diet allowed us to investigate the specific effects of VA; however, it is not the form of typical VA consumption (i.e., dairy products and ruminant meat). Evidence (44, 45) suggests that VA consumed in amounts and foods typically found in the diet is inversely or not associated with CVD risk.

Implications

In 2003, the US Food and Drug Administration ruled that TFA would be required to be listed on the Nutrition Facts Panel of the food label (46). The ruling defines TFA as fatty acids containing one or more isolated double bonds in the trans configuration. Consequently, c9,t11-CLA is excluded from being listed, because it contains conjugated double bonds in the trans configuration; however, other rTFA isomers, such as VA, meet the definition and are required to be included. Dietary regulations and recommendations to reduce TFA have been predominantly based on the body of evidence demonstrating the numerous adverse health effects of iTFA. Data from a recent study suggest that public health efforts to reduce intake of TFA and remove TFA from the food supply in the past decade have been successful, as indicated by a reduction in intake of TFA (i.e., elaidic acid, VA, palmitelaidic acid, and linoleaidic acid) by >50% in the population sampled (47). As a result, the amount of rTFA as a proportion of total TFA has increased and is now a major contributor of TFA in the diet. This trend is expected to continue due to the recent determination by the US Food and Drug Administration that partially hydrogenated oils are no longer “generally recognized as safe” (48).

Questions have been raised as to whether naturally occurring TFA from ruminant sources should be differentiated from iTFA,
with regard to dietary guidance and regulations. The results from the present study demonstrate that at higher than usual US intakes, VA (≥ 10 times the estimated intake) and iTFA (≥ 6 times the intake) both adversely affect atherogenic lipoproteins, whereas c9,11-CLA (≥ 10–18 times the intake) does not adversely affect lipoproteins, when replaced for stearic acid. These results support the current labeling guidelines, with the requirement of VA, but not c9,11-CLA, to be listed under TFA on the Nutrition Facts Panel.

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The authors’ responsibilities were as follows—SKG and DJB: conducted the research, drafted the manuscript, had full access to all data in the study, and took responsibility for the integrity of the data and the accuracy of the data analysis; F Destaillats, F Dionisi, and DJB: designed the research; SKG, RMK, and DJB: acquired the data; SKG, F Destaillats, RMK, and DJB: analyzed and interpreted the data; DJB: had primary responsibility for the final content; and all authors: critically reviewed the manuscript for important intellectual content. F Destaillats and F Dionisi are employees of Nestlé.

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46. Food labeling; trans fatty acids in nutrition labeling; consumer research to consider nutrient content and health claims and possible footnote or disclosure statements; final rule and proposed rule. Fed Regist 2003;68:41433.
