Introduction

There are 2 main dietary sources of trans fatty acids (TFA)\(^9\), an industrial source derived from the partial hydrogenation of vegetable oils (PHVO) and a natural source that originates from ruminant-derived food products. Intake of industrial-derived vegetable oils (PHVO) and a natural source that originates from industrial source derived from the partial hydrogenation of vegetable oil; RA, rumenic acid; TFA, trans fatty acids; PHVO, partially hydrogenated vegetable oil; CI-MS/MS, chemical ionization tandem MS; CLA, conjugated linoleic acid; EA, elaidic acid; IDL, intermediate-density lipoprotein; LC-PUFA, long chain-PUFA; PHVO, partially hydrogenated vegetable oil; RA, rumenic acid; TFA, trans fatty acids; VA, vaccenic acid.

TFA has been associated with the progression of several chronic human diseases, especially coronary heart disease (CHD) (1), and an impairment of essential fatty acid metabolism (2,3). These relationships have not been established for TFA from ruminant products; rather, as reviewed by Lock et al. (4), the available epidemiological data suggest a neutral or potentially beneficial effect of natural TFA on the relative risk of CHD.

The adverse health effects observed with industrial TFA have been traditionally associated with the presence of trans 18:1 fatty acids in PHVO. Trans 18:1 fatty acids comprise nearly one-half of the fatty acids in PHVO and contain a Gaussian distribution of isomers centering on trans-9, -10, -11, and -12 18:1 (5). In contrast, the TFA content of ruminant sources is largely trans-11 18:1 [vaccenic acid (VA)], which is the precursor for endogenous synthesis of cis-9, trans-11 18:2 [rumenic acid (RA)], a fatty acid shown to have anticarcinogenic and antiatherogenic activity in biomedical studies with animal models (6). The possible contribution of individual TFA to overall CHD risk remains largely unknown. The objective of the present study was to investigate the effects of 2 major trans 18:1 isomers, trans-9 18:1 [elaidic acid (EA)] and trans-11 18:1 [vaccenic acid (VA)] on plasma lipid biomarkers of CHD risk.

Thirty-two male Golden Syrian hamsters were randomly assigned to 1 of 4 dietary treatments: 1) control “Western” diet; 2) PHVO supplement; 3) EA supplement; and 4) VA supplement. Fat supplements were incorporated into the respective treatment diets at 2.5 g/100 g of diet. Compared with the control diet, the PHVO diet increased the plasma ratios of total:HDL-cholesterol and non-HDL:HDL-cholesterol by 17 and 23%, respectively. In contrast, these values decreased by 27 and 46% after the EA treatment and 8 and 14% after the VA treatment, respectively, indicating an improvement (reduction) in CHD risk. With regard to liver lipids, the EA diet reduced the content of (n-3) and (n-6) PUFA relative to the other treatments, suggesting an inhibition of enzymes common to the 2 biosynthesis pathways. Overall, results demonstrate that the hypercholesterolemic effects of PHVO are not dependent on the presence of EA or VA and that other bioactive components in PHVO must be responsible for its associated adverse health effects. J. Nutr. 139: 257–263, 2009.

Abstract

Trans fatty acids (TFA) from industrial sources [i.e. partially hydrogenated vegetable oil (PHVO)] have been associated with several chronic human diseases, especially coronary heart disease (CHD). The possible contribution of individual TFA to overall CHD risk remains largely unknown. The objective of the present study was to investigate the effects of 2 major trans 18:1 isomers, trans-9 18:1 [elaidic acid (EA)] and trans-11 18:1 [vaccenic acid (VA)] on plasma lipid biomarkers of CHD risk. Thirty-two male Golden Syrian hamsters were randomly assigned to 1 of 4 dietary treatments: 1) control “Western” diet; 2) PHVO supplement; 3) EA supplement; and 4) VA supplement. Fat supplements were incorporated into the respective treatment diets at 2.5 g/100 g of diet. Compared with the control diet, the PHVO diet increased the plasma ratios of total:HDL-cholesterol and non-HDL:HDL-cholesterol by 17 and 23%, respectively. In contrast, these values decreased by 27 and 46% after the EA treatment and 8 and 14% after the VA treatment, respectively, indicating an improvement (reduction) in CHD risk. With regard to liver lipids, the EA diet reduced the content of (n-3) and (n-6) PUFA relative to the other treatments, suggesting an inhibition of enzymes common to the 2 biosynthesis pathways. Overall, results demonstrate that the hypercholesterolemic effects of PHVO are not dependent on the presence of EA or VA and that other bioactive components in PHVO must be responsible for its associated adverse health effects.

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Abbreviations used: CHD, coronary heart disease; CI-MS/MS, chemical ionization tandem MS; CLA, conjugated linoleic acid; EA, elaidic acid; IDL, intermediate-density lipoprotein; LC-PUFA, long chain-PUFA; PHVO, partially hydrogenated vegetable oil; RA, rumenic acid; TFA, trans fatty acids; VA, vaccenic acid.

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Studies with biomedical models have indicated a relatively neutral effect of trans-9 18:1 (elaidic acid (EA)) compared with VA and cis-9 18:1 (oleic acid) on biomarkers of CHD risk using the Golden Syrian hamster (7,8). However, in both of these studies, trans 18:1 isomers represented the predominant fatty acid in the diet and this does not reflect the normal human intake, where TFA intake rarely exceeds >2% of total energy (9).

In this study, our objective was to examine effects of 2 trans 18:1 isomers, EA, a major fatty acid component of PHVO, and VA, the predominant TFA in ruminant fat, on biomarkers of CHD risk using the cholesterol-fed Golden Syrian hamster as our biomedical model. Diets were rich in saturated fat (primarily 16:0), cis-9 18:1, and 18:2 (n-6) and formulated to approximate the dietary fatty acid composition typically consumed in many Westernized countries (10). PHVO was included as a positive-control treatment for determining if the adverse effects associated with industrial TFA are dependent on the presence of EA or VA.

We are not aware of any previous investigations that have directly compared these trans 18:1 isomers with the effects for PHVO.

Materials and Methods

Hamsters and diets. All procedures involving hamsters were subject to UK Home Office regulations. Thirty-two male Golden Syrian Hamsters (13–14 wk old; weight, 108.2 ± 9.9 g, mean ± SD; Harlan UK) were individually housed and maintained in a controlled environment (21°C; 55% humidity) with a 12-h-light/dark cycle. Following acclimatization, hamsters were randomly assigned to 1 of 4 treatment diets: 1) control “Western” diet; 2) PHVO supplement; 3) EA supplement; and 4) VA supplement. Per 100 g of diet, treatment diets contained 84.8 g of rodent diet (Rat and Mouse Breeding Diet No. 3, Special Diet Services; detailed diet composition has been previously reported by Lock et al. (11) (Supplemental Table 1)), 15.0 g of fat blend, and 0.2 g of crystalline cholesterol (Sigma) (Table 1). The fat blend for the control diet was formulated to approximate the quantity and quality of fat consumed in a typical “Western” diet. The fatty acid composition of the experimental diets is provided online in Supplemental Table 1. Diets were fed for 4 wk, during which hamsters were allowed free access to food and water. Body weights were measured every 3 d and 1 hamster (VA treatment) was removed from the study due to excessive weight loss. At the end of the trial, hamsters were anesthetized using an intraperitoneal injection of sodium pentobarbitone (Sagatal; 1 mL/kg body weight), and blood (3–4 mL) was obtained by cardiac puncture and collected into EDTA tubes.

Livers and 2 adipose tissue depots, perirenal and epididymal, were removed, weighed, and snap frozen in liquid nitrogen. Plasma was isolated by centrifugation at 2500 × g; 20 min at 4°C.

Source of fatty acid supplements. The PHVO supplement was supplied to the diet as triglycerides; smaller amounts of monoglycerides, diglycerides, and FFA were also present in the PHVO supplement (Cargill). The EA and VA supplements were supplied to the diet as FFA. EA and VA supplements were synthesized and purified by proprietary procedures based on methods referenced in Lock et al. (12). Procedures used to characterize were also described previously (12). The EA product (84% purity) was a yellow solid, melting at 42–43°C; the VA product (84% purity) was similar in appearance and melted at 45–46°C.

Separation and analysis of plasma lipoproteins. Lipoprotein fractions were separated by sequential ultracentrifugation. Chylomicrons, VLDL, intermediate-density lipoproteins (IDL), LDL, and HDL were separated according to McAteer et al. (13). Cholesterol and triglyceride concentrations were quantified for plasma total and the individual lipoprotein fractions using enzymatic kits (Infinity kits; Alpha Laboratories) according to the manufacturer’s directions with modifications for microplate analysis (Thermo Scientific).

Hepatic cholesterol and triglyceride analyses. Frozen livers were homogenized in sodium sulfate and total lipids extracted according to Hara and Radin (14) using hexane/isopropanol (3:2) and sodium sulfate. Lipids were analyzed for hepatic concentrations of cholesterol and triglyceride as described for plasma.

Isolation of hepatic total RNA and determination of mRNA abundance. Frozen livers were homogenized on ice in Trizol (Invitrogen) according to the manufacturer’s directions and RNA was subsequently quantified with DNase I (Promega). cDNA synthesis was carried out using reagents from Promega. Quantitative RT-PCR utilized an ABI Prism 7700 sequence detector (Applied Biosystems). Primers and dual-labeled fluorescent probes (Taqman) were designed based on hamster-specific sequences (15).

GC analyses of FAME. Dietary lipids were extracted according to Hara and Radin (14) and transmethylated according to Christie (16) with modifications by Chouinard et al. (17). Fat supplements were methylated using 2% methanolic sulfuric acid and toluene for 4 h at 50°C (18). Liver lipids were extracted as described for hepatic cholesterol analyses and methylated using 2% methanolic sulfuric acid and toluene overnight at 40°C. Following methylation, hepatic FAME were separated from free cholesterol using solid phase extraction with aminopropyl columns (Varian) as described by Agren et al. (19). cDNA synthesis was carried out using reagents from Promega. Quantitative RT-PCR utilized an ABI Prism 7700 sequence detector (Applied Biosystems). Primers and dual-labeled fluorescent probes (Taqman) were designed based on hamster-specific sequences (15).

MS analysis of FAME. Covalent adduct CI-MS/MS of FAME was carried out using acetonitrile on a Varian Star 3400 gas chromatograph (Varian) equipped with a BPX-70 column (60-m × 0.25-mm i.d. with 0.25-μm film; Varian) according to McAteer et al. (20) and Lawrence and Brenna (21). Distribution and quantification of 18:1, 18:2, and 20:1 fatty acids were carried out by plotting modification by Chouinard et al. (17). Fat supplements were methylated using 2% methanolic sulfuric acid and toluene for 4 h at 50°C (18). Liver lipids were extracted as described for hepatic cholesterol analyses and methylated using 2% methanolic sulfuric acid and toluene overnight at 40°C. Following methylation, hepatic FAME were separated from free cholesterol using solid phase extraction with aminopropyl columns (Varian) as described by Agren et al. (19). FAME were quantified by GC (GCD system HP 6890+; Hewlett Packard) equipped with a CP-Sil 88 fused silica capillary column (100-m × 0.25-mm i.d. with 0.25-μm film; Varian) according to Lock et al. (11). Peaks were identified using pure FAME standards (Nu-Chek Prep) and by chemical ionization tandem MS (CI-MS/MS).

Statistical analyses. Statistical analysis was conducted using the Fit Model platform of JMP (2006 SAS Institute, 6.0.2) to fit mixed models and involved the fixed effect of treatment. For gene expression data, mRNA abundance was corrected using the geometric mean of 3 housekeeping genes (β-actin, glyceraldehyde-3-phosphate dehydrogenase, and TATA-box binding protein). Effects were considered significant at P < 0.05. Values are presented as means ± SEM.

TABLE 1 Composition of experimental diets.

<table>
<thead>
<tr>
<th>Diet component</th>
<th>Control</th>
<th>PHVO</th>
<th>EA</th>
<th>VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent diet</td>
<td>84.8</td>
<td>84.8</td>
<td>84.8</td>
<td>84.8</td>
</tr>
<tr>
<td>Western fat blend</td>
<td>15.0</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Tallow</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Tripalmitin</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Rapeseed oil</td>
<td>3.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PHVO supplement</td>
<td>—</td>
<td>2.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EA supplement</td>
<td>—</td>
<td>—</td>
<td>2.5</td>
<td>—</td>
</tr>
<tr>
<td>VA supplement</td>
<td>—</td>
<td>—</td>
<td>2.5</td>
<td>—</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

1 Detailed diet composition previously reported by Lock et al. (11; Supplemental Table 1); Gross macronutrient composition (g/100 g) was as follows: 51.2 carbohydrate, 22.3 crude protein, 4.3 crude oil, 4.5 crude fiber, and 7.7 ash.
The fatty acid composition of the fat supplements (PHVO, EA, and VA) is detailed in Table 2. The content of trans 18:1 fatty acids differed among fat supplements, averaging 52.0, 84.5, and 84.4% of total fatty acids for PHVO, EA, and VA, respectively, and provided an additional 1.1, 2.1, and 2.1 g of trans 18:1 fatty acids per 100 g of diet in the respective dietary treatments. The EA and VA supplements also contained small amounts of 16:0, 18:0, and cis-9 18:1. Approximately 18.7% of fatty acids in the PHVO supplement were cis isomers of 18:1, including cis-8, -9, -10, -11, and -12. The PHVO supplement also contained numerous homoallylic 18:2 isomers accounting for 4.6% of total fatty acids (isomer distribution not defined). Fat supplements also differed in the total content of SFA, averaging 23.0, 6.1, and 6.4% of fatty acids for PHVO, EA, and VA, respectively. However, overall differences in the SFA content of the diets was relatively modest, because the supplements replaced rapeseed oil and comprised only 16% of total added fat (Supplemental Table 1). Furthermore, the amount of sunflower oil in the supplemented diets was increased to ensure similar 18:2(n-6) contents; the major difference between the control diet and supplemented diets was replacement of cis-9 18:1 with trans 18:1 isomers.

Dietary treatment did not affect final body weight or feed intake, which were 109.7 ± 1.4 g and 5.9 ± 0.1 g/d, respectively, across all treatments (Supplemental Table 2). Treatments had a small effect on tissue weights; hamsters fed the control diet had the greatest epididymal adipose weights (percent body weight) but the lowest liver weights (percent body weight). Hepatic concentrations of cholesterol and triglycerides were within the range typically observed for hypercholesterolemic hamsters and were unaffected by treatment, with 301.2 ± 8.9 μmol/liver and 74.9 ± 2.4 μmol/liver, respectively, across all treatments.

### Results

The fatty acid composition of the fat supplements (PHVO, EA, and VA) is detailed in Table 2. The content of trans 18:1 fatty acids differed among fat supplements, averaging 52.0, 84.5, and 84.4% of total fatty acids for PHVO, EA, and VA, respectively, and provided an additional 1.1, 2.1, and 2.1 g of trans 18:1 fatty acids per 100 g of diet in the respective dietary treatments. The EA and VA supplements also contained small amounts of 16:0, 18:0, and cis-9 18:1. Approximately 18.7% of fatty acids in the PHVO supplement were cis isomers of 18:1, including cis-8, -9, -10, -11, and -12. The PHVO supplement also contained numerous homoallylic 18:2 isomers accounting for 4.6% of total fatty acids (isomer distribution not defined). Fat supplements also differed in the total content of SFA, averaging 23.0, 6.1, and 6.4% of fatty acids for PHVO, EA, and VA, respectively. However, overall differences in the SFA content of the diets was relatively modest, because the supplements replaced rapeseed oil and comprised only 16% of total added fat (Supplemental Table 1). Furthermore, the amount of sunflower oil in the supplemented diets was increased to ensure similar 18:2(n-6) contents; the major difference between the control diet and supplemented diets was replacement of cis-9 18:1 with trans 18:1 isomers.

Dietary treatment did not affect final body weight or feed intake, which were 109.7 ± 1.4 g and 5.9 ± 0.1 g/d, respectively, across all treatments (Supplemental Table 2). Treatments had a small effect on tissue weights; hamsters fed the control diet had the greatest epididymal adipose weights (percent body weight) but the lowest liver weights (percent body weight). Hepatic concentrations of cholesterol and triglycerides were within the range typically observed for hypercholesterolemic hamsters and were unaffected by treatment, with 301.2 ± 8.9 μmol/liver and 74.9 ± 2.4 μmol/liver, respectively, across all treatments.

### Effect of diet on plasma lipoproteins

Dietary treatments greatly affected the concentrations of plasma total cholesterol and cholesterol within the individual lipoprotein fractions but did not affect plasma triglycerides (Table 3). Hamsters consuming the PHVO diet had the highest concentrations of plasma total cholesterol and cholesterol in the atherogenic lipoprotein fractions, including VLDL, IDL, and LDL. Conversely, hamsters fed the EA diet had the lowest concentrations of plasma total cholesterol and cholesterol in the atherogenic lipoprotein fractions. Compared with the PHVO and control treatments, con-

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### Table 2

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>PHVO</th>
<th>EA</th>
<th>VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis 10:0</td>
<td>11.9</td>
<td>16.5</td>
<td>16.9</td>
</tr>
<tr>
<td>trans 10:0</td>
<td>9.5</td>
<td>11.6</td>
<td>10.1</td>
</tr>
<tr>
<td>cis 12:0</td>
<td>14.0</td>
<td>8.1</td>
<td>7.7</td>
</tr>
<tr>
<td>trans 12:0</td>
<td>7.7</td>
<td>6.5</td>
<td>6.2</td>
</tr>
<tr>
<td>cis 14:0</td>
<td>1.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>trans 14:0</td>
<td>0.7</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1. Distribution of trans 18:1 isomers in the PHVO supplement as a proportion of total fatty acids (g/100 g) was: 7.8 trans-8 (t8), 9.5 t9, 11.6 t10, 10.1 t11, 7.9 t12, and 5.2 t13. For the EA and VA supplements, the trans 18:1 isomer distribution was comprised solely of the respective trans 18:1 treatment isomer.
2. Distribution of cis 18:1 isomers in the PHVO supplement as a proportion of total fatty acids (g/100 g) was: 2.6 cis-8 (c8), 4.3 c9, 4.5 c10, 4.1 c11, 2.2 c12, and 1.0 c13. The cis 18:1 isomer distribution of the EA and VA supplements was comprised solely of c9.
3. The PHVO supplement contained a large distribution of cis and trans homoallylic 18:2 isomers that overlapped and coeluted considerably. For EA and VA supplements, the major homoallylic isomer was 18:2, t9,t12; smaller amounts of 18:2, c9,t12, and 18:2, t8, t9,c12 were also present.
4. Distribution of 20:1 isomers as a proportion of total 20:1 fatty acids (g/100 g) was: 11.9 t8, 31.1 t9, 27.1 t11, 2.6 t12, 0.9 t13, 2.8 t18, 14.5 c9, and 6.2 c11.
5. Distribution of conjugated linoleic acid isomers as a proportion of total conjugated linoleic acid fatty acids (g/100 g) was: 11.9 11c,13c, 16.5 10c,12c, 16.9 9c,11c or 9t,11t, 14.0 8c,10c, 7.7 9c,11t, 7.2 8c,10t, 6.8 8t,10c, 6.5 11c,13t, 5.7 10c,12t, 3.5 9t,11t, 1.8 7t,9c, and 1.7 10t,12c.
consumption of EA and VA diets resulted in lower cholesterol in the atherogenic lipoprotein fractions.

Compared with the control diet, the PHVO treatment increased plasma total:HDL-cholesterol and nonHDL:HDL-cholesterol ratios by 17 and 23%, respectively (Fig. 1). These values decreased by 27 and 46% after the EA treatment and 8 and 14% after the VA treatment, respectively. For all treatments, changes in plasma cholesterol ratios were most affected by changes in the content of cholesterol in the atherogenic lipoprotein fractions, because the concentrations of HDL-cholesterol were not significantly affected. HDL-cholesterol tended to be higher in hamsters fed the EA diet than in hamsters fed the other diets ($P = 0.07$).

Expression of hepatic LDL-receptor was greatest for hamsters consuming the EA diet and did not differ among other treatments (Fig. 2A). The effect of treatment on plasma LDL-cholesterol correlated with the expression of hepatic LDL-receptor mRNA abundance. Compared with the PHVO diet, hamsters fed the EA diet had lower concentrations of LDL-cholesterol and a greater abundance of LDL-receptor mRNA (Fig. 2B).

**Effect of treatment on liver fatty acids.** Composition of liver fatty acids reflected intake of the experimental diets (Table 4; Supplemental Table 3). Differences existed among treatments for trans 18:1 isomers, ranging from 0.5% of fatty acids for control hamsters to nearly 3.0% of fatty acids for hamsters fed the VA diet. Relative to the control, livers of hamsters fed the PHVO diet had increased cis and trans 18:1 fatty acid isomers in a distribution that reflected the PHVO supplement (Fig. 3). The VA diet resulted in increased RA in liver fatty acids, averaging 1.05% of total fatty acids compared with 0.15% for the hamsters receiving the control diet (Supplemental Table 3).

Compared with the other treatments, hamsters fed the EA diet had greater hepatic levels of 18:2(n-6) and 20:3(n-6) and lower concentrations of several long chain-PUFA (LC-PUFA), including 20:4(n-6), 20:5(n-3), 22:4(n-6), 22:5(n-6), 22:5(n-3), and 22:6(n-3) (Table 4; Supplemental Table 3). Liver content of LC-PUFA did not differ among hamsters fed the other diets.

**Discussion**

Industrial-derived TFA have been associated with the progression of a number of chronic human diseases, especially CHD (1), although the role of individual TFA isomers remains largely unknown. The current study was designed to investigate the effects of specific trans 18:1 fatty acid isomers on biomarkers of CHD risk using the Golden Syrian hamster. This species has often been used as a biomedical model for investigating the effects of dietary fat on CHD risk, because its lipoprotein metabolism, low rates of endogenous cholesterol synthesis, and high level of cholesterol ester transfer protein activity are most similar to humans compared with other rodent species (22,23). Hamsters, similar to humans, also produce VLDL containing apolipoprotein-B100 (24). In the present study, the pattern of effects of the PHVO treatment on the total:HDL-cholesterol and nonHDL:HDL-cholesterol ratios and the changes in plasma cholesterol are as typically observed when humans consume diets rich in PHVO (25), thereby providing further validation of this model.

Plasma ratios of total:HDL-cholesterol and nonHDL:HDL-cholesterol are effective predictors of CHD risk that account for HDL and its role in reverse-cholesterol transport (26). In the present study, PHVO increased plasma total:HDL-cholesterol and nonHDL:HDL-cholesterol ratios by 17 and 23%, respectively, compared with the control diet (Fig. 1). In contrast, the EA and VA diets produced major decreases in the plasma cholesterol ratios, with the EA treatment causing a nearly 50% reduction in the plasma nonHDL:HDL-cholesterol ratio compared with the control diet. The magnitude of these differences was particularly striking, because EA has typically been considered an atherogenic component of PHVO (27,28). However, we are not aware of any investigations with biomedical models that
TABLE 4  Partial listing of fatty acids from total liver lipids in hamsters fed the control diet or diets supplemented with 2.5% PHVO, EA, or VA1

<table>
<thead>
<tr>
<th>Fatty acid2</th>
<th>Control</th>
<th>PHVO</th>
<th>EA</th>
<th>VA</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
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<td></td>
<td></td>
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<tr>
<td>18:0</td>
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<td></td>
</tr>
<tr>
<td>18:1, cis3</td>
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<td>18:1, trans3</td>
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<td>18:2(n-6)</td>
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<td>18:2, cis-9, trans-11</td>
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<tr>
<td>20:3(n-6)</td>
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<tr>
<td>Summation</td>
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</tbody>
</table>

1 Values are least squares means and pooled SEM; n = 8 for PHVO and EA diets; n = 7 for control and VA diets. Means in a row with superscripts without a common letter differ, P < 0.05.
2 A complete fatty acid profile is provided in Supplemental Table 3.
3 Distribution of cis and trans 18:1 isomers is presented in Figure 3.

have reported specific adverse effects of pure EA on plasma lipid biomarkers of CHD risk. Rather, in previous studies with the Golden Syrian hamster, Meijer et al. (8) observed little difference in the effects of EA, VA, or cis-9 18:1 on plasma cholesterol and liver lipids and Woollett et al. (7) demonstrated a relatively neutral effect of EA to regulate activity of the hepatic LDL-receptor compared with cis-9 18:1. In contrast to Woollett et al. (7), we observed that the EA treatment increased hepatic LDL-receptor expression relative to the other diets and was correlated with lower levels of LDL-cholesterol (Fig. 2). Expression of hepatic LDL-receptor mRNA serves as a proxy for determining hepatic LDL uptake and clearance from plasma, with increased expression indicating a reduction in CHD risk (22). Furthermore, several dietary fatty acids have been shown to regulate hepatic LDL-receptor mRNA in a manner that is parallel with changes in LDL-receptor protein abundance (29).

EA and VA treatments had similar effects on the plasma lipoprotein profile, which represented a reduced CHD risk compared with the PHVO and control diets (Table 3); these effects were especially dramatic for the EA treatment. Consistent with previous observations, hamsters fed the EA treatment had a small, but significant improvement (reduction) in the plasma LDL:HDL-cholesterol ratio compared with VA (8). These results are somewhat surprising, because dietary VA has the potential to be converted to RA via Δ9-desaturase and RA has antiatherogenic properties (6). We previously observed that a VA/RA-enriched butter reduced plasma LDL:HDL-cholesterol by 58% of that for the control in cholesterol-fed hamsters (11). More recently, Bauchart et al. (30) demonstrated beneficial effects of VA/RA-enriched butter on plasma lipid biomarkers of CHD risk in rabbits, but treatment with trans-10 18:1-enriched butter tended to increase plasma cholesterol and adversely affect the plasma lipoprotein profile. Thus, effects we observed with PHVO may be related to trans-10 18:1, but we are not aware of any study that has directly examined pure trans-10 18:1 on biomarkers of CHD risk.

PHVO has been shown to adversely affect essential fatty acid metabolism (2) and exacerbate essential fatty acid deficiency (31). In a study with weanling rats, partially hydrogenated soybean oil fed at 20 g/100 g of diet inhibited hepatic Δ5- and Δ6-desaturase activities and resulted in a decreased hepatic content of LC-PUFA (2). In the present study, PHVO did not adversely affect the content of hepatic LC-PUFA, but the supply of PHVO (2.5 g/100 g of diet) may have been too low to elicit inhibitory effects. Conversely, the EA diet reduced the content of (n-6) and (n-3) LC-PUFA in liver lipids, suggesting an inhibitory effect on enzymes common to the 2 pathways. In support of this, EA, but not VA, has been previously shown to directly inhibit Δ5- and Δ6-desaturase activities in human fibroblast cells (32,33). These observations may explain why the content of LC-PUFA in livers of hamsters fed the VA diet remained unaffected. The adverse effects of EA have also been observed in vivo; dietary trieladin fed at 6.75 g/
100 g of diet to rats decreased the content of (n-3) and (n-6) LC-PUFA and increased the content of 18:2(n-6) in heart phospholipids (34). The inhibition of these desaturase enzymes is undesirable, because LC-PUFA are known to offer numerous beneficial effects related to health maintenance and growth and development (35).

There are numerous examples that bioactive fatty acids can elicit dramatic effects when present in small proportions of the diet. The lack of evidence for dietary EA or VA to adversely affect the plasma lipoprotein profile in the present study suggests that other unique fatty acids found as the minor components of PHVO may play a previously unrecognized role with regard to biological mechanisms and effects on CHD risk factors. Furthermore, the adverse health effects associated with PHVO may occur due to the presence of highly bioactive compounds found in minute quantities or the ultimate accumulation of unique fatty acids and their metabolites over time, for which biological mechanisms and effects on CHD risk factors. In conclusion, dietary supplementation with EA or VA reduced plasma concentrations of total cholesterol and cholesterol in the aerotherogenic lipoprotein fractions. Compared with the PHVO and control treatments, these effects translated into reductions in the plasma total:HDL-cholesterol and nonHDL:HDL-cholesterol ratios, indicative of a reduced CHD risk. Industrial-derived TFA have been implicated the progression of CHD; however, few studies have examined the specific effects of individual 18:1 fatty acid components. Data from the present study demonstrate that the hypercholesterolemic effect of PHVO was not dependent on the presence of EA or VA and that other bioactive components of PHVO must be responsible for eliciting these adverse effects.

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