Safflower oil consumption does not increase plasma conjugated linoleic acid concentrations in humans

Barbara K Herbel, Michelle K McGuire, Mark A McGuire, and Terry D Shultz

ABSTRACT Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (LA) with conjugated double bonds. CLA has anticarcinogenic properties and has been identified in human tissues, dairy products, meats, and certain vegetable oils. A variety of animal products are good sources of CLA, but plant oils contain much less. However, plant oils are a rich source of LA, which may be isomerized to CLA by intestinal microorganisms in humans. To investigate the effect of triacylglycerol-esterified LA consumption on plasma concentrations of esterified CLA in total lipids.

Moreover, meat from ruminants has been shown to contain more CLA than meat from nonruminants because of increased conversion of LA to CLA by rumen microorganisms.

In a series of experiments, treatment with CLA partially inhibited initiation of mouse skin carcinogenesis by 7,12-dimethylbenz[a]anthracene and inhibited mouse forestomach tumorigenesis induced by benzo[a]pyrene. A feeding study in rodents conducted by Ip et al (17) revealed that dietary intake of CLA also significantly reduced the number and incidence of mammary tumors. Furthermore, a series of in vitro experiments showed that CLA is cytostatic and cytotoxic to human malignant melanoma, colorectal, and breast cancer cells (18, 19). Tumor growth inhibition may be due to the ability of CLA to inhibit protein and nucleotide biosynthesis (19). To date, the 9c,11r-isomer has been found to be the only CLA isomer incorporated into the phospholipids of mouse forestomach and rat mammary tumors and liver; thus, it is this isoform that is thought to be biologically active (16, 17).

Pertinent to the work reported here is whether CLA can be produced from LA in humans. Several cultures from rat intestines resulted in microbial isolates with the ability to convert LA to CLA, with at least one strain able to produce multiple CLA isomers (20). Data indicate that supplementation of conventional rat diets with 2–6 wk with 2.5% or 5% LA increased CLA concentrations in the rodents’ serum, liver, lung, fat pad, muscle, and kidney tissues in proportion to the amount of LA fed, whereas supplementation of germ-free rat diets with LA did not increase tissue CLA (21). Butyrovibrio fibrisolvens, a gram-negative bacteria found in the digestive tracts of humans and ruminants (22), has been found to isomerize LA, producing CLA (23–25). Thus, bacterial isomerization may be the mechanism responsible for the increase in tissue CLA observed in the aforementioned study using conventional rats (21). However, feeding conventional rats corn oil containing triacylglycerol-esterified LA did not result in...
increased tissue CLA concentrations, suggesting a differential metabolism of LA and esterified LA to CLA.

CLA has been found in human adipose tissue, serum, bile, milk, and duodenal juices, with 9c,11t being the predominant isomer (10, 14, 26–31). Dietary intake of other fatty acids has been shown to be reflected in the fatty acid composition of blood, milk, and adipose tissue (32, 33). For example, consumption of natural diets containing LA for 2–3 wk has been positively associated with the LA content of human serum phospholipids, triacylglycerols, and cholesteryl esters (32, 34, 35). However, little is known about the relation between dietary intake of LA in humans and resulting plasma CLA concentrations in blood lipid components. Because human intestinal microorganisms may convert dietary LA to CLA we tested the hypothesis that consumption of a diet supplemented with triacylglycerol-esterified LA would increase plasma concentrations of esterified CLA in total lipids in normal lipidemic men and women.

SUBJECTS AND METHODS

Subjects
Six male and six female subjects who were healthy and ranged in age from 21 to 36 y (x ± SEM: 28.6 ± 1.4 y) were recruited from the campus of Washington State University. All prospective subjects filled out a medical and demographic questionnaire and were interviewed before their entry into the study. Nine subjects were white, two were Asian, and one was Asian Indian. All participants were within 20% of their ideal body weight and had no previous history of liver, heart, or kidney disease; diabetes; hypertension; alcoholism; or gastrointestinal disorders. Fasting blood samples were drawn before the study to detect metabolic abnormalities. Volunteers taking prescription drugs, including oral contraceptives, or who had recently taken antibiotics were excluded. The study protocol was approved by the Washington State University Institutional Review Board. All subjects signed an informed consent form before they entered the study.

Experimental design
A 100-g salad consisting of 65 g iceberg lettuce, 10 g carrot, and 25 g tomato topped with 21 g safflower oil (containing 16 g triacylglycerol-esterified LA) and 10 g vinegar dressing was the vehicle for delivery of the oil. This daily supplement of 21 g safflower oil provided 10.5 mg CLA/d. Subjects consumed the salads each day during the dietary intervention period at their noon meal under the supervision of the investigating nutritionist. This salad was consumed for 6 wk followed by a 6-wk period in which no salad was consumed. The safflower oil was purchased in one lot from a local grocery store. Other salad ingredients were purchased weekly. French bread (50 g) was provided to remove the remaining salad dressing from the plate and from the dressing container to ensure complete consumption. Subjects were instructed to follow normal dietary patterns during the study so that their intake of meat and dairy products would remain as consistent as possible, because some of these foods contain significant quantities of CLA (5–7). Subjects were weighed at week 0, weekly until week 6, and at week 12.

An a priori power calculation was done to determine an appropriate sample size. Using a level of significance of 0.05 and a power of 0.80, we determined that a sample size of five subjects within this crossover experiment would allow the detection of a 20% increase in plasma CLA concentrations during the experimental period. Data published previously by Huang et al (30) were used to estimate expected mean and intrasubject variance in plasma CLA concentrations (7.1 and 1.2 µmol/L, respectively). Thus, to allow for subject dropout and a possible effect of the sex of the subject on response to dietary intervention, we enrolled six men and six women to participate in this study.

Dietary and anthropometric assessments
Subjects were instructed by study personnel about how to complete a 3-d diet record. Three-day diet records were completed for Sunday through Tuesday at week 0 (before dietary intervention, baseline), week 6 (after dietary intervention) and week 12. Dietary intakes were estimated using NUTRITIONIST IV, a computerized nutrient analysis program (36). Dietary CLA intakes were estimated separately from previously published data (5, 6).

Blood collection
Samples (30 mL) of fasting blood (12 h) were obtained between 0700 and 0800 from the antecubital vein of each subject at weeks 0, 6 and 12. Blood was drawn the morning after the 3-d diet records were completed.

Analytic methods

Biochemical materials
Acetoneitrile, hexane, methanol, chloroform, hydrochloric acid, and acetic acid (all HPLC grade) were obtained from JT Baker (Phillipsburg, NJ). Potassium hydroxide and methyl-tertiary butyl ether (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ). LA and arachidonic acid (AA) (99% pure) were acquired from Sigma Chemical Co (St Louis). Radiolabeled triolein [9,10-3H(N)] was obtained from Du Pont (New England Nuclear, Boston) and was purified with a silica Sep-Pak column (Waters Chromatography Division, Millipore Corp, Milford, MA) and the methyl-tertiary butyl ether:methanol:ammonium acetate solvent system described by Hamilton and Comai (37) was used. CLA was obtained from MW Pariza, Food Research Institute, University of Wisconsin, Madison. CLA was analyzed for purity with a capillary gas chromatography method (7) and found to be 99% pure. The 9c,11t- or 9t,11c- and the 10t,12c-octadecadienoic acids comprised 46% and 50% of the total CLA, respectively.

Lipid analysis

The concentration of LA, as well as that of other fatty acids in the safflower oil, was determined with standard methods (38) (Hazleton Wisconsin, Madison, WI). The safflower oil contained 76.1% LA, 13.5% oleic acid, 6.7% palmitic acid, and 2.6% stearic fatty acid (as a percentage of total fatty acids). The CLA concentration in safflower oil was quantified as outlined below by the method of Werner et al (7). The CLA concentration in two randomly selected bottles of safflower oil ranged from 0.41 to 0.62 mg CLA/g fat (x ± SEM: 0.50 ± 0.02 mg CLA/g fat), and the 9c,11t-form was the only isomer detected.

Plasma (0.25 mL) was extracted by the method of Folch et al (39). After alkaline hydrolysis of plasma concentrations of esterified fatty acids with 1 mL of 1 mol KOH in methanol at 100°C

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for 15 min, the hydrolysate was acidified with 1 mL 2 mol HCl (7). Fatty acids were extracted twice with chloroform, and the fatty acid fraction was isolated by using prepacked silica Sep-Pak columns as described by Hamilton and Comai (37). Fatty acid eluates were evaporated to dryness under nitrogen and solubilized in 1 mL acetonitrile:water:acetic acid (90:10:0.1, by vol).

Aliquots of the eluate (25 μL) were then injected on a reversed-phase HPLC column (Absorbsphere-ODS, 5 μm particle size, 250 × 4.6 mm; Alltech Associates, Inc, Deerfield, IL) fitted with a C18 guard column having packing material similar to the HPLC column described previously (10 × 4.6 mm bed volume; Alltech Associates, Inc). An isocratic mobile phase of the previously mentioned solvent was used (0.5 mL/min). An Altex model 110A HPLC pump (Beckman Instruments, Berkeley, CA), an Isco V4 absorbance detector (Lincoln, NE), and a Hewlett-Packard 3394A integrator (Avondale, PA) were used to conduct and monitor the HPLC analysis. The CLA with unspecified stereochemistry was measured at 234 nm; non-CLA and AA were measured at 205 nm (29, 30, 40). Fatty acids were identified and quantified by comparing retention times and peak areas with standards (Supelco Inc, Bellefonte, PA).

Radiolabeled triolein was used as an internal standard for recovery correction after lipid extraction, hydrolysis and silica Sep-Pak fatty acid isolation and before HPLC injection. The mean (± SEM) recovery of radiolabeled triolein was 94 ± 1% (n = 12). Values were corrected for recovery of radiolabeled triolein. All assays were performed in duplicate. Interassay variabilities for CLA, LA, and AA were 9%, 10%, and 7%, respectively (n = 12 each); intraassay variabilities were 4%, 4%, and 4%, respectively (n = 4 each). Counting efficiency for 3H was 44%.

**Plasma lipid measurements**

Plasma cholesterol, triacylglycerol, and HDL-, LDL-, and VLDL-cholesterol concentrations were determined according to accepted lipid clinical chemistry methodology of the Pathologist’s Regional Laboratory (Lewiston, ID). Total cholesterol and HDL-cholesterol concentrations were ascertained by using an enzymatic method (Boehringer Mannheim Diagnostics, Indianapolis); triacylglycerol concentrations were analyzed by an endpoint determination method (Sysmex; EM Diagnostics, Gibbonstown, NJ). LDL- and VLDL-cholesterol concentrations were determined by calculation from cholesterol and HDL-cholesterol concentrations.

**Statistical analysis**

All statistical analyses were determined by using SAS for Windows version 6.12 (SAS Institute Inc, Cary, NC). Anthropometric measurements, dietary nutrient intakes, plasma esterified CLA in total lipids, LA, AA, cholesterol, triacylglycerol, and HDL-, LDL-, and VLDL-cholesterol concentrations were compared between sexes and among study periods (weeks 0, 6, and 12) using repeated-measures analysis of variance. Initially, a full model including sex, period, and the interaction between sex and period was run for each variable; if the interaction was not significant, it was removed from the model and a reduced model was run to determine the independent effects of sex and period. If a significant interaction was observed (P ≤ 0.10), main effects of sex and period were not considered independently. When the significance of independent main effects was observed (P < 0.05), a least-significant-difference test was performed to evaluate differences between the means. Furthermore, a covariate analysis was conducted to determine whether differences in plasma CLA values were related to differences in dietary CLA intake. Pearson’s product-moment correlation coefficients were computed between average 3-d nutrient intakes of energy, carbohydrate, protein, total fat, oleic acid, LA, CLA, and saturated, monounsaturated, and polyunsaturated fatty acids, and plasma concentrations of CLA, LA, AA, total cholesterol, and lipoprotein cholesterol. Pearson product correlation coefficients were also calculated among plasma CLA, LA, cholesterol, and HDL- and LDL-cholesterol concentrations. Differences and correlations were considered significant at P < 0.05.

**RESULTS**

**Dietary intake and anthropometric measurements**

There was a significant interaction between sex and period on body weight, carbohydrate intake, protein intake, and AA intake (P < 0.10) (Table 1). The mean body weight of the men, but not of the women, decreased significantly (P < 0.01) during the study (Table 1). There was a trend (P = 0.07) toward an increase in dietary carbohydrate intake during the study in the women only. Protein intake increased significantly (P < 0.05) in the women but not in the men between study weeks 6 and 12. AA intake increased significantly (P < 0.001) in the men between weeks 6 and 12 but decreased significantly (P < 0.05) in the women between weeks 0 and 6. Furthermore, an independent effect of study period explained significant variation in dietary intakes of energy, total fat, LA, polyunsaturated fatty acids, and the ratio of polyunsaturated to saturated fatty acids (Table 1); these variables increased during the intervention and postintervention periods.

Note that the data set used in this version of NUTRITIONIST IV is relatively complete for the total fat contents of foods but less complete for specific fatty acid types. Thus, the data generated for total fat intake were relatively accurate, whereas the estimated intakes of polyunsaturated, monounsaturated, and saturated fatty acids were less complete. However, we believe that the data generated do represent any changes in intake by lipid type during the study, and therefore, we included these data in our results.

Proportions of energy obtained from carbohydrate, protein, and lipid are also shown in Table 1; there was no effect of study period on these variables. Further, there was a significant independent effect of sex on polyunsaturated fatty acid intake (18 ± 2 compared with 23 ± 2 g/d for women and men, respectively; P < 0.05).

**Plasma lipids**

Sex and study period interacted significantly to influence only the ratio of LDL to HDL cholesterol (Table 2), such that the men were observed to have lower ratios at both weeks 6 and 12 (as compared with the baseline period), whereas the women were observed to have decreased ratios at week 6 only. Plasma total cholesterol and LDL-cholesterol concentrations also decreased significantly by dietary intervention (Table 2). Study period did not influence plasma triacylglycerol, HDL- and VLDL-cholesterol, CLA, or LA concentrations. There were significant independent effects of sex on plasma HDL-
cholesterol concentration (1.50 ± 0.02 compared with 1.11 ± 0.02 mmol/L for women and men, respectively; \( P < 0.05 \)) and the ratio of total cholesterol to HDL cholesterol (2.88 ± 0.30 compared with 3.79 ± 0.39 for women and men, respectively; \( P < 0.005 \)).

Additionally, a covariate analysis of plasma esterified CLA concentrations in total lipids, taking into account each subject’s dietary intake of CLA, revealed no significant difference among plasma CLA concentrations during the baseline, dietary intervention, or postdietary intervention periods.

Pearson product correlation coefficients calculated between dietary intakes (total fat, oleic acid, LA, CLA, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and cholesterol) and plasma esterified fatty acid concentrations in total lipids (ie, CLA, LA, and AA) revealed no significant correlations during the baseline, dietary intervention, or postdietary intervention periods. There were also no significant correlations of dietary total fat, fatty acid, and cholesterol constituents with plasma cholesterol or HDL- and LDL-cholesterol concentrations.

DISCUSSION

Most naturally occurring substances exhibiting anticarcinogenic activity in experimental models are of plant origin (41–43). However, CLA is an exception to this rule because it is present in high concentrations in foods of animal origin (4–7, 10–15). Plant foods, particularly vegetable oils such as peanut, corn, and safflower, are also possible sources of CLA, but they contain less CLA per unit weight of fat than do animal products (5).

Other potential sources of CLA in human tissues may include the isomerization of LA [18:2(9,12)] to vaccenic acid [18:1(11)] either through CLA [18:2(9,11)] (23–25, 44) or by \( \Delta^\alpha \) desaturation of oleic acid to CLA by liver microsomes (35, 45). Our data indicate that consumption of 16 g triacylglycerol-esterified LA/d for 6 wk did not directly affect the plasma total-esterified CLA concentration. Safflower oil does contain small amounts of oleic acid. However, oleic acid intake during this study did not increase significantly during the dietary intervention period. A previous study by Huang et al (30), in which there was a significant increase in oleic acid intake during a 4-wk dietary intervention, revealed no significant relation between dietary oleic acid and plasma phospholipid-esterified CLA concentration.

In the present study, the average dietary intake of LA and polyunsaturated fatty acids increased during dietary intervention. Data indicate that the amount of LA consumed in this study was approximately twice that of average dietary consumption (30, 46, 47). However, other unidentified dietary sources of CLA may have confounded the results of this study; consequently, a covariate analysis was performed to eliminate this possibility. No significant differences in plasma esterified CLA concentrations in total lipids among baseline, dietary, and postdietary intervention periods were found.

The results of this study further support and extend the previous finding by Chin et al (21) that there was no increase in tissue CLA concentration in rats fed LA in the triacylglycerol-esterified form as corn oil. In the present study, it appears that triacylglycerol-esterified LA may not be a suitable substrate for

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 6</th>
<th>Week 12</th>
<th>Pooled SEM</th>
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</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>88.3(a)</td>
<td>87.4(ab)</td>
<td>85.6(b)</td>
<td>0.4</td>
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<tr>
<td>Men</td>
<td>56.7</td>
<td>57.3</td>
<td>57.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Women</td>
<td>43.2</td>
<td>41.1</td>
<td>40.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>9498(a)</td>
<td>10368(ab)</td>
<td>10694(b)</td>
<td>410</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>329</td>
<td>345</td>
<td>327</td>
<td>5</td>
</tr>
<tr>
<td>Men</td>
<td>264</td>
<td>271</td>
<td>327</td>
<td>5</td>
</tr>
<tr>
<td>Women</td>
<td>152</td>
<td>161</td>
<td>150</td>
<td>5</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>65</td>
<td>65</td>
<td>52</td>
<td>1</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>34</td>
<td>35</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td>Men</td>
<td>94</td>
<td>99</td>
<td>92</td>
<td>5</td>
</tr>
<tr>
<td>Women</td>
<td>66(a)</td>
<td>65(a)</td>
<td>84(b)</td>
<td>5</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>75(a)</td>
<td>95(b)</td>
<td>94(b)</td>
<td>6</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>30</td>
<td>35</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td>Arachidonic acid (g)</td>
<td>614(ab)</td>
<td>552(a)</td>
<td>679(b)</td>
<td>24</td>
</tr>
<tr>
<td>Men</td>
<td>733(a)</td>
<td>649(b)</td>
<td>673(ab)</td>
<td>24</td>
</tr>
<tr>
<td>Women</td>
<td>20</td>
<td>22</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Oleic acid (g)</td>
<td>10(b)</td>
<td>25(b)</td>
<td>12(b)</td>
<td>1</td>
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<tr>
<td>Linoleic acid (g)</td>
<td>127</td>
<td>151</td>
<td>139</td>
<td>18</td>
</tr>
<tr>
<td>Conjugated linoleic acid (mg)</td>
<td>26</td>
<td>29</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Saturated fatty acids (g)</td>
<td>26</td>
<td>28</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>Monounsaturated fatty acids (g)</td>
<td>15(a)</td>
<td>29(b)</td>
<td>17(a)</td>
<td>2</td>
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<tr>
<td>Polyunsaturated fatty acids (g)</td>
<td>0.69(a)</td>
<td>1.22(b)</td>
<td>0.62(a)</td>
<td>0.11</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>242</td>
<td>218</td>
<td>280</td>
<td>29</td>
</tr>
</tbody>
</table>

\( ^1 \) \( n = 6 \) men and 6 women. Least-squares means in the same row with different superscript letters are significantly different, \( P < 0.05 \).

\( ^2 \) Significant sex-by-period interaction, \( P < 0.10 \).

\( ^3 \) Ratio of polyunsaturated to saturated fatty acids.
the conversion of LA to CLA in human intestines. Although mean CLA concentrations among study periods were not significantly different, one male and three female subjects did have increased (4–31%) plasma total lipid-esterified CLA concentrations after safflower oil feeding. This observation suggests that increased (4–31%) plasma total lipid-esterified CLA concentrations after safflower oil feeding. This observation suggests that increased (4–31%) plasma total lipid-esterified CLA concentrations may reside in the human intestine merits further examination.

Britton et al (48) identified foods high in CLA and directed 14 subjects to consume self-selected diets for 3 wk that were either high or low in CLA content. Seven-day diet histories recorded by the subjects were used to estimate CLA intake. The serum phospholipid-esterified CLA concentration and the molar ratio of CLA to LA increased significantly in the group consuming the high-CLA diet and decreased significantly in the group consuming the low-CLA diet (48). Huang et al (30) conducted a dietary intervention study with nine healthy male subjects. Cheddar cheese (112 g/d) containing 178.5 mg CLA was added to the subjects’ diet for 4 wk. These investigators reported that the amount of plasma phospholipid-esterified CLA concentration increased significantly (19–27%) after dietary intervention. The molar ratio of CLA to LA also increased significantly after dietary intervention, confirming the results obtained by Britton et al (48). However, consumption of safflower oil in the present study did not increase plasma esterified CLA concentrations in total lipids. Daily CLA consumption by the US population is estimated to be at least several hundred milligrams per person (4). The amount of CLA consumed in this study approximated the aforementioned estimate, and to our knowledge this is the first published report of dietary CLA intakes derived from diet records.

The results reported herein support a previous rodent study (21) and suggest that intake of triacylglycerol-esterified LA does not significantly increase plasma esterified CLA concentrations in humans. Because the presence of CLA in biological fluids may represent a protective factor against cancer, further studies are warranted to investigate specific foods as sources of CLA.

### Table 2

**Plasma lipid and fatty acid concentrations during baseline (week 0), high–linoleic acid intake (week 6), and low–linoleic acid intake (week 12) periods**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 6</th>
<th>Week 12</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.19</td>
<td>3.90</td>
<td>4.24</td>
<td>0.08</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>0.85</td>
<td>0.95</td>
<td>0.90</td>
<td>0.13</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.29</td>
<td>1.24</td>
<td>1.32</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.51</td>
<td>2.20</td>
<td>2.46</td>
<td>0.05</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.39</td>
<td>0.44</td>
<td>0.47</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL:HDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>2.42</td>
<td>2.20</td>
<td>2.13</td>
<td>0.06</td>
</tr>
<tr>
<td>Women</td>
<td>1.74</td>
<td>1.53</td>
<td>1.73</td>
<td>0.06</td>
</tr>
<tr>
<td>Total: HDL cholesterol</td>
<td>3.4</td>
<td>3.3</td>
<td>3.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Conjugated linoleic acid (μmol/L)</td>
<td>7.1</td>
<td>6.4</td>
<td>7.3</td>
<td>0.33</td>
</tr>
<tr>
<td>Linoleic acid (mmol/L)</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Arachidonic acid (mmol/L)</td>
<td>614</td>
<td>552</td>
<td>689</td>
<td>23.6</td>
</tr>
<tr>
<td>Women</td>
<td>731</td>
<td>649</td>
<td>673</td>
<td>23.6</td>
</tr>
</tbody>
</table>

1. n = 6 men and 6 women. Least-squares means in the same row with different superscript letters are significantly different, *P* < 0.05.

2. Significant sex-by-period interaction, *P* < 0.10.

### REFERENCES


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