Greater enrichment of triacylglycerol-rich lipoproteins with apolipoproteins E and C-III after meals rich in saturated fatty acids than after meals rich in unsaturated fatty acids

Kim G Jackson, Emma J Wolstencroft, Paul A Bateman, Parveen Yaqoob, and Christine M Williams

ABSTRACT
Background: Although there is considerable interest in the post-prandial events involved in the absorption of dietary fats and the subsequent metabolism of diet-derived triacylglycerol-rich lipoproteins, little is known about the effects of meal fatty acids on the composition of these particles.
Objective: We examined the effect of meal fatty acids on the lipid and apolipoprotein contents of triacylglycerol-rich lipoproteins.
Design: Ten normolipidemic men received in random order a mixed meal containing 50 g of a mixture of palm oil and cocoa butter [rich in saturated fatty acids (SFAs)], safflower oil [n−6 polyunsaturated fatty acids (PUFAs)], or olive oil [monounsaturated fatty acids (MUFAs)] on 3 occasions. Fasting and postprandial apolipoproteins B-48, B-100, E, C-II, and C-III and lipids (triacylglycerol and cholesterol) were measured in plasma fractions with Svedberg flotation rates (Sf) >400, Sf 60–400, and Sf 20–60.
Results: Calculation of the composition of the triacylglycerol-rich lipoproteins (expressed per mole of apolipoprotein B) showed notable differences in the lipid and apolipoprotein contents of the SFA-enriched particles in the Sf > 400 and Sf 60–400 fractions. After the SFA meal, triacylglycerol-rich lipoproteins in these fractions showed significantly greater amounts of triacylglycerol and of apolipoproteins C-II (Sf 60–400 fraction only), C-III, and E than were found after the MUFA meal (P < 0.02) and more cholesterol, apolipoprotein C-III (Sf > 400 fraction only), and apolipoprotein E than after the PUFA meal (P < 0.02).
Conclusions: Differences in the composition of Sf > 400 and Sf 60–400 triacylglycerol-rich lipoproteins formed after saturated compared with unsaturated fatty acid–rich meals may explain differences in the metabolic handling of dietary fats. Am J Clin Nutr 2005;81:25–34.

KEY WORDS Apolipoprotein B-100, apolipoprotein B-48, apolipoprotein C-II, n−6 polyunsaturated fatty acids, monounsaturated fatty acids

INTRODUCTION
Increased postprandial concentrations of triacylglycerol-rich lipoproteins, which circulate after a meal, are positively correlated with coronary heart disease risk (1, 2). As a result, there is considerable interest in the events involved in the absorption of dietary fats and the subsequent postprandial metabolism of the diet-derived triacylglycerol-rich lipoproteins (3). The type of fat consumed in a meal has been shown to influence the fatty acid composition of chylomicron particles and the subsequent postprandial triacylglycerol response (4). This finding suggests that meal fatty acid composition may influence the absorption, synthesis, secretion, and subsequent metabolism of dietary triacylglycerol. Unsaturated fatty acids have been shown to increase the size of chylomicron particles compared with saturated fatty acids (SFAs) (5, 6), with animal studies observing a more rapid hydrolysis of polyunsaturated fatty acid (PUFA)-enriched particles by lipoprotein lipase (LPL; EC 3.1.1.34) (7) and clearance of remnant particles by the liver (8). However, in those studies, differences in the rates of clearance of PUFA-enriched compared with monounsaturated fatty acid (MUFA)-enriched and SFA-enriched particles from the circulation could not be explained by differences in the activities of LPL and hepatic lipase (HL; EC 3.1.1.3) alone.

The metabolic handling of triacylglycerol-rich lipoproteins is known to be influenced by their apolipoprotein (apo) C and apo E composition, because these proteins have several regulatory functions. Apo C-II, which is synthesized in the liver and intestine, activates LPL, which hydrolyzes triacylglycerol within the core of the lipoprotein particles and plays a key role in the regulation of triacylglycerol clearance (9). Apo C-III, which is also synthesized in the liver and intestine, is thought to play several roles in the metabolism of triacylglycerol-rich lipoproteins. Increased concentrations of apo C-III have been shown to inhibit the binding and hydrolysis of lipoproteins by LPL and HL (9, 10) and to inhibit the recognition and uptake of lipoproteins by the liver (11, 12). Apo E, which is synthesized in the liver, plays a crucial role in mediating the hepatic recognition and uptake of the remnant particles by receptor-mediated processes (13). In addition, the overexpression of apo E on lipoproteins has been shown to inhibit the LPL hydrolysis of triacylglycerol-rich emulsions in vivo and in vitro (14). It has been suggested that, during their lifetime in the circulation, triacylglycerol-rich lipoproteins will...

contain multiple copies of these exchangeable apolipoproteins (15–17), the content of which will determine their metabolic fate in the circulation.

Although the apo C and E composition of different VLDL subfractions has been determined after a single oral fat load (18, 19), little is known about the compositional characteristics of triacylglycerol-rich lipoproteins after meals enriched with different fatty acids. Of the few studies conducted to date, apolipoprotein concentrations have been determined after emulsions enriched in PUFA and MUFA (apo C-II) (20) and after meals enriched in SFA and PUFA (21–23).

The aim of the present study was to quantify lipid and apolipoprotein (B, C-II, C-III, and E) concentrations in lipid fractions enriched in SFA and PUFA (21–23). Lipoprotein concentrations have been determined after emulsions different fatty acids. Of the few studies conducted to date, apolipoprotein content of triacylglycerol-rich lipoproteins after meals enriched with different fatty acid compositions could underlie differences in their metabolic handling after meals of various fatty acid compositions.

SUBJECTS AND METHODS

Subjects

Ten healthy middle-aged men [age (x ± SD): 48 ± 9 y; body mass index (in kg/m²) 25 ± 3] were studied on 3 occasions. Ethical consent was provided by The University of Reading Ethics Committee, and written informed consent was obtained from the subjects before the study began. Subjects were excluded if they had any metabolic disorders (eg, diabetes or any other endocrine or liver diseases), were taking dietary supplements (triacylglycerol: 1.3 ± 0.4 mmol/L; cholesterol: 5.0 ± 0.7 mmol/L; glucose: 5.7 ± 0.4 mmol/L). The subjects were asked to maintain their usual exercise patterns and to abstain from alcohol and organized exercise regimens for 24 h before each postprandial investigation. A low-fat evening meal was consumed on the evening before each study day.

The design of the study was a single-blind, within-subject crossover in which the subjects attended an investigation unit at The University of Reading on 3 separate occasions separated by ≥1 mo. Three test meals of different fatty acid composition were given to the subjects at ~0800 in the form of a warm chocolate drink containing the test oil and toasted white bread with strawberry jam. This meal contained 53 g fat. The fatty acid compositions of the test meals were adjusted by substituting 50 of the 53 g fat with different dietary oils. These were as follows: 1) a mixture of palm oil (29 g) and cocoa butter (21 g) rich in SFAs (palm oil supplied by Anglia Oils Limited, Hull, United Kingdom, and cocoa butter supplied by ADM Cocoa, Hull, United Kingdom); 2) safflower oil rich in n–6 PUFAs (Anglia Oils Limited); and 3) olive oil rich in n–9 MUFAs (Tesco, Cheshunt, United Kingdom). The nutrient and fatty acid compositions of the test meals are shown in Tables 1 and 2, respectively.

After a 12-h overnight fast, the subjects attended the investigation unit, where an indwelling cannula was inserted into the antecubital vein of the forearm under local anesthetic (1% lidocaine), and 2 fasting blood samples were taken. The test meal was given and consumed within 20 min. Blood samples were collected 60, 180, 300, 360, and 480 min after the test meal. No food was allowed during the 480-min test period, and decaffeinated sugar-free drinks were consumed ad libitum. The test meals were well tolerated by the subjects without any unpleasant side effects.

Plasma separation and analytic methods

Blood samples were transferred to heparin-containing tubes for the analysis of cholesterol, triacylglycerol, apo B-48, apo B-100, apo C-II, apo C-III, and apo E in the S₄₅ > 400, S, 60–400, and S₄₂₀–60 triacylglycerol-rich lipoprotein fractions. Plasma was separated by centrifugation at 1700 × g for 15 min in a bench-top centrifuge at 4 °C. EDTA (0.5 mol/L), phenylmethylsulfonyl fluoride (10 mmol/L, dissolved in propan-2-ol), and aprotinin (10 000 kallikrein inactivator units/mL; TrasyloL; Bayer plc, Newbury, United Kingdom) were added immediately to the isolated plasma to prevent the proteolytic degradation of the apolipoproteins. The plasma was stored overnight at 4 °C until isolation of the triacylglycerol-rich lipoprotein fractions by using density gradient ultracentrifugation (25) as previously described (26). After the triacylglycerol-rich lipoprotein fractions were collected (~1 mL), they were divided into portions and stored at −20 °C until analyzed. To further protect the apolipoproteins from proteolytic cleavage, another preservative cocktail was added to the appropriate tubes before addition of the lipoprotein fractions to give a final concentration of 5% by vol (27).

Triacylglycerol and cholesterol concentrations were measured with an ILAB 600 clinical chemistry analyzer (Instrumentation Laboratory, Warrington, United Kingdom) by using
enzyme-based colorimetric kits supplied by Instrumentation Laboratory. Apo C-II, C-III, and E were measured by the use of turbidimetric immunoassay using kits supplied by Alpha Laboratories (Eastleigh, Hants, United Kingdom). All samples for each subject were analyzed within a single batch, and the interassay CVs were <6%.

Apo B-48 was measured by a specific competitive enzyme-linked immunosorbent assay (ELISA; 28) as previously described (26). [Results with the apo B-48 heptapeptide standard yield apo B-48 concentrations that are ≈2.5 fold lower than those obtained by using human lymph as the standard.] Apo B-100 was measured by using a specific in-house sandwich ELISA. The inner 60 wells of a Nunc Maxisorp microtitre plate (Nunc, Roskilde, Denmark) were coated with 1 mg/mL of a 1:1 mixture of the apo B-100 monoclonal antibodies 4G3 and 5E11 (Ottawa Heart Institute Research Corporation, University of Ottawa Heart Institute, Ottawa) in 0.1 mol bicarbonate-carbonate buffer/L (pH 9.6) for 16 h at 4 °C. After washing with 0.02 mol phosphate-buffered saline (PBS)/L containing 0.05% (by vol) Tween 20 and 0.1% bovine serum albumin (PBSBT), the plate was blocked with 150 μL of 0.02 mol PBS/L, 0.025% (by vol) Tween 20, and 3% bovine serum albumin at 37 °C for 1 h. The wells in the microtitre plate were then emptied to receive the sample, and subsequent incubations were at 37 ºC unless otherwise stated; all washing steps used PBSBT.

A 9-point standard curve was prepared by serial dilution of human LDL (density = 1.019–1.063 g/mL; Autogen Bioclear UK Limited, Wiltshire, United Kingdom) in PBSBT, thus producing a concentration range of apo B-100 from 1.25 μg/mL to 5 ng/mL. Pooled fasting plasma samples were collected to prepare the quality controls. The triacylglycerol-rich samples from the Sf > 400 lipoprotein fraction were diluted 1:6, Sf 60–400 samples were diluted 1:1000, and Sf 20–60 samples were diluted 1:2000 in PBSBT before addition to the plate. Standards, samples, and quality controls (100 μL) were added in duplicate to the appropriate wells on the microtitre plate and were incubated for 90 min. The plates were washed, 100 μL of goat anti–apo B antibody (Guildhay Ltd, Surrey, United Kingdom) was added to the plate at a final dilution of 1:50 000, and the plate was incubated for a further 90 min. The plates were washed before the addition of the third antibody (anti–sheep-goat antibody conjugated to horseradish peroxidase; The Binding Site, Birmingham, United Kingdom) at a final dilution of 1:10 000. The plate was incubated for a further 90 min, was washed, and 100 μL of 3,3′,5,5′-tetramethylbenzidine substrate (Sigma, St Louis) was added for 30 min. After the reaction had been stopped by the addition of 50 μL of 1 mol HCl/L, absorbance was read on an automated ELISA plate spectrophotometer (Tecan, Theale, United Kingdom) at 450 nm. A standard curve was constructed, and the amount of apo B-100 within the quality controls and the samples was determined. The interassay CVs for both the apo B-48 and the apo B-100 analyses were <10%.

**Calculations**

The number of apolipoprotein and lipid molecules per apo B particle was calculated by dividing apolipoprotein and lipid concentrations in each lipoprotein fraction by their respective molecular mass (apo B-100 = 549 kD, apo B-48 heptapeptide = 0.98 kD, apo C-II = 8.9 kD, apo C-III = 8.9 kD, and apo E = 36.5 kD). Because each of the triacylglycerol-rich lipoprotein fractions contained a mixture of apo B-48 and apo B-100—containing lipoproteins, the molarity of the apolipoproteins and lipids was then divided by the molarity of total apo B in each lipoprotein fraction (apo B-48 and apo B-100 concentrations combined).

**Statistical analysis**

The data were analyzed by using SPSS version 11 (SPSS Inc, Chicago). The results presented in postprandial time courses and in the tables are mean values ± SEMs. The area and incremental area under the curve (AUC and IAUC, respectively) were calculated by using the trapezoidal rule (29). Fasting concentrations, AUCs, and IAUCs were analyzed by using one-way repeated-measures analysis of variance (ANOVA). The triacylglycerol, apo B-48, and apo B-100 responses and the lipid and apolipoprotein compositions of the Sf > 400, Sf 60–400, and Sf 20–60 lipoprotein fractions after the 3 test meals were analyzed by two-factor repeated-measures ANOVA with interaction. A Bonferroni correction was used for the post hoc detection of significant pairwise differences. The data were checked for normality and were log transformed where necessary to render their distribution normal before statistical analysis. P values < 0.05 were taken as significant.

**RESULTS**

**Triacylglycerol and apo B responses in the Sf > 400, Sf 60–400, and Sf 20–60 fractions**

Fasting triacylglycerol, apo B-48, and apo B-100 concentrations in the Sf > 400, Sf 60–400, and Sf 20–60 fractions were not significantly different among study days and are shown in Tables 3 and 4. The triacylglycerol responses in the Sf > 400, Sf 60–400, and Sf 20–60 fractions after the SFA, PUFA, and MUFA meals are shown in Figure 2. In the Sf > 400 fraction, the PUFA meal resulted in a significantly lower AUC and IAUC than did the SFA or MUFA meal (P < 0.009; Table 3). Although there were no significant differences in the incremental triacylglycerol responses in the Sf 20–60 fraction, the AUC after the SFA meal was significantly greater than that after the PUFA meal (P = 0.02; Table 3). In both the Sf > 400 and Sf 60–400 fractions, there were significant differences in the patterns of lipemic response, with the PUFA meal showing a biphasic response compared with the MUFA meal (P = 0.001; Figure 1).

After the test meals, apo B-48 concentrations rose in the Sf > 400 fraction, with the MUFA meal resulting in significantly higher apo B-48 concentrations than did the PUFA meal (P = 0.02) and a different pattern of response than the SFA and PUFA meals (P = 0.001; Figure 2A). The MUFA meal also showed a significantly higher AUC compared with the PUFA meal (P = 0.005) and significantly higher IAUC compared with the SFA and PUFA meals (P < 0.02; Table 4). In the Sf 60–400 fraction, the apo B-48 IAUC for the PUFA meal was significantly lower than that for the MUFA meal (P = 0.01). There was a significant difference in the patterns of apo B-48 response between the test meals, with the MUFA meal showing a different pattern of response than the SFA and PUFA meals (P = 0.001; Figure 2B).

There were no significant differences in the Sf 20–60 apo B-48 responses after the SFA, PUFA, and MUFA meals (Figure 2C).

There were no significant differences in apo B-100 responses between the SFA, PUFA, and MUFA meals in the Sf > 400 and Sf 60–400 fractions, and the rise in apo B-100 concentrations was negligible in the Sf > 400 fraction compared with the Sf 60–400 and Sf 20–60 fractions (Figure 2). In the Sf 20–60...
TABLE 3
Summary measures for fasting and postprandial triacylglycerol responses in plasma fractions with Svedberg flotation rates (Sf) > 400, 60–400, and 20–60 after meals enriched with different fatty acids

<table>
<thead>
<tr>
<th>Fraction</th>
<th>SFA meal</th>
<th>PUFA meal</th>
<th>MUFA meal</th>
<th>P for ANOVA</th>
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<tr>
<td>Sf &gt; 400</td>
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</tr>
<tr>
<td>Fasting (μmol/L)</td>
<td>8.0 ± 2.5</td>
<td>11.2 ± 5.0</td>
<td>5.4 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>AUC (μmol - 480 min/L)</td>
<td>135.6 ± 16.9</td>
<td>76.5 ± 11.5</td>
<td>127.8 ± 21.2</td>
<td>0.002</td>
</tr>
<tr>
<td>IAUC (μmol - 480 min/L)</td>
<td>131.7 ± 16.4</td>
<td>71.1 ± 10.7</td>
<td>125.2 ± 21.1</td>
<td>0.001</td>
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<tr>
<td>Sf 60–400</td>
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</tr>
<tr>
<td>Fasting (μmol/L)</td>
<td>581.9 ± 142.4</td>
<td>441.5 ± 72.6</td>
<td>462.7 ± 84.2</td>
<td>NS</td>
</tr>
<tr>
<td>AUC (μmol - 480 min/L)</td>
<td>345.3 ± 81.5</td>
<td>218.1 ± 32.8</td>
<td>279.4 ± 45.6</td>
<td>NS</td>
</tr>
<tr>
<td>IAUC (μmol - 480 min/L)</td>
<td>66.0 ± 18.8</td>
<td>6.2 ± 25.6</td>
<td>57.3 ± 22.8</td>
<td>NS</td>
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<tr>
<td>Sf 20–60</td>
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</tr>
<tr>
<td>Fasting (μmol/L)</td>
<td>238.2 ± 27.4</td>
<td>183.3 ± 26.0</td>
<td>201.1 ± 25.3</td>
<td>NS</td>
</tr>
<tr>
<td>AUC (μmol - 480 min/L)</td>
<td>122.5 ± 14.8</td>
<td>96.6 ± 10.9</td>
<td>109.6 ± 10.1</td>
<td>0.047</td>
</tr>
<tr>
<td>IAUC (μmol - 480 min/L)</td>
<td>8.2 ± 5.9</td>
<td>8.6 ± 6.0</td>
<td>13.0 ± 11.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM; n = 10. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; AUC, area under the curve; IAUC, incremental area under the curve.
2,3 Significantly different from the PUFA meal (one-way repeated-measures ANOVA followed by Student’s t test with Bonferroni correction): 2P < 0.001, 3P < 0.05.

fraction, the AUC for the apo B-100 response after the SFA meal was significantly higher than that after the PUFA meal (P = 0.02; Table 4).

Apolipoprotein composition of fasting and postprandial Sf > 400, Sf 60–400, and Sf 20–60 apo B–containing particles

The apolipoprotein contents of the triacylglycerol-rich lipoproteins in the Sf > 400, Sf 60–400, and Sf 20–60 fractions after the 3 test meals are shown in Figure 3. Fasting apolipoprotein contents were not significantly different before consumption of the test meals in any of the lipoprotein fractions.

Sf > 400 Fraction

The patterns of the apo C-II content of the lipoproteins were significantly different after the test meals. The particles released after the PUFA meal displayed a biphasic pattern of apo C-II enrichment compared with the SFA and MUFA meals (P < 0.02; Figure 3), although there were no significant differences in apo C-II contents. However, there was a marked difference in the apo C-III contents of the triacylglycerol-rich lipoproteins after the test meals, with the SFA-rich lipoproteins containing significantly more apo C-III per particle than did the MUFA- and PUFA-rich lipoproteins (P < 0.005; Figure 3). Although the MUFA- and PUFA-rich lipoproteins showed similar apo C-III contents, the pattern of postprandial apo C-III enrichment of the lipoproteins was significantly different (P = 0.009). The triacylglycerol-rich lipoproteins released after the SFA meal also contained significantly more apo E (≈5-fold) and exhibited a different pattern of postprandial apo E enrichment than did the lipoproteins released after the MUFA and PUFA meals (P < 0.001; Figure 3).

TABLE 4
Summary measures for fasting and postprandial apolipoprotein B-48 and B-100 responses in plasma fractions with Svedberg flotation rates (Sf) > 400, 60–400, and 20–60 after meals enriched with different fatty acids

<table>
<thead>
<tr>
<th>Fraction</th>
<th>SFA meal</th>
<th>PUFA meal</th>
<th>MUFA meal</th>
<th>SFA meal</th>
<th>PUFA meal</th>
<th>MUFA meal</th>
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<tbody>
<tr>
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<tr>
<td>Fasting (nmol/L)</td>
<td>3.3 ± 0.3</td>
<td>3.8 ± 0.7</td>
<td>2.8 ± 0.4</td>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
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<tr>
<td>AUC (nmol - 480 min/L)</td>
<td>3782 ± 428</td>
<td>3693 ± 714</td>
<td>5773 ± 831</td>
<td>69.0 ± 19.4</td>
<td>39.4 ± 6.1</td>
<td>57.1 ± 11.0</td>
</tr>
<tr>
<td>IAUC (nmol - 480 min/L)</td>
<td>2177 ± 364</td>
<td>1878.0 ± 492</td>
<td>4433 ± 753</td>
<td>51.8 ± 14.3</td>
<td>29.1 ± 5.4</td>
<td>45.0 ± 9.8</td>
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<tr>
<td>Sf 60–400</td>
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<tr>
<td>Fasting (nmol/L)</td>
<td>7.7 ± 1.0</td>
<td>9.2 ± 1.6</td>
<td>12.1 ± 3.5</td>
<td>36.8 ± 8.3</td>
<td>29.6 ± 4.8</td>
<td>30.0 ± 4.3</td>
</tr>
<tr>
<td>AUC (nmol - 480 min/L)</td>
<td>7124 ± 743</td>
<td>7043 ± 1113</td>
<td>13 337 ± 2141</td>
<td>19 368 ± 4353</td>
<td>13 294 ± 1608</td>
<td>16 263 ± 1846</td>
</tr>
<tr>
<td>IAUC (nmol - 480 min/L)</td>
<td>3440 ± 450</td>
<td>2618 ± 589</td>
<td>7505 ± 1983</td>
<td>1695 ± 1325</td>
<td>¬892 ± 1792</td>
<td>1844 ± 1319</td>
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<tr>
<td>Sf 20–60</td>
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<tr>
<td>Fasting (nmol/L)</td>
<td>11.4 ± 1.7</td>
<td>12.3 ± 2.1</td>
<td>10.5 ± 0.9</td>
<td>36.8 ± 5.4</td>
<td>27.8 ± 3.6</td>
<td>31.8 ± 3.2</td>
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<tr>
<td>AUC (nmol - 480 min/L)</td>
<td>6443 ± 616</td>
<td>7659 ± 1159</td>
<td>7622 ± 546</td>
<td>17 967 ± 2478</td>
<td>12 930 ± 1568</td>
<td>16 766 ± 1480</td>
</tr>
<tr>
<td>IAUC (nmol - 480 min/L)</td>
<td>983 ± 424</td>
<td>1765 ± 532</td>
<td>2585 ± 417</td>
<td>292 ± 760</td>
<td>¬398 ± 855</td>
<td>1495 ± 1296</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM; n = 10. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; AUC, area under the curve; IAUC, incremental area under the curve.
2,3 Significantly different from the MUFA meal (one-way repeated-measures ANOVA followed by Student’s t test with Bonferroni correction): 2P < 0.001, 3P < 0.05.
4 Significantly different from the PUFA meal, P < 0.05 (one-way repeated-measures ANOVA followed by Student’s t test with Bonferroni correction).
The postprandial apo C-II and C-III contents of the Sf 60–400 lipoproteins were significantly different, with the triacylglycerol-rich particles released after the SFA meal containing more apo C-II and C-III than did the particles released after the MUFA meal (P = 0.005; Figure 3). The triacylglycerol-rich lipoproteins released after the SFA meal contained significantly more apo E than did the lipoproteins released after the MUFA and PUFA meals (P < 0.005). The pattern of postprandial apo E enrichment was also significantly different compared with the MUFA meal (P = 0.001; Figure 3); apo E content gradually increased with time postprandially for the SFA meal, whereas the apo E content showed a gradual decline with time for the MUFA meal.

**Sf 20–60 Fraction**

There were no significant differences in apo C-II, C-III, or E contents of the lipoproteins contained within the Sf 20–60 fraction after the SFA, PUFA, and MUFA meals. Although the lipoproteins released after the MUFA and SFA meals contained similar amounts of apo E, the pattern of the postprandial apo E enrichment of the particles was significantly different after the SFA meal (P = 0.007; Figure 3), with the enrichment of these particles with apo E increasing with time compared with a gradual decline in enrichment after the PUFA and MUFA meals.

**Lipid composition of fasting and postprandial triacylglycerol-rich lipoproteins**

The lipid contents of the triacylglycerol-rich lipoproteins within the Sf > 400, Sf 60–400, and Sf 20–60 fractions after the SFA, PUFA, and MUFA meals are shown in Figure 4. The fasting triacylglycerol and cholesterol contents of the lipoproteins within the different fractions were not significantly different among postprandial days.

**Sf > 400 Fraction**

The postprandial triacylglycerol contents of the lipoproteins differed significantly between the meals, with the lipoproteins released after the SFA meal carrying significantly more triacylglycerol (≈1.5–2-fold) than those released after the MUFA meal (P = 0.01; Figure 4). The cholesterol content of the lipoproteins was also significantly different after the test meals; the lipoproteins released after the SFA meal contained significantly more cholesterol than did the lipoproteins released after the PUFA meal (P = 0.018) and had a significantly different pattern of postprandial cholesterol enrichment than did the lipoproteins released after the MUFA meal (P = 0.002; Figure 4).

**Sf 60–400 Fraction**

The postprandial triacylglycerol contents of the lipoproteins released after the SFA meal were significantly higher than the content of the lipoproteins released after the MUFA meal (P = 0.002; Figure 4). There were no significant differences in the cholesterol contents of the lipoproteins released after the SFA, PUFA, and MUFA meals, although the SFA meal showed a tendency for higher postprandial cholesterol contents (Figure 4).

**Sf 20–60 Fraction**

There was no significant effect of meal fatty acid composition on the postprandial triacylglycerol and cholesterol contents of the Sf 20–60 lipoproteins (Figure 4).

**DISCUSSION**

The present study reports marked qualitative and quantitative differences in lipid and apolipoprotein responses to meals of different fatty acid composition that, we suggest, may be important in determining differences in the metabolic processing and subsequent atherogenicity of lipoproteins formed during the postprandial state. The triacylglycerol responses in both the Sf > 400 and Sf 60–400 fractions were similar for the SFA and MUFA meals.
meals, whereas responses to PUFA meals were lower. Estimation of the triacylglycerol:apo B ratios for the 2 fractions showed the ratios to be similar for the MUFA and PUFA meals but to be higher for the SFA meal, indicating triacylglycerol-rich lipoproteins of larger particle size. Most notably, the SFA meal resulted in S\textsubscript{f} \textgreater 400 and S\textsubscript{f} 60–400 particles that contained significantly more triacylglycerol, apo C-III, and apo E than did particles formed after the MUFA meal and more apo C-III (S\textsubscript{f} > 400 fraction only) and apo E compared with particles formed after the PUFA meal. These data suggest that although the MUFA and PUFA meals generated different numbers of triacylglycerol-rich lipoprotein particles (being considerably greater in response to the MUFA meal), the compositional characteristics of the circulating triacylglycerol-rich lipoproteins formed were similar. Conversely, the number of particles generated in response to the SFA and PUFA meals was similar, although the composition of the particles differed. In particular, the particles formed after the SFA meal were markedly enriched with apo E; in the S\textsubscript{f} > 400 fraction, enrichment with apo E was 4-fold higher, and in the S\textsubscript{f} 60–400 fraction enrichment was 2-fold higher, after the SFA than after the MUFA or PUFA meal.
Few previous studies have compared postprandial apolipoprotein contents of triacylglycerol-rich lipoproteins after meals of different fatty acid composition. Consistent with the present study, the study by Brouwer et al (20) showed no apparent effects of PUFA- and MUFA-enriched emulsions on apo C-II content, and the study by Mero et al (21) showed that compared with PUFA, a SFA-rich mixed meal showed a nonsignificant tendency for higher apo E concentrations in the S_f > 400 fraction postprandially. Interestingly, the other SFA-rich meal (cream) in that study resulted in lower apo E concentrations 6–8 h postprandially than after the mixed SFA-rich meal. This finding suggests that the vehicle used to introduce the fat into the test
meal, ie, mixed food versus oils, influences the metabolism of the triacylglycerol-rich lipoproteins in the enterocyte or circulation. Other authors have shown differences in the postprandial triacylglycerol response when dairy fats of identical fat composition but provided in different food matrices are given (30). The use of a single fat source as opposed to combinations of SFA-, PUFA-, and MUFA-rich foods in the test meals of the present study may underlie our findings and may also explain the differences in the patterns of $S_f > 400$, $S_f 60–400$, and $S_f 20–60$ fractions. For the changes in TG and cholesterol contents in the $S_f > 400$ fraction, there was a significant time effect ($P < 0.001$) and a significant meal $\times$ time interaction ($P < 0.005$) for TG.

Greater numbers of triacylglycerol-rich lipoprotein particles, reflected by a greater increase in postprandial apo B-48 in the $S_f > 400$ and $S_f 60–400$ fractions, were a notable feature of the response to the MUFA meal. A more marked increase in apo B-48 after MUFA than after SFA or PUFA meals was previously reported by ourselves (26, 31) and others (34, 35) and suggests that ingestion of MUFA-rich meals causes the formation of a greater number of large ($S_f > 400$) and moderately sized ($S_f 60–400$) triacylglycerol-rich lipoproteins. We have proposed that this response reflects a greater capacity to promote chylomicron formation and secretion from the enterocyte after exposure to MUFAs, a proposition that is supported by studies using the Caco-2 cell model (36–41).

A greater number of circulating triacylglycerol-rich lipoprotein particles might be considered to be an adverse postprandial response and appears to conflict with the widely reported cardioprotective effects of MUFA-rich diets and meals. However, a notable feature of the triacylglycerol and apo B-48 postprandial profiles for the MUFA meals is the rapidly declining concentrations between 360 and 480 min. These declining concentrations result in the return of the particle number to fasting levels. This suggests that, whatever the origin of the greater rise in particle number, the particles do not persist within the circulation and are therefore less likely to have adverse atherogenic consequences.

The marked decline in apo C-III content in the late postprandial period for the MUFA meal may also be relevant, because apo C-III inhibits remnant removal.

The major compositional differences in triacylglycerol-rich lipoproteins observed in response to SFA-rich meals raise important questions as to the origin of these differences and their
consequences for the metabolism and subsequent atherogenicity of the apolipoprotein-rich particles. Detailed studies in animals have shown that meal fatty acid composition influences the metabolism of the triacylglycerol-rich lipoproteins in the circulation and their subsequent uptake by the liver. The rate of hydrolysis of the particles by LPL and HL and internalization of the particles are influenced by fatty acid composition, with PUFA-rich particles being more rapidly hydrolyzed and MUFAs and PUFA remnants particles being more rapidly internalized by the liver than are SFA-enriched particles (7, 8, 42). It is generally assumed that these metabolic differences relate to differences in the lipolytic activity of LPL against particles of different fatty acid composition. We suggest that our present findings indicate that at least some of the differences in particle composition and lipid content could be secondary to differences in the apolipoprotein composition of the Sf > 400 and Sf 60–400 lipoproteins.

The factors that influence the amounts of the different apolipoproteins that are incorporated into triacylglycerol-rich lipoproteins have not been fully elucidated. A recent study by Kovar and Havel (43) suggested that particle surface area is a major determinant of the content of apolipoproteins on triacylglycerol-rich lipoproteins. Of relevance to the findings of the present study, work using in vitro triacylglycerol emulsions showed that the binding densities of apo C-II, C-III, and E were greater for larger lipid emulsions than for smaller ones (44). Taking account of previous work showing greater hydrolysis of particles containing unsaturated fatty acids, we suggest that slower initial rates of hydrolysis of SFA-rich particles may lead to greater accumulation of apo C-II, C-III, and E, and that the accumulation of apo C-III prevents the rapid clearance of the SFA-rich particles by the liver.

In the present study, triacylglycerol:apo B ratios were highest for the SFA meal and less for the MUFAs and PUFA meals, which suggests that in the later postprandial period, the SFA-rich particles were larger than the MUFA- or PUFA-rich particles. However, we have only been able to estimate the size of the particles within the circulation, and this may not be the most relevant measure. The size of the nascent chylomicrons in the intestinal lymph may be more important for the acquisition of apolipoproteins, and this may be the most important determinant of their apolipoprotein composition. The increased apo E content of the large triacylglycerol-rich lipoproteins after the SFA meal would normally indicate that such particles would be rapidly cleared from the circulation (45). However, these particles also show greater apo C-III content, which would impair the hydrolysis of particles by LPL and HL and subsequent particle uptake by the liver. This apolipoprotein has also been shown to increase the activity of the cholesterol ester transfer protein, and the accumulation of the SFA-enriched particles may affect the metabolism and atherogenicity of the Sf 60–400 and Sf 20–60 fraction lipoproteins.

In conclusion, our study of the effect of meal fatty acids on the lipid and apolipoprotein composition of triacylglycerol-rich lipoprotein fractions has generated novel findings. In this group of middle-aged men, we found that the ingestion of an SFA-enriched meal markedly increases the triacylglycerol, cholesteryl, apo C-III, and apo E content of large (Sf > 400) and the apo E content of moderately sized (Sf 60–400) triacylglycerol-rich lipoproteins compared with PUFA and MUFAs meals. We suggest that these compositional differences may have adverse atherogenic consequences that are additional to those resulting from the LDL-raising effects of SFAs. Second, the magnitude of the apo B-48 responses in the Sf > 400 and Sf 60–400 fractions were influenced by the meal fatty acids, with a more marked accumulation and rapid decline in particle number for the MUFA meal. We suggest that the rapid removal of MUFAs-rich particles indicated by the rapid change in apo B-48 and apo C-III concentrations in the declining part of the postprandial response offers antiatherogenic benefits additional to the LDL-reducing effects of MUFAs.

This study was designed by CMW, PY, and KGJ, and the data were collected and analyzed by EJW, PAB, and KGJ. The manuscript was written by KGJ and CMW. The authors had no conflict of interests.

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