Contribution of postprandial lipemia to the dietary fat–mediated changes in endogenous lipoprotein-cholesterol concentrations in humans\textsuperscript{1–3}

Byung-Hong Chung, BH Simon Cho, Ping Liang, Steve Doran, Laura Osterlund, Robert A Oster, Betty Darnell, and Frank Franklin

ABSTRACT

Background: Dietary fats alter LDL and HDL cholesterol while serving as precursors of postprandial triacylglycerol-rich lipoproteins (TRLs).

Objective: We hypothesized that the saturated fatty acid (SFA)–mediated increase and the polyunsaturated fatty acid (PUFA)–mediated decrease in endogenous lipoprotein cholesterol are promoted by postprandial TRLs.

Design: We performed a 16-d crossover diet study to examine the effect of PUFA-rich \textsuperscript{[ratio of PUFAs to SFAs (P:S) = 2.0]} and SFA-rich \textsuperscript{[P:S = 0.25]} diets on fasting and postprandial plasma lipid and lipoprotein-cholesterol concentrations in 16 normolipidemic subjects.

Results: Fasting plasma cholesterol decreased significantly after a PUFA-rich diet because of a decrease in LDL \textsuperscript{[−12.3%; \(P < 0.05\)]} and HDL \textsuperscript{[−3.8%; NS]}, but did not change after an SFA-rich diet. The appearance of postprandial TRLs in plasma at 4 h was linked to a significant lowering of both LDL \textsuperscript{[−7.4%]} and HDL \textsuperscript{[−4.8%]} after a PUFA-rich diet; no such effect was observed after the SFA-rich diet. At 7 h, LDL and HDL cholesterol returned to near fasting concentrations without postprandial TRL accumulation after a PUFA-rich diet but with a significant postprandial TRL accumulation after an SFA-rich diet. Thus, the in vivo postprandial clearance of cholesterol in LDL+HDL was greater after a PUFA-rich diet than after an SFA-rich diet. The appearance of postprandial TRLs in plasma increased the cholesteryl ester transfer protein–mediated transfer of cholesteryl ester from LDL+HDL to TRLs in vitro without a significant influence from dietary fat.

Conclusion: Dietary fat–mediated alterations in the rate of hepatic removal of postprandial TRLs, which carry cholesterol accepted from LDL+HDL via cholesteryl ester transfer protein in vivo, may contribute to the dietary fat–mediated change in endogenous lipoprotein cholesterol.

KEY WORDS Chylomicrons, VLDL, LDL, HDL, lecithin:cholesterol acyltransferase, cholesteryl ester transfer protein, postprandial lipemia, reverse cholesterol transport, polyunsaturated fat, saturated fat

INTRODUCTION

It is well established that dietary fat composition affects the concentration and distribution of cholesterol among lipoproteins in fasting plasma and the risk of developing cardiovascular disease (CVD) \textsuperscript{1–3}. A diet rich in polyunsaturated fatty acids (PUFA) lowers fasting plasma cholesterol by decreasing LDL and HDL cholesterol and thereby reduces CVD risk, whereas a diet rich in saturated fatty acids (SFA) has the opposite effect \textsuperscript{1–3}. There is a consensus that the SFA- and PUFA-mediated changes in fasting cholesterol concentrations are due to a change in the concentration and activities of hepatic LDL receptors \textsuperscript{4, 5}. However, the SFA- and PUFA-mediated simultaneous changes in LDL and HDL cholesterol cannot be fully explained by changes in the concentration and activities of the LDL receptors alone because those changes may not affect and so do not explain changes in HDL cholesterol. Most long-term studies of dietary fat–induced changes in plasma cholesterol have focused on endogenous lipoprotein-cholesterol concentrations in fasting plasma even though dietary fats are direct precursors of postprandial TRL but do not directly contribute to the formation of endogenous lipoproteins. Postprandial lipemia is due to an increase in both intestine-derived apolipoprotein (apo) B-48 containing chylomicrons and liver-derived apo B-100 containing VLDL (6, 7). SFA-induced postprandial lipemia is more pronounced and of a longer duration than is PUFA-induced postprandial lipemia \textsuperscript{(8–11)}.

Recent studies indicate that plasma LDL-cholesterol concentrations negatively correlate with the removal rate from plasma of chylomicron-like emulsion particles \textsuperscript{(12)} and that the pharmacologic reduction of plasma LDL cholesterol is associated with an increase in the clearance of postprandial triacylglycerol-rich lipoproteins (TRLs) or chylomicron-like emulsion particles in humans \textsuperscript{(13–16)}. Conversely, an association between elevated

\textsuperscript{1} From the Atherosclerosis Research Unit (B-HC, PL, SD, and LO), the General Clinical Research Center (RAS and BD), and the Departments of Medicine (B-HC, PL, SD, and LO), Pediatrics (FF), and Nutrition (FF), Medical School, University of Alabama at Birmingham, and the Moore Heart Research Foundation, University of Illinois, Champaign, IL (BHSC).

\textsuperscript{2} Supported by the US Public Health Service (grant NIH HL 60936) and by the General Clinical Research Center, University of Alabama at Birmingham (grant RR0032).

\textsuperscript{3} Address reprint requests to B-H Chung, Department of Nutrition Science, Webb Building 248, University of Alabama, Birmingham, AL 35294. E-mail: bhchung@uab.edu. Received September 18, 2003. Accepted for publication May 7, 2004.
LDL-cholesterol concentrations and delayed chylomicron remnant clearance has been observed in the elderly (17). The above in vivo observations suggest that LDL-cholesterol concentrations in plasma can be altered by the change in the clearance rate of postprandial TRLs. However, whether the PUFA- and SFA-mediated changes in LDL- and HDL-cholesterol concentrations in fasting plasma are linked to alterations in the clearance rate of postprandial TRLs has not been fully explored. Many studies have shown that the induction of postprandial lipemia is accompanied by a transient increase in plasma lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activities (18–20), a net decrease in cholesteryl ester (CE) concentrations in both LDL and HDL as well as in plasma as a whole (11, 21, 22-24) and a shift in the distribution of CE from LDL and HDL to chylomicron (25). In plasma, LCAT and CETP promote the esterification of unesterified cholesterol (UC) and the reciprocal exchange of CE and triacylglycerol between the cholesterol-rich lipoproteins (LDL and HDL) and TRLs (VLDL + chylomicron) (20, 26). The possibility then exists, but has not been fully explored, that the postprandial enhancement of LCAT and CETP activities could possibly lower the concentrations of LDL and HDL cholesterol by transferring CE from LDL and HDL to postprandial TRLs and by subsequently clearing postprandial TRLs.

We previously provided evidence that postprandial TRLs act as potent acceptors both of cholesterol from endogenous LDL and HDL and of that released from cell membranes via LCAT and CETP activities in vitro (27). In another recent study (28), we showed that the alcohol-mediated increase in postprandial TRLs further enhanced the postprandial reduction of LDL and HDL cholesterol in vivo and the acceptance of cholesterol by TRLs from endogenous lipoproteins and cell membranes via LCAT and CETP in vitro. Given that postprandial TRLs are cleared from plasma mostly by hepatic uptake (29), our data suggest that postprandial TRLs can serve as vehicles for transporting cholesterol from endogenous LDL and HDL and cell membranes to the liver via LCAT and CETP. Because dietary fat can alter the concentration and clearance rate of postprandial TRLs in vivo (8–11) and may even affect the potencies of postprandial TRLs to serve as acceptors of LCAT and CETP reaction products, it could potentially alter the concentration of endogenous lipoprotein cholesterol and the rate of reverse cholesterol transport in vivo.

To determine whether dietary fat alters endogenous lipoprotein cholesterol and CVD risk by affecting the potency and rate of postprandial TRLs to carry cholesterol accepted from endogenous LDL and HDL and from cell membranes via LCAT and CETP to the liver for its removal, we examined the acute and chronic effects of the consumption of a normal PUFA-rich diet (P:S = 2.0) and SFA-rich (P:S = 0.25) diet on the concentrations of lipid and lipoprotein cholesterol in fasting and postprandial plasma. Subjects adopted each of the 2 diets for a period of 20 d, with a 3–4 wk return to their habitual ad libitum diet in between. Both diets provided 15% of energy from proteins, 50% of energy from carbohydrates, and 35% of energy from fat and contained 175 mg cholesterol/1000 kcal. SFAs, monounsaturated fatty acids, and PUFAs provided 7.5%, 12%, and 15.5% of total energy in the PUFA-rich diet, respectively, and 18.8%, 11.5%, and 4.7% of total energy in the SFA-rich diet, respectively. All meals were prepared by the University of Alabama GCRC research kitchen and were provided daily to the subjects. To examine the postprandial lipemic response, a fat-loading test was performed on day 16 of each diet by offering a breakfast that had the same P:S as did the chronic experimental diet. Another fat-loading study was performed on the last day (day 20) of each diet to examine the acute effect of changing a meal’s fat composition on the postprandial lipemic response. In the studies on day 20, the subjects consuming a chronic SFA-rich diet were given a PUFA-rich breakfast, whereas subjects consuming a chronic PUFA-rich diet were given a SFA-rich breakfast.

To maximize the postprandial lipemic response, the challenge meal contained a slightly higher concentration of fat (40% of energy from fat) than did the background diet (35% of energy from fat). Briefly, on the evening before the fat-loading test (day 16 and day 20), the subjects were admitted to the GCRC, where a dinner meal was prepared for them. After they fasted overnight at the GCRC, the subjects were offered a challenge breakfast meal. Because the subjects would not eat anything else for ≥7 h, the challenge meal consisted of whole foods that provided 50% of the total daily calories. Fasting and postprandial blood samples were collected from the subjects immediately before the meal (40 mL) and 4 h (80 mL) and 7 h (40 mL) after the meal.

**Diet and blood collection**

A randomized crossover diet study was performed to determine the chronic and acute effects of a normal PUFA-rich [ratio of PUFAs to SFAs (P:S) = 2.0] and SFA-rich (P:S = 0.25) diet on the concentrations of lipid and lipoprotein cholesterol in fasting and postprandial plasma. Subjects adopted each of the 2 diets for a period of 20 d, with a 3–4 wk return to their habitual ad libitum diet in between. Both diets provided 15% of energy from proteins, 50% of energy from carbohydrates, and 35% of energy from fat and contained 175 mg cholesterol/1000 kcal. SFAs, monounsaturated fatty acids, and PUFAs provided 7.5%, 12%, and 15.5% of total energy in the PUFA-rich diet, respectively, and 18.8%, 11.5%, and 4.7% of total energy in the SFA-rich diet, respectively. All meals were prepared by the University of Alabama GCRC research kitchen and were provided daily to the subjects. To examine the postprandial lipemic response, a fat-loading test was performed on day 16 of each diet by offering a breakfast that had the same P:S as did the chronic experimental diet. Another fat-loading study was performed on the last day (day 20) of each diet to examine the acute effect of changing a meal’s fat composition on the postprandial lipemic response. In the studies on day 20, the subjects consuming a chronic SFA-rich diet were given a PUFA-rich breakfast, whereas subjects consuming a chronic PUFA-rich diet were given a SFA-rich breakfast.

**Treatment of blood and plasma samples**

Blood samples were collected in tubes containing EDTA (0.1%) and immediately placed in an ice bath; plasma was separated from red blood cells by centrifuging the blood at 2620 ×
solvent mixtures, the partially delipidated TRLs that had adhered without agitation of the tubes. After gentle aspiration of the brief (5 min) to cold hexane:ethanol (3:1, by vol) mixtures, and the dried TRLs adhering to the tubes were exposed to the walls of the tubes were then dissolved in an SDS-treatment buffer and loaded on to 4–20% SDS gradient gels (Invitrogen Co, Carlsbad, CA) to separate apo B-100, apo B-48, and other apolipoproteins on TRLs. Apo B-100 prepared from purified LDL was used as a standard. After the gels were stained with gel code blue stain (Pierce Co, Rockford, IL), the concentrations of fasting and postprandial TRL apo B-100 and apo B-48 in the gel were then determined based on an apo B-100 standard by using a computer-driven UVP gel imaging system (Quest Scientific Co, Cumming, GA). Postprandial changes in the concentrations of apo B-100 and apo B-48 associated with TRLs were examined by determining the percentage of postprandial plasma TRL apo B-48 and apo B-100 and fasting VLDL apo B-48 relative to fasting plasma VLDL apo B-100 and by performing the statistical analysis on the extent (percentage) of postprandial increases in concentrations of apo B-48 and apo B-100 relative to those in fasting plasma.

**Measurement of plasma lipid, lipoprotein-cholesterol, and triacylglycerol concentrations**

Concentrations of plasma total cholesterol, UC, and triacylglycerol were measured by using enzymatic cholesterol, triacylglycerol, and UC assay reagents purchased from Sigma Co (St Louis), Boehringer Mannheim Co (Indianapolis), and Waco Diagnostics Co (Richmond, VA). The distribution of cholesterol and triacylglycerol in VLDL, LDL, and HDL density fractions in each fresh fasting plasma sample obtained on day 1 and in fasting and 4-h and 7-h plasma samples obtained on day 16 and day 20 was determined by the modified lipoprotein cholesterol auto-recorder method developed in this laboratory (30, 31). This method involves 1) short (150 min) single-spin density-gradient ultracentrifugal separation of the major lipoprotein fraction in plasma in a swing-out rotor (model SW 50.1; Beckman Co), 2) continuous-flow online mixing of effluent from density-gradient tubes with enzymatic cholesterol or triacylglycerol reagent, 3) online incubation and online measurement of absorbance produced, and 4) computerized calculation of lipoprotein-cholesterol and triacylglycerol concentrations (31). To determine the overall dietary fat–mediated changes in the density spectrum of lipoprotein particles as well as in their concentrations in fasting plasma, an aliquot (0.5 mL) of each frozen fasting plasma sample, collected from all study subjects (n = 16) on day 1 and day 16, was taken and pooled. The lipoprotein-cholesterol profiles of pooled plasma were then determined by the lipoprotein-cholesterol auto-recorder method. We showed previously that a single freezing and thawing of fasting plasma produces no appreciable change in its lipoprotein-cholesterol profile (30).

**Measurement of TRL apo B-100 and apo B-48**

A modified version of the sodium dodecyl sulfate (SDS) gel electrophoresis method described by Kotite et al (32) was used to examine the postprandial changes in concentrations of apo B-100 and apo B-48 associated with TRLs. Briefly, 4 mL fasting and postprandial plasma containing protease inhibitor mixtures (Roche Co, Indianapolis) were placed in the bottom of a Beckman SW 50.1 ultracentrifuge tube containing 1 mL buffered saline (0.01 mol tris/L, 1.15 mol NaCl/L, pH 7.4) with the use of an underlayering method. After the ultracentrifugation of plasma at 269 971 × g (49 000 rpm) for 16 h at 4 °C, TRLs floated to the top of the tubes (in a total volume of 0.8 mL) was quantitatively removed. Once the triacylglycerol and cholesterol concentration of fasting VLDLs had been determined, a fasting VLDL solution containing 10 μg triacylglycerol and 4-h and 7-h TRL solutions with the same volume as the fasting VLDL solution were placed in a conical centrifuge tube containing transferrin as an internal standard. The rotary vacuum evaporator rapidly dried the samples, and the dried TRLs adhering to the tubes were exposed briefly (5 min) to cold hexane:ethanol (3:1, by vol) mixtures without agitation of the tubes. After gentle aspiration of the solvent mixtures, the partially delipidated TRLs that had adhered to the walls of the tubes were then dissolved in an SDS-treatment buffer and loaded on to 4–20% SDS gradient gels (Invitrogen Co, Carlsbad, CA) to separate apo B-100, apo B-48, and other apolipoproteins on TRLs. Apo B-100 prepared from purified LDL was used as a standard. After the gels were stained with gel code blue stain (Pierce Co, Rockford, IL), the concentrations of fasting and postprandial TRL apo B-100 and apo B-48 in the gel were then determined based on an apo B-100 standard by using a computer-driven UVP gel imaging system (Quest Scientific Co, Cumming, GA). Postprandial changes in the concentrations of apo B-100 and apo B-48 associated with TRLs were examined by determining the percentage of postprandial plasma TRL apo B-48 and apo B-100 and fasting VLDL apo B-48 relative to fasting plasma VLDL apo B-100 and by performing the statistical analysis on the extent (percentage) of postprandial increases in concentrations of apo B-48 and apo B-100 relative to those in fasting plasma.

**Measurement of the concentration and activity of plasma CETP**

The CETP concentrations in fasting and postprandial plasma were determined by using a CETP enzyme-linked immunosorbent assay kit purchased from Waco Diagnostic Co. The plasma CETP activities were assayed by measuring the rate and extent of transfer of [3H]CE from LDL+HDL to TRLs in fasting and postprandial plasma in vitro. Briefly, [3H]CE-labeled LDL+HDL was prepared by using a TRL-free plasma fraction (density > 1.006 g/mL) as described by Thomas and Rudel (33). The distribution pattern of [3H]CE for LDL and HDL in the prepared samples mirrored that of LDL and HDL cholesterol. After isolation of [3H]CE-labeled LDL and HDL by one-step ultracentrifugation (269 971 × g, 150 min, 4 °C), equal (trace) amounts of [3H]CE-labeled LDL+HDL were added to fresh fasting and postprandial plasma, and the mixtures were incubated for 3–9 h at 37 °C. After incubation, TRLs were separated quantitatively from LDL and HDL by short (150 min), single-spin, density-gradient ultracentrifugation in a SW 50.1 swing-out rotor (31). The level of [3H]CE radioactivity transferred from LDL+HDL into TRLs was measured by counting the radioactivity associated with the TRL fraction. The increases in cholesterol mass in TRLs after in vitro incubation of plasma for 16 h at 37 °C was determined by analyzing the change in the lipoprotein-cholesterol profiles of plasma (31). To determine whether a CETP-mediated reciprocal transfer of CE and triacylglycerol occurs between TRLs and the LDL+HDL fraction during postprandial lipemia in vivo, we measured the changes in the fatty acyl chain composition of triacylglycerol associated with TRLs and the LDL+HDL fraction after quantitatively separating them from fresh and LCAT- and CETP-reacted fasting and postprandial plasma using the gas chromatographic method as previously described (34).

**Statistical analysis**

Quantitative variables were expressed as means ± SDs. Statistical tests were applied to compare the concentration of plasma lipids, lipoprotein cholesterol, and TRL apo B-100 and apo B-48 in fasting plasma and in postprandial plasma obtained after subjects consumed an SFA- or PUFA-rich diet or a meal or after an...
in vitro reaction of LCAT and CETP in plasma. These comparisons were performed by using mixed-models repeated-measures analysis of variance, assuming an unstructured covariance matrix. Tukey’s multiple comparisons test was then used to determine which specific pairs of means were significantly different (P < 0.05). All statistical tests were two-sided and were performed at a 5% significance level (ie, P < 0.05). All statistical analyses were performed with the use of SAS software (version 9.0; SAS Institute Inc, Cary, NC).

RESULTS

Chronic effect of a PUFA-rich and SFA-rich diet on changes in plasma lipid and lipoprotein-cholesterol concentrations and lipoprotein-cholesterol profiles of fasting plasma

The concentrations of lipids and lipoprotein cholesterol in fasting plasma obtained from all study subjects at entry (day 1) and after 16 days of a PUFA-rich or an SFA-rich diet are shown in Table 1. The overall PUFA- and SFA-mediated changes in the density spectrum of cholesterol associated with lipoproteins in fasting plasma are shown in Figure 1. Consumption of a PUFA-rich diet significantly decreased fasting plasma total cholesterol and CE concentrations owing to a significant reduction in LDL cholesterol with no significant reduction in HDL cholesterol (Table 1 and Figure 1). In contrast, consumption of an SFA-rich diet caused no significant change in concentrations of fasting plasma total cholesterol and CE or of either LDL or HDL cholesterol (Table 1 and Figure 1). Neither diet significantly altered the concentrations of fasting plasma triacylglycerol, UC, or VLDL cholesterol (Table 1). When concentrations of lipids and lipoprotein cholesterol in fasting plasma obtained on day 16 of a PUFA- or an SFA-rich diet were compared, the concentrations of fasting plasma total cholesterol, CE, and LDL cholesterol were significantly lower with the PUFA-rich diet than with the SFA-rich diet (Table 1). The abovementioned PUFA- and SFA-rich diet-mediated changes in the concentration and distribution of cholesterol in lipoproteins (Table 1) are evident from the lipoprotein-cholesterol profiles of pooled fasting plasma obtained on day 1 and day 16 of each diet (Figure 1). The LDL peak center appeared to have shifted slightly into a denser density region after an SFA-rich diet but not after a PUFA-rich diet (Figure 1).

Table 1

<table>
<thead>
<tr>
<th>Lipids and lipoproteins</th>
<th>PUFA-rich diet Day 1</th>
<th>Day 16</th>
<th>SFA-rich diet Day 1</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TG</td>
<td>1.07 ± 0.43</td>
<td>1.03 ± 0.43</td>
<td>1.06 ± 0.39</td>
<td>1.12 ± 0.45</td>
</tr>
<tr>
<td>Plasma total C</td>
<td>4.61 ± 1.06</td>
<td>4.22 ± 1.06</td>
<td>4.64 ± 0.96</td>
<td>4.81 ± 1.01</td>
</tr>
<tr>
<td>Plasma UC</td>
<td>1.06 ± 0.21</td>
<td>1.01 ± 0.20</td>
<td>1.02 ± 0.23</td>
<td>1.04 ± 0.22</td>
</tr>
<tr>
<td>Plasma CE</td>
<td>3.56 ± 0.89</td>
<td>3.20 ± 0.92</td>
<td>3.62 ± 0.85</td>
<td>3.78 ± 0.97</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.29 ± 0.20</td>
<td>0.32 ± 0.20</td>
<td>0.31 ± 0.22</td>
<td>0.34 ± 0.22</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.94 ± 0.93</td>
<td>2.59 ± 0.86</td>
<td>2.99 ± 0.89</td>
<td>3.07 ± 0.91</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.37 ± 0.41</td>
<td>1.32 ± 0.38</td>
<td>1.37 ± 0.37</td>
<td>1.40 ± 0.34</td>
</tr>
</tbody>
</table>

1 All values are x ± SD; n = 16. Means in a row with different superscript letters are significantly different, P < 0.05 (repeated-measures ANOVA with Tukey’s post hoc test). C, cholesterol; TG, triacylglycerol; UC, unesterified cholesterol; CE, cholesteryl ester.

FIGURE 1. Lipoprotein-cholesterol profiles in pooled fasting plasma samples obtained from 16 normolipidemic subjects before (day 1) and after 16 d of consuming diets rich in polyunsaturated (PUFA) or saturated (SFA) fatty acids.
Chronic effect of PUFA- and SFA-rich diets on postprandial changes in concentrations of plasma lipids, lipoprotein-cholesterol, TRL apo B-100 and apo B-48, and lipoprotein-cholesterol and triacylglycerol profiles

Postprandial change in plasma lipid and lipoprotein-cholesterol concentrations, lipoprotein-triacylglycerol profiles, composition and concentration of VLDL apo B, and lipoprotein-cholesterol profiles of fasting plasma after 16 d of either a PUFA- or an SFA-rich diet are shown in Table 2, Figure 2, and Figure 3.

Postprandial changes in fasting plasma triacylglycerol and TRL apo B-100 and apo B-48 concentrations

With both diets, fasting plasma triacylglycerol concentrations increased significantly at 4 h (Table 2). At 7 h, the elevated triacylglycerol concentrations decreased to fasting concentration with the PUFA-rich diet but only decreased partially with the SFA-rich diet (Table 2). The postprandial changes in plasma lipoprotein-triacylglycerol profiles seen in a typical subject after a PUFA-rich diet and an SFA-rich diet (Figure 2, A) indicate that postprandial increases and decreases in plasma triacylglycerols are primarily due to changes in concentrations of the triacylglycerols associated with the TRL (VLDL density) fraction. Substantial accumulation of postprandial plasma triacylglycerol in TRls 7 h after consumption of an SFA-rich but not after consumption of a PUFA-rich diet was evident from postprandial changes in the triacylglycerol profiles (Figure 2, A). Separation and quantification of TRL apo B-100 and apo B-48 with SDS gradient gels showed that the postprandial increase in TRL-triacylglycerol seen at 4 h with both diets was due to a significant increase in the concentration of both apo B-100–containing VLDLs and apo B-48–containing chylomicrons (Figure 2, A and B). We detected a small variable amount of apo B-48 in most fasting VLDLs obtained from our normolipidemic study subjects (Figure 2, B). The mean concentration of TRL apo B-48 in fasting plasma after an SFA-rich diet (6.2% of fasting VDL apo B-100) was not different from that after a PUFA-rich diet (4.7% of fasting VLDL apo B-100) (Figure 2, B).

A recent study (35) showed that normolipidemic subjects contained widely variable amounts of apo B-48–containing lipoprotein particles in their fasting plasma. The significant postprandial increases in TRL apo B-100 (158%) or apo B-48 (261%) 4 h after consumption of an SFA-rich diet were not significantly different from those increases noted after a PUFA-rich diet (TRL apo B-100 (147%) and TRL apo B-48 (197%)) (Figure 2, B). At 7 h, TRL apo B-48 decreased to its fasting concentration with both the PUFA-rich diet and the SFA-rich diet (Figure 2, B). With the SFA-rich diet, the concentration of apo B-100—containing TRls at 7 h was still significantly greater than the fasting concentration (Figure 2, B). We determined further molar concentrations of fasting VLDL apo B-100 and apo B-48 on the basis of the concentration of fasting plasma VLDL triacylglycerol and the content of apo B-100 and apo B-48 per 10 µg VLDL triacylglycerol and using a molecular weight of 555 486 for apo B-100 and a molecular weight of 260 416 for apo B-48. The molar concentrations of fasting VLDL apo B-100 and apo B-48 were 61.5 ± 22.7 and 6.8 ± 5.35 mmol/L with the PUFA-rich diet and 63.5 ± 24.9 mmol/L and 8.8 ± 4.3 mmol/L with the SFA-rich diet, respectively. The concentrations of fasting VLDL apo B-100 and apo B-48 with the PUFA-rich diet were not significantly different from those with the SFA-rich diet.

Postprandial changes in cholesterol concentrations of plasma and lipoproteins and the lipoprotein-cholesterol profile

With both diets, fasting plasma UC concentrations increased significantly at 4 h without a significant change in plasma total cholesterol, which resulted in a net decrease in the plasma cholesterol concentration (Table 2). The postprandial decrease in plasma CE

### Table 2

<table>
<thead>
<tr>
<th>Lipids and lipoproteins</th>
<th>Fasting</th>
<th>4 h</th>
<th>7 h</th>
<th>Fasting</th>
<th>4 h</th>
<th>7 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic effect of diets (day 16)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma TG</td>
<td>1.03 ± 0.43</td>
<td>1.64 ± 0.71</td>
<td>1.01 ± 0.45</td>
<td>1.12 ± 0.45</td>
<td>2.02 ± 0.89</td>
<td>1.49 ± 0.52</td>
</tr>
<tr>
<td>Plasma total C</td>
<td>4.22 ± 1.06</td>
<td>4.15 ± 1.08</td>
<td>4.16 ± 0.99</td>
<td>4.81 ± 1.01</td>
<td>4.85 ± 1.03</td>
<td>4.85 ± 0.95</td>
</tr>
<tr>
<td>Plasma UC</td>
<td>1.01 ± 0.20</td>
<td>1.11 ± 0.28</td>
<td>1.03 ± 0.22</td>
<td>1.04 ± 0.22</td>
<td>1.16 ± 0.26</td>
<td>1.10 ± 0.29</td>
</tr>
<tr>
<td>Plasma CE</td>
<td>3.21 ± 0.92</td>
<td>3.04 ± 0.89</td>
<td>3.13 ± 0.85</td>
<td>3.77 ± 0.97</td>
<td>3.69 ± 0.98</td>
<td>3.75 ± 0.88</td>
</tr>
<tr>
<td>TRL-C</td>
<td>0.32 ± 0.20</td>
<td>0.48 ± 0.25</td>
<td>0.34 ± 0.19</td>
<td>0.34 ± 0.22</td>
<td>0.54 ± 0.35</td>
<td>0.45 ± 0.22</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.59 ± 0.86</td>
<td>2.42 ± 0.87</td>
<td>2.52 ± 0.82</td>
<td>3.07 ± 0.91</td>
<td>2.95 ± 0.81</td>
<td>3.00 ± 0.81</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.32 ± 0.38</td>
<td>1.25 ± 0.38</td>
<td>1.30 ± 0.37</td>
<td>1.40 ± 0.34</td>
<td>1.36 ± 0.35</td>
<td>1.39 ± 0.36</td>
</tr>
</tbody>
</table>

**Acute effects of a meal (day 20)**

<table>
<thead>
<tr>
<th>Lipids and lipoproteins</th>
<th>Fasting</th>
<th>4 h</th>
<th>7 h</th>
<th>Fasting</th>
<th>4 h</th>
<th>7 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TG</td>
<td>1.00 ± 0.38</td>
<td>1.57 ± 0.53</td>
<td>1.27 ± 0.49</td>
<td>1.11 ± 0.42</td>
<td>1.88 ± 0.77</td>
<td>1.48 ± 0.50</td>
</tr>
<tr>
<td>Plasma total C</td>
<td>4.23 ± 1.04</td>
<td>4.27 ± 1.06</td>
<td>4.29 ± 1.00</td>
<td>4.80 ± 0.98</td>
<td>4.77 ± 0.93</td>
<td>4.78 ± 0.97</td>
</tr>
<tr>
<td>Plasma UC</td>
<td>1.01 ± 0.23</td>
<td>1.10 ± 0.25</td>
<td>1.05 ± 0.23</td>
<td>1.04 ± 0.21</td>
<td>1.15 ± 0.26</td>
<td>1.10 ± 0.28</td>
</tr>
<tr>
<td>Plasma CE</td>
<td>3.22 ± 0.90</td>
<td>3.17 ± 0.90</td>
<td>3.22 ± 0.89</td>
<td>3.76 ± 0.91</td>
<td>3.62 ± 0.89</td>
<td>3.67 ± 0.84</td>
</tr>
<tr>
<td>TRL-C</td>
<td>0.31 ± 0.19</td>
<td>0.47 ± 0.23</td>
<td>0.40 ± 0.22</td>
<td>0.35 ± 0.19</td>
<td>0.52 ± 0.26</td>
<td>0.44 ± 0.22</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.60 ± 0.91</td>
<td>2.56 ± 0.98</td>
<td>2.59 ± 1.01</td>
<td>3.04 ± 0.90</td>
<td>2.90 ± 0.79</td>
<td>2.93 ± 0.82</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.31 ± 0.37</td>
<td>1.26 ± 0.36</td>
<td>1.29 ± 0.36</td>
<td>1.40 ± 0.32</td>
<td>1.37 ± 0.34</td>
<td>1.40 ± 0.32</td>
</tr>
</tbody>
</table>

*All values are ± SD; n = 16. Means in a row with different superscript letters are significantly different, P < 0.05 (repeated-measures ANOVA with Tukey’s post hoc test). C, cholesterol; TG, triacylglycerol; UC, unesterified cholesterol; CE, cholesteryl ester; TRL, triacylglycerol-rich lipoproteins (VLDL in fasting plasma or VLDL and chylomicrons in postprandial plasma).
concentrations at 4 h was significant with the PUFA-rich diet but not with the SFA-rich diet (Table 2). The increased concentrations of plasma UC with both diets at 4 h and decreased concentrations of plasma CE at 4 h with the PUFA-rich diet returned to near fasting concentrations at 7 h (Table 2). The chronic effects of a diet rich in PUFAs or SFAs on the postprandial changes in the concentrations of cholesterol in VLDL-, LDL-, and HDL-density fractions were further examined by determining the lipoprotein-cholesterol profiles in fasting and postprandial plasma. On the basis of postprandial changes in the cholesterol profile of fasting plasma obtained from a subject with a brisk postprandial lipemic response (Figure 3, A and B), induction of postprandial lipemia after both diets caused an increase in cholesterol concentrations in TRLs and a concomitant decrease in cholesterol concentrations in LDL and HDL in 4 h plasma. Overall, the postprandial increase in TRL cholesterol at 4 h was statistically significant with both diets, but the postprandial decrease in concentrations of cholesterol in LDL and HDL at 4 h was statistically significant only with the PUFA-rich diet (Table 2). The postprandial increase in cholesterol in TRLs with a concomitant postprandial decrease in the concentration of LDL and HDL cholesterol and the lack of significant changes in plasma total cholesterol suggests a shift in the distribution of cholesterol from LDL and HDL to TRLs. We observed that the postprandial decrease in LDL and HDL cholesterol occurs with little or no postprandial increase in TRL cholesterol in subjects with a high HDL-cholesterol concentration (data not shown). Because plasma UC increased significantly at 4 h (Table 2), the postprandial decreases in cholesterol in LDL and HDL were observed to be due to decreases in their CE concentration (data not shown).
The postprandial changes in lipoprotein-cholesterol concentrations (Table 2) indicate further that the mean postprandial decrease in cholesterol concentrations of the LDL/HDL fraction (0.24 mmol/L) exceeded the mean postprandial increase in cholesterol concentrations of TRLs (0.17 mmol/L) after a PUFA-rich diet; in contrast, the mean postprandial increase in TRL cholesterol (0.21 mmol/L) exceeded the mean postprandial decrease in cholesterol of the LDL/HDL fraction (0.16 mmol/L) after an SFA-rich diet (Table 2). Because the extent of the postprandial increase in TRL-cholesterol concentration at 4 h with both diets was about equal, the PUFA-mediated net decrease and the SFA-mediated net increase in plasma cholesterol were attributed primarily to the differences in the extent of the postprandial decrease in LDL and HDL cholesterol. At 7 h, the lowered plasma concentrations of LDL and HDL cholesterol at 4 h increased back to near fasting concentrations with both the PUFA- and SFA-rich diets, but the significant accumulation of postprandial TRLs in the plasma after the SFA-rich diet was not observed after the PUFA-rich diet (Table 2 and Figure 3, A and B).

Acute effects of substituting a meal of the alternate diet on day 20 of the experimental diet on the postprandial changes in plasma lipid and lipoprotein-cholesterol concentrations and lipoprotein-cholesterol profiles

The concentrations of lipids and lipoprotein cholesterol in fasting plasma obtained on day 20 of the experimental diet were not significantly different from those obtained on day 16 (Table 2; acute and chronic effects). The substitution of a PUFA-rich meal for an SFA-rich meal or vice versa did not significantly alter the extent of postprandial increases in plasma triacylglycerol and UC concentrations in 4 h plasma (Table 2; acute effects). The extent of postprandial plasma triacylglycerol accumulated in 7 h plasma significantly increased after the substitution of an SFA-rich meal for a PUFA-rich meal in the subjects who consumed the PUFA-rich diet but was not altered after the reverse substitution (Table 2; acute effects). The effect of substituting an SFA-rich meal for a PUFA-rich meal or vice versa on the postprandial changes in lipoprotein-cholesterol concentration was determined further by examining the alteration in postprandial changes in lipoprotein-cholesterol profiles (Figure 3). It is evident that in a subject with a brisk postprandial lipemic response, the substitution of an SFA-rich meal for a PUFA-rich meal in subjects consuming a chronic PUFA-rich diet diminished the postprandial lipemia-mediated reduction of LDL and HDL cholesterol at 4 h and increased the accumulation of postprandial TRLs at 7 h (Figure 3, A and C). It was also evident that the reverse substitution in subjects consuming a chronic SFA-rich diet only minimally altered postprandial lipoprotein-cholesterol profiles (Figure 3, B and D). In the subjects who consumed the chronic PUFA-rich diet, the postprandial lipemia produced after substitution of an SFA-rich meal for a PUFA-rich meal was associated with no significant reduction in plasma CE and LDL and HDL cholesterol at 4 h and a significant accumulation of postprandial TRL cholesterol at 7 h (Table 2; acute effects). This finding is in contrast with a significant reduction in plasma CE and LDL and HDL cholesterol at 4 h and no significant accumulation of postprandial TRL cholesterol 7 h after a PUFA-rich meal (Table 2; chronic effects). No statistically significant difference in postprandial changes in plasma lipid and lipoprotein-cholesterol concentrations were noted in subjects consuming a chronic SFA-rich diet when a PUFA-rich meal was acutely given (Table 2; chronic and acute effects).

Comparison of the chronic and acute effects of PUFA-rich and SFA-rich diets on the extent of postprandial changes in plasma lipid and lipoprotein-cholesterol concentrations

The effects of acute and chronic SFA- and PUFA-mediated postprandial lipemia on the percentage change in plasma lipids and lipoprotein cholesterol are shown in Table 3. The extent of the postprandial increase in plasma triacylglycerol and TRL cholesterol 4 h after a chronic PUFA-rich diet did not differ from that...
after a chronic SFA-rich diet and was not altered by the substitution of a PUFA-rich for an SFA-rich meal or vice versa (Table 3). The extent of accumulation of postprandial plasma triacylglycerol and LDL cholesterol at 7 h increased significantly after the substitution of an SFA-rich meal for a PUFA-rich meal (Table 3) but was not altered by the substitution of a PUFA-rich meal for an SFA-rich meal (Table 3). Postprandial lipemia at 4 and 7 h tended to cause a net decrease and a net increase in plasma cholesterol after a chronic PUFA-rich diet and SFA-rich diet, respectively; however, this difference was not statistically significant (Table 3).

The substitution of an SFA-rich meal for a PUFA-rich meal caused a statistically significant net increase in cholesterol in both 4 h and 7 h plasma, whereas the reverse substitution caused no significant change (Table 3). The postprandial lipemia-mediated percentage reduction in LDL cholesterol at 4 h tended to be greater after a chronic PUFA-rich diet than after a chronic SFA-rich diet (Table 3). The substitution of an SFA-rich meal for a PUFA-rich meal caused a statistically significant lowering of the postprandial decrease in LDL cholesterol (Table 3). The reverse substitution, however, did not significantly alter the extent of the postprandial lipemia-mediated reduction in LDL cholesterol (Table 3). The percentage change in HDL cholesterol at 4 and 7 h was not significantly altered by either a chronic diet or an acute meal. The above data indicated that an acute SFA-rich meal can blunt the chronic effect of a PUFA-rich diet-mediated change in postprandial plasma lipids and lipoprotein-cholesterol concentration; however, an acute PUFA-rich meal does not have the same blunting effects on chronic SFA-mediated changes.

Effect of in vitro reactions of endogenous LCAT and CETP in fasting and postprandial plasma on LDL cholesterol concentration

Table 3 Comparison of changes in the percentage of plasma lipid and lipoprotein-cholesterol concentrations after a diet rich in polyunsaturated (PUFA) or saturated (SFA) fatty acids (chronic effect) or a single meal (acute effect)

<table>
<thead>
<tr>
<th>Lipids and lipoprotein C</th>
<th>SFA-rich diet</th>
<th>PUFA-rich diet</th>
<th>PUFA-rich diet</th>
<th>SFA-rich diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TG</td>
<td>82.7 ± 48.8</td>
<td>70.4 ± 50.2</td>
<td>53.5 ± 25.8</td>
<td>55.4 ± 34.8</td>
</tr>
<tr>
<td>Plasma C</td>
<td>0.6 ± 3.2b</td>
<td>−1.0 ± 3.7b</td>
<td>−2.3 ± 4.8a</td>
<td>0.7 ± 5.2b</td>
</tr>
<tr>
<td>TRL-C</td>
<td>61.6 ± 34.2</td>
<td>48.5 ± 28.6</td>
<td>58.0 ± 37.2</td>
<td>60.4 ± 36.4</td>
</tr>
<tr>
<td>LDL-C</td>
<td>−4.2 ± 5.0b</td>
<td>−4.9 ± 6.4b</td>
<td>−1.7 ± 5.7a</td>
<td>−3.4 ± 6.0b</td>
</tr>
<tr>
<td>HDL-C</td>
<td>−2.9 ± 5.9</td>
<td>−2.8 ± 4.0</td>
<td>−4.8 ± 5.0</td>
<td>−3.6 ± 6.7</td>
</tr>
</tbody>
</table>

Changes at 4 h (4 h vs fasting)

Changes at 7 h (7 h vs fasting)

Downloaded from https://academic.oup.com/ajcn/article-abstract/80/5/1145/4690416 by guest on 16 January 2019
right panel A). After incubation with red blood cells, the percentage increase in the cholesterol content of TRLs in fasting plasma, 4 h plasma, and 7 h plasma obtained after an SFA-rich diet did not differ significantly from that in TRLs obtained after a PUFA-rich diet (Figure 4, right panel C). The above data indicate that the potencies to accept cholesterol released from red blood cells by TRL particles in fasting and postprandial plasma were not affected by dietary fat composition.

Effects of postprandial lipemia on plasma CETP concentrations and activity

We further examined whether the postprandial decrease in cholesterol in the LDL/HDL fraction and the concomitant increase in TRL cholesterol were due to a postprandial lipemia-mediated increase in plasma CETP concentration and activity (Figure 5). As indicated in Figure 5 (panel A), the CETP concentration in fasting plasma was not affected by either the consumption of a PUFA-rich diet for 16 d or by the induction of postprandial lipemia. Similarly, no significant change in the plasma CETP concentration was observed when subjects consumed the SFA-rich diet for 16 d and when postprandial lipemia was subsequently induced (data not shown). We examined CETP activity by incubating fresh fasting and postprandial plasma with an equal amount of [3H]CE-labeled LDL and HDL and then measured the amount of [3H]CE that appeared in TRLs. This study showed that the rate and extent of transfer of [3H]CE from the LDL/HDL fraction to TRLs were much greater in 4 h plasma than in fasting plasma (Figure 5, B). The decrease in TRL concentrations in 7 h plasma via the clearance of postprandial TRLs in vivo largely eliminated the earlier increase in CETP activity in
Further study showed that the transfer of [3H]CE from the LDL+HDL fraction to TRLs in 4 h plasma was markedly reduced by the centrifugal removal of chylomicrons from 4 h plasma but was increased by the enrichment of 4 h plasma with autologous chylomicron before its incubation (Figure 5, C). Because postprandial lipemia did not change CETP concentrations (Figure 5, A) and because CETP is responsible for the intraplasma transfer of CE (20), it is possible that postprandial lipemia increases the transfer of CE from LDL or HDL to TRLs in vivo by increasing the concentration of chylomicrons that serve as acceptors of the CETP reaction. We observed also that the appearance of postprandial TRLs in 4 h plasma significantly increased the partitioning of [3H]CE formed from [3H]UC in plasma by LCAT into TRLs with a concomitant significant decrease in its partitioning into the LDL+HDL fraction (data not shown). The above observations suggest that the CETP-mediated transfer of endogenous CE in LDL and HDL and in LCAT-produced CE to TRLs may be increased by the appearance of postprandial TRLs in plasma.

Effects of postprandial lipemia and in vitro reactions of LCAT and CETP on the changes in the fatty acyl chain composition of triacylglycerol associated with TRLs or the LDL+HDL fractions

CETP in plasma catalyzes the reciprocal transfer of CE and triacylglycerol between TRLs and the LDL+HDL fractions (20). To determine whether the CETP-mediated transfer of triacylglycerol from postprandial TRLs to the LDL+HDL fraction occurs in vivo, we examined the effect of postprandial lipemia on the appearance of dietary fat–derived triacylglycerol on postprandial TRLs and the LDL+HDL fraction by analyzing the postprandial change in the fatty acyl chain composition (P:S) of triacylglycerol associated with TRLs and the LDL+HDL fraction.

We previously observed that the P:S of triacylglycerol associated with TRLs in fasting plasma obtained from subjects on their habitual diets increased significantly after the intake of a meal rich in PUFAs (34). Thus, we obtained fresh fasting and postprandial plasma from the subjects while they were consuming their habitual diets (day 1) and before and after they consumed a PUFA-rich meal (P:S = 2.0) was fed acutely to the same subjects on their habitual diet (data not shown). The above observations suggest that the triacylglycerol composition of chylomicron and VLDL in postprandial plasma reflects the fat composition of an acutely ingested meal.

The triacylglycerol P:S of the LDL+HDL fraction in 4 h plasma was also significantly higher than that in fasting plasma, whereas the triacylglycerol P:S of the LDL+HDL fraction was higher in 7 h plasma than in 4 h plasma and was not significantly
different from the triacylglycerol P:S of TRLs (Table 4). After incubation of fasting and postprandial plasma, the triacylglycerol concentration of the LDL+HDL fraction increased significantly (2.2- to 2.7-fold) because of the CETP-mediated transfer of triacylglycerol from TRLs to the LDL+HDL fraction; however, this incubation minimally altered the P:S of triacylglycerol associated with the LDL+HDL fraction (Table 4). The above data indicate that, at 4 h, a near equilibrium existed between triacylglycerol in TRLs and the LDL+HDL fraction, which suggested that the CETP-mediated reciprocal transfer of triacylglycerol and CE between the postprandial TRLs and LDL+HDL fractions occurred in vivo. The occurrence of a significant postprandial change in the triacylglycerol P:S on the LDL+HDL fraction without a significant change in its concentration at 4 h further suggested that triacylglycerol accepted by LDL and HDL from postprandial TRLs are rapidly hydrolyzed by hepatic lipase in vivo.

DISCUSSION

We examined the effects of the appearance and clearance of postprandial TRLs serving as acceptors of LCAT and CETP reaction products on the postprandial changes in endogenous lipoprotein-cholesterol concentrations in normolipidemic subjects who have followed PUFA- or SFA-rich diets for 20 d. Our study showed that both diets affected not only the cholesterol concentrations of lipoproteins in fasting plasma but also the postprandial changes in the distribution of cholesterol among lipoproteins in plasma. LDL and HDL cholesterol in fasting plasma decreased after a PUFA-rich diet but increased after a SFA-rich diet (Table 1), which further confirmed the known cholesterol-lowering effect of a PUFA-rich diet and the cholesterol-raising effect of an SFA-rich diet in humans (1–3).

Many previous studies conducted in human volunteers showed that subjects who consumed an SFA-rich diet had higher concentrations of postprandial TRLs and their remnants and that these postprandial TRL remnants cleared more slowly than in subjects who consumed a PUFA-rich diet (8–11). Consistent with reports from other investigators (8–11), we observed in the current study that at 7 h, postprandial TRL cholesterol, apo B-100, apo B-48, and plasma triacylglycerol accumulated to a greater degree after an SFA-rich diet than after a PUFA-rich diet (Table 2 and Figures 2 and 3). The SFA-rich background diet delayed the clearance of apo B-100–containing TRLs more than it did the clearance of apo B-48–containing TRLs and was found to have a more potent influence on the clearance of postprandial TRLs than did a single meal (Table 2, Figures 2 and 3), which confirmed the previous findings of Bergeron and Havel (11). The increased accumulation of postprandial TRLs in plasma 7 h after an SFA-rich diet or meal could possibly have been due to the delayed hepatic clearance of the lipolytic remnants of postprandial TRLs or to the delayed appearance of postprandial TRLs in plasma because SFA is less well absorbed than is PUFA (36, 37).

Our study showed that the induction of postprandial lipemia after both types of diets resulted in a significant increase in TRL cholesterol and triacylglycerol and a concomitant transient decrease in LDL and HDL cholesterol (Table 2 and Figure 3). Although the abovementioned postprandial lipemia-mediated changes in lipoprotein-cholesterol concentrations have been reported by many other investigators (11, 21–25), the mechanism responsible for these changes has not been fully explored in other studies. The simultaneous postprandial increase in concentrations of TRL-associated triacylglycerol and cholesterol in 4 h plasma (Figures 2 and 3) could be due to increased secretions of postprandial TRLs from the intestines or liver induced by ingested triacylglycerol and cholesterol. However, many studies (38, 39) showed that dietary cholesterol (deuterated), when provided as a single fat-containing meal, does not simultaneously appear with triacylglycerol but is secreted in intestinally derived chylomicrons during several subsequent phases in healthy humans. The maximal dietary (deuterated) cholesterol appearing in postprandial chylomicron at the postprandial lipemic peak was only 1% of ingested cholesterol (38), which indicated that the bulk of cholesterol associated with chylomicrons at the postprandial lipemic peak was derived from endogenous sources. Because the postprandial increase in TRL cholesterol occurred concomitantly with a decrease in LDL and HDL cholesterol (Table 2 and Figure 3), postprandial enhancement in the CETP-mediated transfer of CE from LDL and HDL to TRLs in vivo could have been the cause. The CETP-mediated transfer of cholesterol from endogenous lipoproteins to TRLs in fasting plasma in vitro increased with the appearance of postprandial TRLs in plasma at 4 h without a change in plasma CETP mass (Figure 4). This observation suggests that the postprandial enhancement of CETP activity may be due to the appearance of postprandial TRLs, which serve as acceptors of a CETP reaction. Furthermore, the postprandial lipemia-mediated decrease in cholesterol in the LDL+HDL fraction was associated with an increase in the P:S of

**TABLE 4**

<table>
<thead>
<tr>
<th>Plasma fraction</th>
<th>Fasting plasma</th>
<th>Incubated</th>
<th>4 h plasma</th>
<th>Incubated</th>
<th>7 h plasma</th>
<th>Incubated</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:S of TG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRL</td>
<td>0.82 ± 0.18^a</td>
<td>—</td>
<td>1.17 ± 0.14^b</td>
<td>—</td>
<td>1.29 ± 0.14^c</td>
<td>—</td>
</tr>
<tr>
<td>CM</td>
<td>—</td>
<td>—</td>
<td>1.71 ± 0.17</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LDL+HDL</td>
<td>0.88 ± 0.13^a</td>
<td>0.90 ± 0.18^a</td>
<td>1.07 ± 0.08^a</td>
<td>1.11 ± 0.14^b</td>
<td>1.20 ± 0.18^c</td>
<td>1.24 ± 0.14^c</td>
</tr>
<tr>
<td>TG concentration (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL+HDL</td>
<td>0.32 ± 0.07^a</td>
<td>0.71 ± 0.22^b</td>
<td>0.32 ± 0.08^a</td>
<td>0.89 ± 0.27^c</td>
<td>0.33 ± 0.08^a</td>
<td>0.76 ± 0.26^c</td>
</tr>
</tbody>
</table>

All values are ± SD; n = 7. Means in a row with different superscript letters are significantly different, P < 0.05 (repeated-measures ANOVA with Tukey’s post hoc test). Fasting and postprandial plasma samples were obtained after the habitual (home) diet on day 1 followed by consumption of the PUFA-rich meal. CM, chylomicrons; TRL indicates the VLDL in fasting plasma or VLDL and CM in postprandial plasma.
triacylglycerol in the LDL+HDL fraction that mirrors that of the fatty acids in the ingested meal (Table 4). This postprandial increase in the P:S of triacylglycerol in the LDL+HDL fraction was likely a consequence of the CETP-mediated reciprocal exchange of CE and triacylglycerol between the TRL and LDL+HDL fractions in vivo, because dietary triacylglycerol is not directly incorporated into LDL and HDL during a short postprandial lipemic period. Studies that examined the fate of [3H]CE on HDL in humans and in rabbits, with active CETP in plasma, showed that [3H]CE on HDL, injected intravenously, appeared in both VLDL and LDL (40, 41). In rabbits, the peak activity of [3H]CE in injected HDL was seen in VLDL and LDL within 30–60 min and 60–120 min, respectively (41). These findings indicated that the CETP-mediated transfer of CE from HDL to TRLs in vivo is a rapid process. Under normal conditions, the rate of the CETP-mediated transfer of CE from HDL to TRLs in vivo was higher than the catabolic rate of endogenous lipoproteins and, thus, the pools of CE among lipoproteins in the fasting state approach equilibrium in vivo (42). However, it is probable that the CETP reaction during the postprandial lipemic state, which lasts several hours, would be sufficient to alter the equilibrium of CE between TRLs and endogenous lipoproteins in vivo because postprandial TRLs serving as additional acceptors of the CETP reaction clear at a faster rate than that of the CETP reaction or endogenous lipoprotein catabolism.

Oliviera et al (43) reported that, in chylomic patients, 58% and 18% of CE in lymph chylomicrons derived from HDL and LDL, respectively. This finding further supports the hypothesis that the CETP-mediated CE transfer from LDL and HDL to postprandial chylomicrons occurs in humans in vivo. Thus, the CETP-mediated transfer of CE from LDL and HDL to postprandial TRLs and the clearance of postprandial TRLs via hepatic uptake in vivo could lead to the net decrease in concentrations of CE in plasma and cholesterol in both LDL and HDL observed in this study (Table 2 and Figure 3). In a comparison of the effects of a PUFA- and an SFA-rich diet on postprandial changes in plasma lipid and lipoprotein-cholesterol concentrations (Figure 3, Tables 2 and 3), we observed that the SFA- and PUFA-mediated induction of postprandial lipemia produced qualitatively similar, but significantly different, quantitative postprandial changes in plasma lipid and lipoprotein-cholesterol concentrations. These quantitative differences include 1) the net postprandial decrease in plasma total cholesterol 4 h after a PUFA-rich diet compared with the net increase 4 h after an SFA-rich diet; 2) the significant postprandial reduction in the concentrations of plasma CE, LDL cholesterol, and HDL cholesterol 4 h after a PUFA-rich diet compared with a nonsignificant reduction after an SFA-rich diet, and 3) the near complete clearance of postprandial TRLs 7 h after a PUFA-rich diet compared with the significant accumulation of postprandial TRLs after an SFA-rich diet (Table 2 and Figure 3). In earlier studies performed in young men, the extent of postprandial reductions in concentrations of cholesterol in LDL and HDL (Δmg/dL) observed with a PUFA-rich diet and meal were shown to be similar to those observed with an SFA-rich diet and meal (11, 24). Because LDL- and HDL-cholesterol concentrations in fasting plasma were significantly greater after an SFA-rich diet than after a PUFA-rich diet (Table 1), the postprandial lipemia-mediated percentage reduction in LDL cholesterol 4 h after the PUFA-rich diet tended to be greater than that after a chronic SFA-rich diet; the change was significantly minimized by the substitution of an SFA-rich meal for a PUFA-rich meal (Table 3). The mechanisms responsible for the above differences in the extent of postprandial changes in the percentage of endogenous lipoprotein cholesterol and plasma CE in vivo are not entirely clear but could be the result of different clearance rates for postprandial TRLs. Because the ability of TRLs to serve as acceptors of cholesterol from LDL and HDL after the in vitro reaction of LCAT and CETP was not affected by the type of dietary fat (Figure 4, left panel), the rapid clearance of postprandial TRLs carrying CE accepted from LDL and HDL via LCAT and CETP in vivo could cause a significant postprandial reduction in the concentration of plasma CE and LDL and HDL cholesterol and a net decrease in plasma cholesterol at 4 h with the PUFA-rich diet. Because humans are mostly in the postprandial lipemic state during the day because of the consumption of meals at regular intervals, the CETP-mediated transfer of CE from LDL and HDL to postprandial TRLs and the subsequent rapid clearance of postprandial TRLs during the consumption of a PUFA-rich diet would lead to the reduction of both LDL and HDL cholesterol in fasting plasma. For the diet rich in SFA, the lack of a significant postprandial lipemia-mediated reduction in plasma CE and LDL and HDL cholesterol and significant accumulation of postprandial TRLs at 7 h (Table 2) could have led to an increase in cholesterol in plasma LDL, HDL, and total cholesterol. Fielding and Fielding (44) reported that the generation of CE by LCAT, in situ, inhibited LCAT activity, provided that the product of LCAT activity remained in the HDL. It is possible that the accumulation of cholesterol accepted from LDL and HDL by postprandial TRLs in plasma, due to an SFA-mediated delayed clearance, could limit the CETP-mediated transfer of CE from LDL and HDL to TRLs via a feedback inhibition mechanism. Thus, rapid removal of postprandial TRLs carrying cholesterol accepted from LDL and HDL via CETP could be of potential importance in lowering endogenous lipoprotein-cholesterol concentrations. It is possible that the extent of flux of cholesterol in LDL and HDL via postprandial TRLs may be substantially lower with an SFA-rich than with a PUFA-rich diet because of a delayed hepatic removal of postprandial TRLs with an SFA-rich diet. Our current study also showed that postprandial TRLs appearing in plasma can serve as a potent acceptor of cholesterol released from cell membranes via LCAT and CETP and that dietary fat composition had no significant influence on the potency of postprandial TRLs to accept cholesterol released from red blood cells via LCAT and CETP (Figure 4, right panel). Because postprandial TRLs will be cleared from circulating plasma primarily via hepatic uptake (29), the LCAT- and CETP-mediated transfer of cholesterol from cell membranes to postprandial TRLs may lead to the removal of that cholesterol by the liver. It is possible that the transport of cholesterol on cell membranes to the liver via postprandial TRLs is enhanced with a PUFA-rich diet because of the rapid clearance of postprandial TRLs and are impaired with an SFA-rich diet because of the delayed clearance of postprandial TRLs. Many case control studies have shown that the delayed clearance of postprandial TRLs is more prevalent in CVD patients than in control subjects (45–47). Thus, the SFA-mediated increase or PUFA-mediated decrease in the risk of CVD (2) could be mediated by differences in their respective clearance rates of postprandial TRLs carrying cholesterol accepted from cell membranes via LCAT and CETP. In summary, data obtained from our current and previous studies (27, 28) suggest that postprandial lipemia in humans is associated with an active transfer of CE from endogenous lipoproteins or
cell membranes to postprandial TRLs via LCAT and CETP. Our current study also indicates that the clearance rate of postprandial TRLs is influenced by dietary fat composition. With a chronic PUFA-rich diet, the rapid clearance of postprandial TRLs, which carry cholesterol accepted from LDL and HDL via CETP and LCAT, may lead to a decrease in endogenous lipoprotein cholesterol in fasting plasma. Conversely, with a chronic SFA-rich diet, the delayed clearance of postprandial TRLs, which carry cholesterol accepted from cell membranes, may lead to an increase in both LDL and HDL cholesterol. Thus, the clearance rate of postprandial TRLs may play an important role in regulating fasting plasma cholesterol concentrations. Because postprandial TRLs act as potent ultimate acceptors of cholesterol released from cell membranes via LCAT and CETP, dietary fat–mediated alterations in the clearance rate of postprandial TRL carrying cholesterol accepted from cell membranes could affect the risk of CVD by altering the rate at which cholesterol is transported to the liver for excretion, a process often referred to as reverse cholesterol transport.

B-HC and FF developed the study concept. LO recruited and characterized the study subjects and monitored the subjects’ adherence to the test diet. BD formulated and prepared the test meals and diets. SD and PL measured the plasma lipids and lipoproteins. BHSC analyzed the fatty acyl chain composition of dietary fat and lipoprotein triacylglycerols. RAO performed the statistical analysis. B-HC drafted the manuscript. None of the authors had any financial interest in this work or with the sponsors of this work.

REFERENCES

37. Emken EA. Metabolism of dietary stearic acid relative to other fatty acids in human subjects. Am J Clin Nutr 1994;60(suppl):1023S–8S.


