Alcohol-mediated enhancement of postprandial lipemia: a contributing factor to an increase in plasma HDL and a decrease in risk of cardiovascular disease[1–3]

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ABSTRACT

Background: Moderate alcohol consumption increases plasma HDL and lowers cardiovascular disease risk while transiently enhancing postprandial lipemia.

Objective: We hypothesized that the alcohol-mediated increase in postprandial triacylglycerol-rich lipoproteins (TRLs) and their clearance elevate HDL cholesterol and reverse cholesterol transport.

Design: We determined the effect in normolipidemic humans (n = 14) of postprandial lipemia produced 4 h after a test meal (M) or a test meal + 0.5 g alcohol/kg body wt (M+A) on postprandial changes in plasma lipids and on the balance of cholesterol between TRL and the cholesterol-rich LDL and HDL fractions (CRL) or red blood cells (RBCs) in fresh and incubated plasma or blood.

Results: Postprandial lipemia after the M and M+A test meals caused a 56% and 89% increase in plasma triacylglycerol, a 30% and 74% increase in TRL cholesterol, and a 3.8% and 6.6% decrease in CRL cholesterol, respectively. In vitro reaction of endogenous lecithin:cholesterol acyltransferase (EC 2.3.1.43) and cholesteryl ester transfer proteins via incubation of fasting plasma samples and postprandial M and M+A plasma samples for 16 h increased TRL cholesterol by 22.8% (0.08 mmol/L), 32.6% (0.16 mmol/L), and 45.8% (0.28 mmol/L) in plasma and by 71.1% (0.27 mmol/L), 89.4% (0.45 mmol/L), and 112.5% (0.70 mmol/L) in RBC-enriched blood, respectively. After the in vitro lipolysis of TRL, the elevation of HDL cholesterol in postprandial M+A plasma, but not in postprandial M plasma, was significantly greater than in fasting plasma.

Conclusion: The alcohol-mediated increase in postprandial TRL flux and the hepatic removal of postprandial TRL after the acceptance of cholesterol from CRL and cell membranes contribute to increased HDL cholesterol and enhancement of reverse cholesterol transport in humans. *Am J Clin Nutr* 2003;78:391–9.

KEY WORDS Alcohol, postprandial lipemia, triacylglycerol-rich lipoproteins, high-density lipoproteins, cholesteryl ester transfer proteins, reverse cholesterol transport

INTRODUCTION

Numerous case-control and prospective epidemiologic studies from many countries with diverse populations have established that a moderate alcohol intake lowers the risk of cardiovascular disease (CVD) (1–5). Regular intake of any alcoholic drink (wine, liquor, or beer) is associated with lower CVD risk (4), indicating that ethyl alcohol may protect against CVD. However, the mechanisms of this protective effect are not well established. A recent meta-analysis (6) suggests that intake of 30 g alcohol/d can reduce the risk of CVD by 25% by altering plasma lipids, hemostatic components associated with platelets, and endothelial cell function. Because alcohol intake raises plasma HDL cholesterol (5, 7), and because high HDL-cholesterol concentrations have a protective effect against CVD (8), the HDL-raising effect of alcohol was estimated to contribute > 50% of alcohol's protective action (6). In the Cooperative Lipoprotein Phenotyping Study, an inverse linear relation was found between alcohol intake and LDL-cholesterol concentrations (7). This suggests that part of alcohol's protective effect against CVD may also be attributed to a lowering of LDL cholesterol.

It is well established that alcohol consumption accentuates the postprandial lipemic response to a meal (9). Postprandial lipemia causes a transient increase in the plasma activities of lecithin: cholesterol acyltransferase (LCAT; EC 2.3.1.43) and cholesteryl ester transfer protein (CETP) (10, 11), which promotes the esterification of unesterified cholesterol and the reciprocal exchange or transfer of triacylglycerol and cholesteryl ester between triacylglycerol-rich lipoprotein (TRL) and cholesterol-rich lipoprotein (CRL) (11, 12). Although the plasma activities of LCAT and CETP are key intraplasma factors that regulate the rate of reverse cholesterol transport in vivo (11, 12), whether alcohol-mediated enhancement of postprandial lipemia affects the rate of reverse cholesterol transport in vivo has not been evaluated. In transgenic mice, an increase in LCAT or CETP activity by overexpression of the individual genes encoding LCAT and CETP or an increase in postprandial TRL by overexpression of the apolipoprotein (apo) C-III gene enhances diet-induced atherosclerosis (13–15). However, simultaneous increases in both LCAT and CETP activities or increases in both CETP activity and postprandial TRL concentrations protect against diet-induced

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atherosclerosis (14, 16). This transgenic mouse model suggests that a coordinated postprandial increase of LCAT and CETP activities and postprandial TRL may protect humans against CVD, but this effect has not been evaluated.

We previously reported that postprandial chylomicrons appearing after a fatty meal are the most potent ultimate acceptors of cholesterol released from cell membranes via the LCAT and CETP reactions (17). In the current study, we examined the effect of accentuated postprandial lipemia after a meal containing alcohol on the balance of cholesterol between TRL and CRL in vivo and on the ability of plasma to promote reverse cholesterol transport in vivo by examining the potencies of TRL to accept cholesterol released from red blood cell (RBC) membranes via LCAT and CETP in vitro. To determine whether the alcohol-mediated increase in the flux of postprandial TRL in vivo was a factor responsible for the alcohol-mediated increase in plasma HDL cholesterol, we examined the effect of in vitro lipolysis of TRL in fasting and postprandial plasma on the extent of change in plasma HDL cholesterol.

SUBJECTS AND METHODS

Human subjects and fat-loading test

Healthy normolipidemic men and women were recruited for the study. Interested volunteers underwent a screening examination at the General Clinical Research Center, University of Alabama at Birmingham Medical Center. Examinations included documentation of a brief medical history and measurement of body weight, height, and fasting plasma lipid and lipoprotein concentrations. Heavy drinkers and subjects with a fasting triacylglycerol concentration above the 75th percentile or a fasting plasma cholesterol concentration above the 95th percentile of their respective age and sex group were excluded from the study. The Institutional Review Board approved the experimental protocol for this study.

Eleven men (10 white and 1 Asian) aged 24–59 y ($\bar{x} \pm$ SD: 37.6 ± 10.2 y) and 3 women (2 white and 1 Asian) aged 32–39 y (39.3 ± 10.6 y) participated in the study. The mean body mass indexes (BMIs; in kg/m^2) of the men and women in this study were 24.3 ± 2.4 and 23.0 ± 2.6, respectively.

A randomized crossover feeding trial was performed to study the acute effect of providing a meal with and without alcohol on postprandial changes in plasma lipoprotein cholesterol and on intraplasma metabolic activities. Study subjects were admitted to the General Clinical Research Center in the evening before the fat-loading test (day 1). To minimize the possible effect of the subjects' last meal (ie, dinner) on the postprandial lipemic response to the test meal, the study subjects were provided with a low-fat dinner. The pretest low-fat meal provided 20% of energy from fat, 40% of energy from carbohydrates, and 40% of energy from proteins, and it contained 100 mg cholesterol/1000 kcal. To induce postprandial lipemia, each subject, who fasted overnight (12 h), was given a test breakfast meal (M) on day 2 and a test breakfast meal plus 0.5 g white wine alcohol/kg body weight (M+A) on day 3. The test meals used on days 2 and 3 consisted of 40% of energy from fat, 40% of energy from carbohydrates, and 20% of energy from proteins and contained 175 mg cholesterol/1000 kcal. The test meal was formulated to provide 8.5%, 14%, and 17.5% of energy from saturated, monounsaturated, and polyunsaturated fatty acids, respectively, and a ratio of polyunsaturated to saturated fatty acids of 2.0. The mean ratio of polyunsaturated to saturated fatty acids of the test meals provided was determined to be 2.16. The meal was calculated on the basis of 50 g fat/m^2 body surface area. Samples of fasting (40 mL) and postprandial (80 mL) blood were collected from the study subjects just before and 4 h after the M and M+A test meals.

Treatment and analysis of blood and plasma samples

Blood samples were collected in tubes containing EDTA (0.1%) and were immediately placed in an ice bath. After blood samples were spun at 291 × *g* for 10 min in a precooled (4 °C) centrifuge, about two-thirds of the plasma in the tube separated from the RBCs and one-third was trapped within the packed RBCs. The upper plasma fraction was divided into 2 portions. One aliquot of plasma was kept in an ice bath, and the other aliquot of plasma and the plasma trapped in the packed RBCs (RBC-enriched blood) were placed in a 37 °C water bath for 16 h to allow lipoproteins in plasma to interact with endogenous LCAT, CETP, or RBCs. After incubation, the plasma trapped within the packed RBCs was separated by centrifuging the blood samples at 2620 × *g* for 25 min at room temperature.

The concentration and distribution of cholesterol or triacylglycerol among the VLDL, LDL, and HDL fractions in fresh and incubated fasting and postprandial plasma were measured by use of a modified lipoprotein cholesterol autoprofiler method developed in this laboratory (18). This method involves *1*) rapid separation of VLDL, LDL, and HDL particles by short-spin density gradient ultracentrifugation in a rotor with swingout buckets (Beckman SW 50.1; Beckman Coulter Inc, Fullerton, CA); *2*) continuous online mixing of the effluent from density gradient tubes with an enzymatic assay reagent of cholesterol or triacylglycerol; and *3*) online incubation and measurement of absorbance. Enzymatic assay kits were used to measure concentrations of total cholesterol (Sigma Co, St Louis), triacylglycerol (Wako Diagnostic Co, Richmond, VA), and unesterified cholesterol (Boehringer Mannheim Co, Indianapolis) in plasma, and an enzyme-linked immunosorbent assay kit (Wako Diagnostic Co) was used to measure CETP in plasma. Postprandial changes in concentrations of apo B-100 and apo B-48 associated with TRL were examined after *1*) quantitative isolation of TRL from plasma, *2*) separation of apo B-100 and apo B-48 associated with TRL on a 4–12% sodium dodecyl sulfate gradient gel, and *3*) quantification of apo B-100 and apo B-48 in the gel with the use of a UVP gel imaging system (Quest Scientific Co, Cumming, GA). The above analyses of fasting plasma samples were performed after pooling an equal volume of fasting plasma obtained on days 2 and 3.

To determine the effect of postprandial lipemia on the in vitro transfer of cholesteryl ester from CRL to TRL, LDL and HDL in VLDL-free fasting plasma—after the removal of VLDL by ultracentrifugation—were labeled with [^{3}H]cholesteryl ester according to the procedure described by Thomas and Rudel (19). The distribution of [^{3}H]cholesteryl ester between LDL and HDL in the prepared VLDL-free fasting plasma was similar to the distribution of cholesterol between LDL and HDL. After the addition of a trace amount of isolated [^{3}H]cholesteryl ester–labeled LDL and HDL into fresh fasting and postprandial plasma and incubation for 4 h at 37 °C, the [^{3}H]cholesteryl ester transferred from LDL and HDL to TRL was measured after quantitative separation of TRL from LDL and HDL by density gradient ultracentrifugation (18). To study the effect of in vitro metabolism of TRL on the change in HDL cholesterol, TRL in fasting and postprandial plasma was lipolyzed in vitro by incubating whole fasting and postprandial plasma with purified bovine milk lipoprotein lipase according to the previously described procedure (20). The extent of a lipolysis-mediated change in the amounts of cholesterol on TRL and HDL in fasting and postprandial plasma

TABLE 1
Plasma and lipoprotein triacylglycerols and cholesterol in fasting plasma and in postprandial (PP) plasma collected 4 h after a test meal (M) or 4 h after a test meal + 0.5 g alcohol/kg body wt (M+A)[1]

Plasma lipids and lipoproteins	Fasting plasma[2]	M plasma	M+A plasma	*P*
	mmol/L	*mmol/L*	*mmol/L*	
Triacylglycerol				
Plasma	1.20 ± 0.61[a]	1.86 ± 0.81[b]	2.26 ± 0.83[c]	0.0001
PP plasma − CM	—	1.34 ± 0.65[a]	1.49 ± 0.63[b]	0.0224
CM	—	0.51 ± 0.36[a]	0.77 ± 0.30[b]	0.0004
Cholesterol				
Plasma	4.38 ± 0.75	4.33 ± 0.77	4.41 ± 0.83	0.6060
TRL	0.41 ± 0.26[a]	0.53 ± 0.29[b]	0.71 ± 0.29[c]	0.0001
LDL	2.76 ± 0.75[a]	2.67 ± 0.69[a]	2.59 ± 0.69[b]	0.0006
HDL	1.19 ± 0.19[a]	1.16 ± 0.21[a,b]	1.12 ± 0.19[b]	0.0001
CRL	3.95 ± 0.70[a]	3.83 ± 0.71[a,b]	3.71 ± 0.71[b]	0.0014
(% of PP changes)	—	−3.77 ± 5.81	−6.64 ± 4.66	0.0695

[1] $\bar{x}$ ± SD; n = 14. CM, chylomicron; TRL, triacylglycerol-rich lipoprotein (VLDL in fasting plasma or VLDL and CM in PP plasma); CRL, cholesterol-rich lipoproteins (LDL + HDL). Means in a row with different superscript letters are significantly different, $P < 0.05$.
[2] Pooled plasma from days 2 and 3.

was measured by using the lipoprotein-cholesterol autoprofiler method described above (18).

Statistical analysis

Quantitative variables are expressed as means ± SDs. Statistical tests were applied to compare the concentrations of lipoproteins or lipids in fasting and postprandial plasma obtained after the M and M+A test meals and in control and LCAT- and CETP-reacted plasma. Mixed-models repeated-measure analysis of variance, assuming an unstructured covariance matrix, was used to perform these comparisons. Tukey's multiple comparison test was then used to determine which specific pairs of means were significantly different. All statistical tests were two-sided and were performed at a 5% significance level (ie, $\alpha = 0.05$). All statistical analyses were performed with the use of SAS software (version 8.2; SAS Institute Inc, Cary, NC).

RESULTS

The changes in plasma lipid and lipoprotein concentrations and in the lipoprotein triacylglycerol and cholesterol profiles after the induction of postprandial lipemia by the M and M+A test meals are shown in **Table 1** and **Figure 1**. Postprandial lipemia was associated with a significant increase in plasma triacylglycerol concentrations (Table 1) as a result of the elevation in triacylglycerol associated with TRL (Figure 1A). The extent of the increase in plasma triacylglycerol was significantly greater after the M+A test meal than after the M test meal (Table 1 and Figure 1A). Postprandial lipemia also caused a small increase in the concentrations of triacylglycerol associated with HDL, and the extent of the postprandial increase in HDL triacylglycerol was greater after the M+A test meal than after the M test meal (Figure 1A). Further examination of postprandial plasma triacylglycerol after centrifugal removal of large intact chylomicrons indicated that 76% and 72% of the postprandial increase after the M and M+A test meals, respectively, was due to the presence of large chylomicrons particles (Table 1). Thus, the amounts of large chylomicrons in postprandial plasma were significantly greater after the M+A test meal than after the M test meal. Plasma triacylglycerol concentrations in postprandial plasma after the removal of chylomicrons were also significantly higher after the M+A test meal than after the M test meal (Table 1).

The separation by sodium dodecyl sulfate gradient gel electrophoresis of apo B-100 and apo B-48 on TRL and the quantification of apo B-100 and apo B-48 by gel scanning indicated that the TRL apo B-100 in fasting plasma increased by 38% and 80% at 4 h after the M and M+A test meals, respectively (**Figure 2**). A small variable amount of apo B-48, detectable in most fasting TRL (6.7% of fasting TRL apo B-100), increased by 90% and 170% 4 h after the M and M+A test meals, respectively (Figure 2). These data indicate that alcohol intake boosted the postprandial production of both apo B-100–containing VLDL and apo B-48–containing chylomicron particles in response to a meal. As the SD ranges of the mean values indicate (Figure 2), the postprandial increases of TRL apo B-48 and TRL apo B-100 were more variable among subjects after the M+A test meal than after the M test meal. The postprandial increase in VLDL, but not in chylomicrons, was significantly higher after the M+A test meal than after the M test meal.

Postprandial lipemia after either test meal altered the distribution of cholesterol among lipoproteins without a significant change in plasma total cholesterol (Table 1 and Figure 1B). Postprandial lipemia produced a significant increase in plasma TRL cholesterol; the extent of the postprandial increase in TRL cholesterol was significantly greater after the M+A test meal than after the M test meal (Table 1 and Figure 1B). The postprandial increases in cholesterol on TRL were associated with a concomitant decrease in cholesterol on both LDL and HDL; these postprandial decreases in cholesterol on LDL and HDL were significant after the M+A test meal but not after the M test meal (Table 1).

To examine whether the postprandial lipemia-mediated decrease in cholesterol on CRL was due to an increase in CETP activity, we examined CETP concentrations and activities. CETP concentrations in plasma collected 4 h after the M (1.37 ± 0.24 μg/mL) or M+A (1.41 ± 0.29 μg/mL) test meal did not differ significantly from those in fasting plasma (1.48 ± 0.27 μg/mL; n = 8). However, after the incubation of fasting and postprandial plasma containing an equal (trace) amount of [^{3}H]cholesteryl ester–labeled LDL and HDL, the extent of transfer of [^{3}H]cholesteryl ester from LDL and HDL to TRL was significantly greater in postprandial M plasma than in fasting plasma and significantly greater in postprandial M+A plasma than in postprandial M plasma (**Figure 3**). The transfer of [^{3}H]cholesteryl ester from LDL and HDL to TRL was markedly reduced by the centrifugal removal of chylomicrons from postprandial plasma (data not

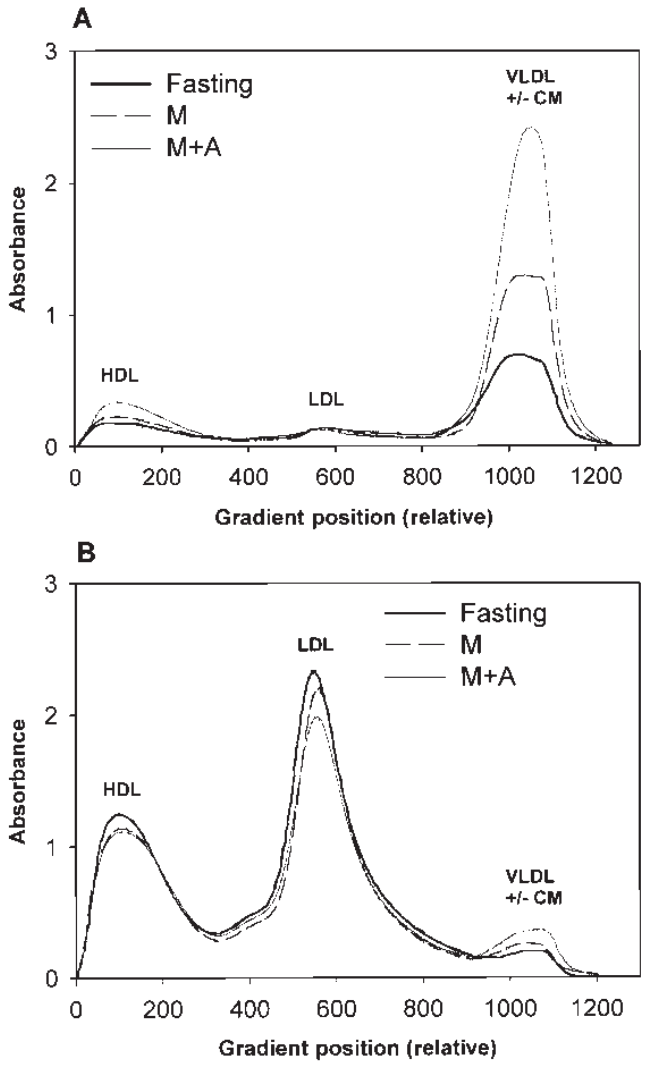

FIGURE 1. Lipoprotein triacylglycerol (A) and cholesterol (B) profiles of fasting plasma and postprandial plasma collected 4 h after a test meal (M) or 4 h after a test meal + 0.5 g alcohol/kg body wt (M+A) from a normolipidemic subject. "VLDL +/− CM" indicates a peak containing only VLDL (fasting plasma) or a peak containing both VLDL and chylomicrons (postprandial plasma). The triacylglycerol concentrations in VLDL, LDL, and HDL of fasting plasma, postprandial M plasma, and postprandial M+A plasma were 0.36, 0.09, and 0.10 mmol/L; 0.58, 0.1, and 0.12 mmol/L; and 0.95, 0.10, and 0.18 mmol/L, respectively. The cholesterol concentrations in VLDL, LDL, and HDL of fasting plasma, postprandial M plasma, and postprandial M+A plasma were 0.18, 2.76, and 1.29 mmol/L; 0.23, 2.58, and 1.24 mmol/L; and 0.33, 2.49, and 1.21 mmol/L, respectively.

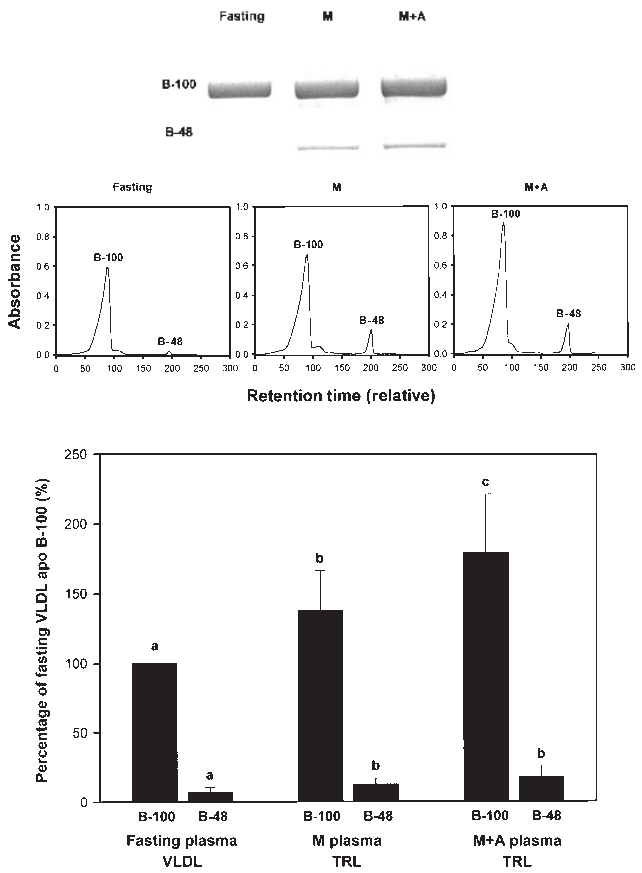

FIGURE 2. Sodium dodecyl sulfate gradient gels (top panel) and densitometric scans of the gels (middle panel) showing the separation and quantification of VLDL apolipoprotein (apo) B-100 and chylomicron apo B-48 in fasting plasma triacylglycerol-rich lipoprotein (TRL) and in postprandial plasma TRL collected 4 h after a test meal (M) or 4 h after a test meal + 0.5 g alcohol/kg body wt (M+A). Also shown (bottom panel) is the mean (± SD) percentage of chylomicron apo B-48 and apo B-100 relative to VLDL apo B-100 in fasting plasma. $n = 14$. Bars with different letters are significantly different, $P < 0.05$.

shown). These observations indicate that the increased CETP activity in postprandial plasma was caused by an increase in postprandial TRL.

In a further study, we examined the effect of incubating fasting and postprandial plasma in the absence and presence of RBCs, an exogenous source of cell membranes, on the change in the cholesterol content of TRL and CRL in plasma (**Figure 4** and **Table 2**). Incubation of fasting and postprandial plasma at 37 °C, which allows further interaction of LCAT and CETP with lipoproteins, increased the cholesterol content of TRL and HDL by decreasing the cholesterol content of LDL (Figure 4 and Table 2). The net and percentage increases in TRL cholesterol were significantly lower in fasting plasma than in postprandial M or M+A plasma (Table 2). The net and percentage increases in TRL cholesterol in postprandial M+A plasma tended to be greater than that in postprandial M plasma, but the difference was not significant. The net and percentage decreases in LDL cholesterol were significantly greater in postprandial M+A plasma than in fasting plasma. In postprandial M plasma, the percentage decrease but not the net decrease in LDL cholesterol was significantly greater than in fasting plasma but significantly less than that in postprandial M+A plasma (Table 2). The percentage increase in HDL cholesterol was significantly less in postprandial M+A plasma than in fasting plasma or postprandial M plasma (Table 2).

When RBCs were included during the incubation of fasting and postprandial plasma, the cholesterol content of all lipoproteins in plasma increased as a result of an elevation of cholesteryl ester via the activities of LCAT and CETP (Table 2). The net increase in the cholesterol content of LDL after the incubation of fasting

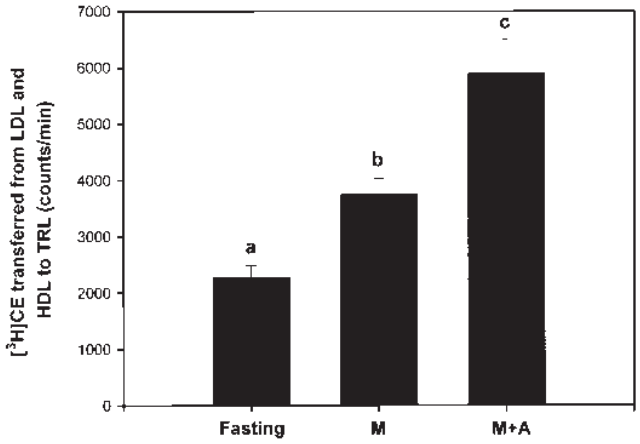

FIGURE 3. Mean (± SD of triplicates) cholesteryl ester transfer protein (CETP) activity in fasting plasma and in postprandial plasma collected 4 h after a test meal (M) or 4 h after a test meal + 0.5 g alcohol/kg body wt (M+A). CETP activity was measured by quantifying the amount of [^{3}H]cholesteryl ester ([^{3}H]CE) transferred from LDL and HDL to triacylglycerol-rich lipoprotein (TRL) after the incubation of fresh fasting plasma (0.68 mmol triacylglycerol/L), postprandial M plasma (1.02 mmol triacylglycerol/L), and postprandial M+A plasma (1.3 mmol triacylglycerol/L) obtained from a normolipidemic subject with an equal (trace) amount of [^{3}H]CE-labeled LDL and HDL (250 000 counts/min). Bars with different letters are significantly different, $P < 0.05$.

and postprandial plasma in the presence of RBCs (Figure 4 and Table 2), in contrast with its decrease in the absence of RBCs, suggests that the LDL cholesterol transferred to TRL and HDL was fully replenished by cholesterol released from RBC membranes.

After the incubation of plasma with RBCs, the net and percentage increases in cholesterol in TRL were significantly greater in postprandial M plasma than in fasting plasma and were significantly greater in postprandial M+A plasma than in postprandial M plasma. However, the net and percentage increases in cholesterol in LDL and HDL in fasting plasma and postprandial M and M+A plasma did not differ significantly (Table 2). As also shown in Table 2, the percentage increase in the cholesterol content of TRL in fasting plasma, postprandial M plasma, and postprandial M+A plasma was 3.9 times, 5.2 times, and 8.0 times the cholesterol content of LDL and 2 times, 2.6 times, and 3.5 times the cholesterol content of HDL, respectively. These postprandial changes in the potency of TRL to accept cholesterol from endogenous CRL and RBCs are evident from changes in the lipoprotein cholesterol profiles of fasting and postprandial plasma after their incubation with and without RBCs (Figure 4).

Lipolytic surface remnants of TRL have been proposed to be a precursor of circulating HDL (21). Thus, we examined whether the alcohol-mediated increase in postprandial TRLs and their clearance contributed to the HDL-raising effect of alcohol. To study this possibility, the changes in lipoprotein cholesterol profiles of fasting and postprandial M and M+A plasma were examined after the in vitro lipolysis of TRL in plasma. Incubation of fasting and postprandial plasma with purified bovine milk lipoprotein lipase at 37 °C for 90 min resulted in the hydrolysis of most (> 85%) of the triacylglycerol associated with TRL in both

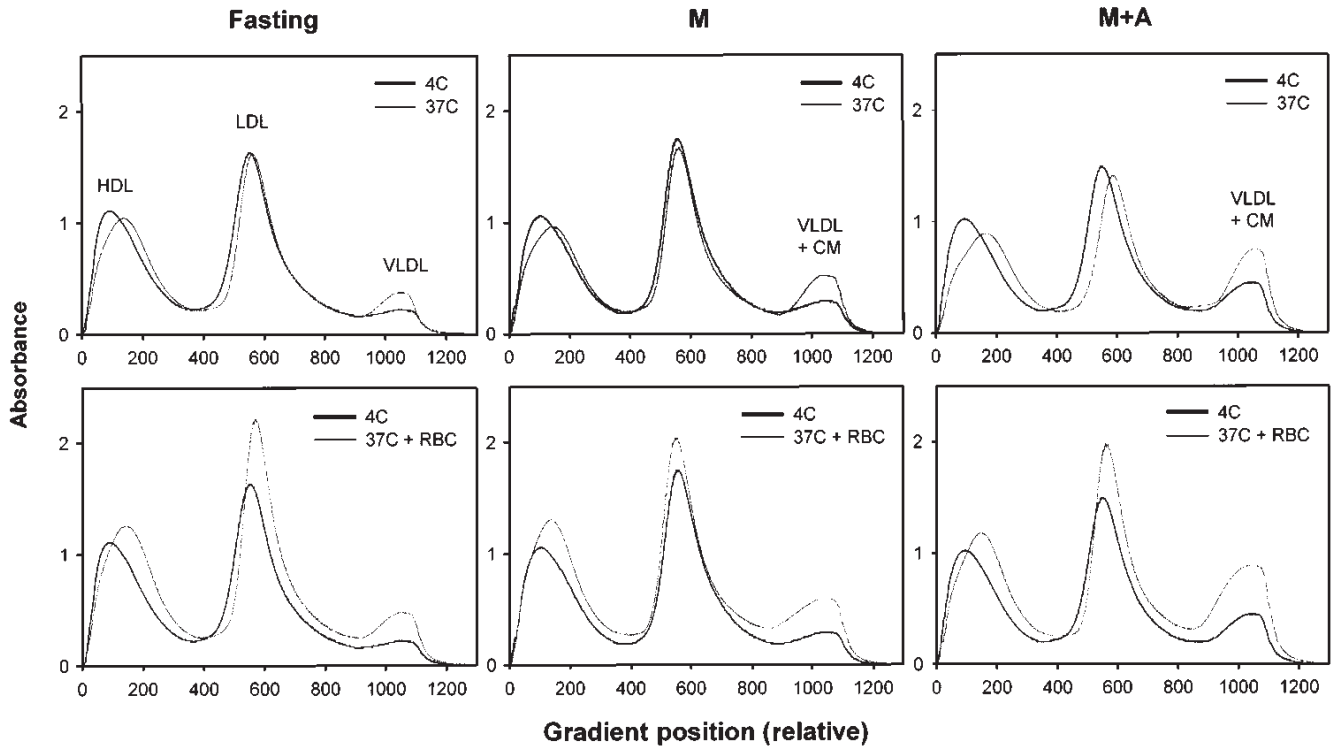

FIGURE 4. Lipoprotein cholesterol profiles of fresh (4C) fasting plasma and fresh postprandial plasma collected 4 h after a test meal (M) or 4 h after a test meal + 0.5 g alcohol/kg body wt (M+A) and of fasting and postprandial plasma incubated in vitro at 37 °C for 16 h in the absence (37C) or presence (37C + RBC) of red blood cells. CM, chylomicron.

TABLE 2
Net and percentage changes in cholesterol content of triacylglycerol-rich lipoprotein (TRL), LDL, and HDL after in vitro reaction with endogenous lecithin-cholesterol acyltransferase and cholesteryl ester transfer protein in the absence or presence of red blood cells (RBCs)[1]

Lipoproteins	Fasting plasma[2]	M plasma	M+A plasma	*P*
Change after incubation without RBCs				
TRL				
(mmol/L)	0.08 ± 0.03[a]	0.16 ± 0.13[b]	0.28 ± 0.15[b]	0.0068
(% change)	22.8 ± 12.3[a]	32.6 ± 13.7[b]	45.8 ± 16.9[b]	0.0010
LDL				
(mmol/L)	−0.17 ± 0.06[a]	−0.22 ± 0.16[a,b]	−0.30 ± 0.14[b]	0.0194
(% change)	−5.9 ± 2.4[a]	−8.2 ± 2.7[b]	−11.6 ± 4.1[c]	0.0001
HDL				
(mmol/L)	0.08 ± 0.05	0.06 ± 0.05	0.03 ± 0.04	0.0538
(% change)	7.2 ± 3.1[a]	5.3 ± 3.9[a]	1.6 ± 3.0[b]	0.0001
Change after incubation with RBCs				
TRL				
(mmol/L)	0.27 ± 0.13[a]	0.45 ± 0.23[b]	0.70 ± 0.18[c]	0.0001
(% change)	71.1 ± 14.9[a]	89.4 ± 18.2[b]	112.5 ± 24.8[c]	0.0001
LDL				
(mmol/L)	0.51 ± 0.15	0.46 ± 0.22	0.36 ± 0.19	0.1089
(% change)	18.0 ± 3.3	17.1 ± 4.6	14.0 ± 5.7	0.1835
HDL				
(mmol/L)	0.42 ± 0.15	0.39 ± 0.18	0.37 ± 0.15	0.4904
(% change)	34.7 ± 9.0	33.8 ± 9.3	31.8 ± 6.8	0.0834

[1] $\bar{x}$ ± SD; $n = 9$. M, postprandial plasma collected 4 h after a test meal; M+A, postprandial plasma collected 4 h after a test meal + 0.5 g alcohol/kg body wt. TRL designates VLDL in fasting plasma or VLDL and CM in postprandial plasma. Means in a row with different superscript letters are significantly different, $P < 0.05$.

[2] Pooled plasma from days 2 and 3.

fasting and postprandial plasma (data not shown) and in the transfer of most (>90%) of the cholesterol on TRL to the LDL and HDL fractions (**Figure 5**). The extent of the lipolysis-mediated increase in HDL cholesterol was significantly greater in postprandial M+A plasma than in fasting plasma. The lipolysis-mediated elevation in HDL cholesterol in postprandial M plasma tended to be higher than that in fasting plasma and lower than that in postprandial M+A plasma, but these differences were not significant. The above in vitro data suggest that an alcohol-mediated increase in concentrations of postprandial TRLs and their clearance in vivo may contribute to the alcohol-mediated elevation in plasma HDL cholesterol.

DISCUSSION

Elevated postprandial TRL concentrations are prevalent in patients with CVD (22–24). The results of the present study show that alcohol intake significantly boosts the postprandial lipemic response to a meal by increasing concentrations of both gut-derived apo B-48–containing chylomicrons and liver-derived apo B-100–containing VLDL (Figure 2). However, whether the alcohol-mediated enhancement of postprandial lipemia increases the risk of CVD is uncertain because a moderate alcohol intake exerts a protective effect against CVD (1–6). A recent study showed that moderate alcohol consumption is associated with a reduced risk of CVD in patients with diabetes, even though alcohol intake might worsen these patients' hypertriglyceridemia (25). Moreover, the protective effect of alcohol was greater when alcohol was consumed with a meal than without meal (26), even though the postprandial lipemic response after the ingestion of alcohol together with a meal was much (1.8 times) greater than the sum effect of either given alone (9, 27). Several studies have shown that the incidence or progression of atherosclerosis is related to the concentration of apo B-48–containing small remnants (S_f: 20–60) or to triacylglycerol concentrations in the late postprandial state but not to concentrations of large chylomicrons or to plasma triacylglycerol concentrations in the postprandial lipemic peak (28, 29). These observations suggest that the delayed clearance of postprandial TRL, but not the increase in concentrations of postprandial TRL, may be an atherogenic factor.

The postprandial increase in TRL concentrations in plasma is associated with a transient change in the concentration and composition of endogenous lipoproteins (30) and increased plasma LCAT and CETP activity (10, 11). Franceschini et al (31) reported that ingestion of a meal with alcohol results in a significant increase in HDL-triacylglycerol concentrations (57%), a decrease in HDL-cholesterol concentrations (10%), and no significant change in apo A-I concentrations and total plasma cholesterol. In contrast, a meal without alcohol results in a small increase in HDL-cholesterol concentrations and no change in HDL-triacylglycerol concentrations. Van Tol et al (32) showed that an alcohol-mediated increase in postprandial TRL concentrations is associated with an increase in the net mass transfer of cholesteryl ester from HDL to apo B–containing lipoproteins (LDL and TRL) during in vitro incubation of plasma. In this study, we showed that induction of postprandial lipemia after a meal with or without alcohol was associated with a significant lowering of cholesterol concentrations of both LDL and HDL, with a concomitant increase in the TRL-cholesterol concentration (Figure 1 and Table 1). The extent of the postprandial increase in TRL-cholesterol concentrations and the postprandial decrease in CRL-cholesterol concentrations was greater after the M+A test meal than after the M test meal (Table 1).

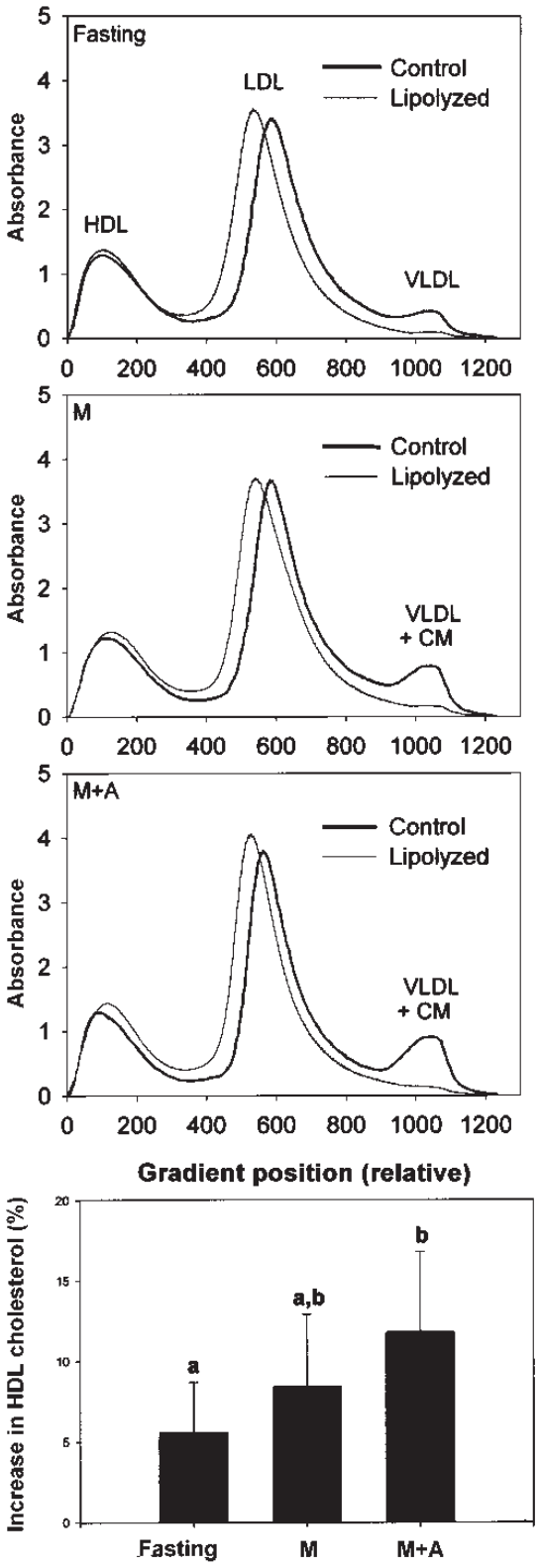

FIGURE 5. Lipoprotein cholesterol profiles of prelipolysis (control) and postlipolysis (lipolyzed) samples of fasting plasma and of postprandial plasma collected 4 h after a test meal (M) or 4 h after a test meal + 0.5 g alcohol/kg body wt (M+A) from one subject and the mean (±SD; n = 9) increase in HDL-cholesterol concentration after in vitro lipolysis of triacylglycerol-rich lipoprotein in fasting and postprandial plasma. Bars with different letters are significantly different, $P < 0.05$.

The simultaneous postprandial increase in concentrations of triacylglycerol and cholesterol associated with TRL after the M or M+A test meal (Figure 1) suggests that the postprandial increase in TRL-cholesterol concentrations could be due to an increase in TRL secreted from the intestines and liver through the utilization of ingested dietary fat and cholesterol. However, the results of a previous study showed that dietary cholesterol, supplied in a single fat-containing meal to healthy humans, does not simultaneously appear with triacylglycerol on chylomicrons (33). After the ingestion of a meal containing labeled (deuterated) cholesterol, only ≈1% of ingested cholesterol appeared at the postprandial lipemic peak with postprandial triacylglycerol (33). This indicates that most of the cholesterol associated with chylomicrons in the postprandial lipemic peak derived from an endogenous source. Our observations in the present study (Table 1 and Figure 1) suggest that the significant postprandial increase in TRL-cholesterol concentrations and the concomitant decrease in cholesterol concentrations on LDL and HDL after the intake of a meal with or without alcohol could be due to the CETP-mediated reciprocal transfer of cholesteryl ester and triacylglycerol between postprandial TRL and endogenous CRL fractions in vivo.

Lally and Barter (34) showed that after the intravenous injection of HDL containing [^{3}H]cholesteryl ester into rabbits, that possess active CETP in plasma, [^{3}H]cholesteryl ester appeared in VLDL with peak activity within 60 min. This indicates that the CETP-mediated transfer of cholesteryl ester from HDL to TRL in vivo is a rapid process. Oliviera et al (35) reported that 58% and 15% of cholesteryl ester on lymph chylomicrons in chyluric patients was derived from HDL and LDL, respectively. It is probable that enhanced CETP activity during postprandial lipemia lasting several hours is sufficient to shift the balance of cholesterol between the acceptor (TRL) and the donors (LDL and HDL) of the CETP reaction. The postprandial enhancement of the transfer of [^{3}H]cholesteryl ester or cholesterol mass from CRL to TRL during in vitro reaction of endogenous LCAT and CETP (Figures 3 and 4 and Table 2) supports the hypothesis that the CETP-mediated transfer of cholesteryl ester from LDL and HDL to postprandial TRL may occur actively in humans during postprandial lipemia.

Our study showed that the extent of the increase in cholesterol concentrations on TRL by the decrease in cholesterol concentrations on CRL after the induction of postprandial lipemia or after in vitro reaction of endogenous LCAT and CETP was greater in postprandial M+A plasma than in postprandial M plasma (Table 1 and Table 2). The CETP-mediated transfer of [^{3}H]cholesteryl ester and cholesterol mass from LDL and HDL to TRL was also greater in postprandial M+A plasma than in postprandial M plasma, although the plasma CETP concentration was not affected by the induction of postprandial lipemia (Figures 3 and 4 and Table 2). The above data suggest that the alcohol-mediated increase in concentrations of TRL, which serves as an acceptor in the CETP reaction, enhances CETP activity in vivo.

Castro and Fielding (36) suggested that the increase in postprandial lipoproteins as acceptors of cholesteryl ester stimulates reverse cholesterol transport. Whether alcohol-mediated enhancement of postprandial lipemia increases the potency of plasma to promote reverse cholesterol transport has not been evaluated previously. Recently, van der Gaag et al (37) reported that consumption of various alcoholic beverages for 3 wk was linked to *1*) a significant increase in HDL-cholesterol concentrations, *2*) cholesterol esterifying activity, and *3*) an increase in the capacity of

fasting plasma to promote cholesterol efflux from cultured cells. That study indicated that alcohol intake increases the potencies of fasting plasma to promote the efflux of cholesterol from cell membranes as a result of a change in the concentration and composition of HDL. We previously reported that postprandial TRL was the most potent acceptor of cholesterol released from RBC membranes via LCAT and CETP (17). The results of the current study show that alcohol intake increases postprandial TRL concentrations, thereby increasing their acceptance of cholesterol from cell membranes while lowering cholesterol accepted by LDL and HDL (Table 2 and Figure 4). Because postprandial TRL clears rapidly from plasma primarily by hepatic uptake (38), the alcohol-mediated increase in postprandial TRL concentrations and the subsequent hepatic removal of postprandial TRL carrying cholesterol accepted from cell membranes via LCAT and CETP reactions may enhance reverse cholesterol transport in vivo. It is likely that the transport of cholesterol derived from cell membranes via postprandial TRL is the most rapid and efficient way for cholesterol to be delivered to the liver.

Heavy alcohol intake delays the clearance of postprandial TRL (9), and a delay in the clearance of postprandial TRL has been shown to increase the risk of coronary artery disease (28, 29). However, whether a moderate intake, which has been defined as the daily consumption of < 3 standard drinks (12 g alcohol per drink, or ≈ 0.17 g alcohol/kg in a subject weighing 70 kg) (39), delays the clearance of postprandial TRL is not clear. We observed that in many but not all subjects, the concentrations of postprandial plasma triacylglycerol or postprandial TRL remaining in plasma 7 h after the M+A test meal were greater than after the M test meal (data not shown). Indirect evidence suggests that alcohol intake is associated with reduced lipolysis of intestinally derived chylomicrons (27). However, several other studies have shown that activities of postheparin lipoprotein lipase or hepatic triacylglycerol lipase are not altered by the intake of alcohol (9, 40) or by the addition of alcohol to postheparin plasma in vitro (9), and that in vivo turnover of VLDL was accelerated in chronic alcohol users (41). Our present data show further that postprandial TRLs produced after the M and M+A test meals were equally susceptible to in vitro lipolysis (Figure 5). Whether a moderate alcohol load affects the clearance rate of postprandial TRL remains to be determined.

A moderate alcohol intake appears to protect against CVD primarily by raising plasma HDL-cholesterol concentrations (5, 6). Alcohol may increase plasma HDL by *1*) increasing the synthesis or decreasing the catabolism of apo A-I (42, 43), *2*) reducing the concentration and activity of CETP (44), or *3*) accelerating VLDL turnover (41). We showed in the present study that in vitro metabolism of TRL caused an increase in plasma HDL-cholesterol concentrations (Figure 5), because surface remnants produced during the lipolysis of postprandial TRL can serve as a precursor of circulating HDL (21). The extent of the increase in the HDL-cholesterol concentration after in vitro lipolysis of TRL was greater in postprandial M+A plasma than in postprandial M plasma or fasting plasma (Figure 5). This observation suggests that the alcohol-mediated increase in the flux of postprandial TRL could be a factor responsible for the alcohol-mediated increase in HDL-cholesterol concentrations in vivo. A controlled diet study performed by Rumpler et al (45) showed that alcohol had a significant effect in decreasing LDL-cholesterol concentrations and increasing cholesterol concentrations on plasma HDL_2 when consumed with a high-fat diet (38% of energy from fat), but had no such effect when consumed with a low-fat diet (18% of energy from fat). This observation suggests that the alcohol-mediated alteration in cholesterol concentrations of LDL and HDL may be mediated via increases in postprandial TRLs.

In summary, our data suggest that postprandial TRLs appearing in the blood after a meal can accept cholesterol from endogenous lipoproteins and cell membranes via the activities of LCAT and CETP in vivo. Alcohol stimulates an increase in the postprandial concentrations of TRL, which serves as an ultimate acceptor of the cholesterol released from cell membranes via the LCAT and CETP reactions. The clearance of TRL by the liver may enhance reverse cholesterol transport in humans. The alcohol-mediated increase in the plasma HDL-cholesterol concentration could be a metabolic marker of an increased flux of postprandial TRL, rather than a protective factor against CVD.

B-HC was responsible for the experimental design; LO was responsible for recruiting and characterizing the study subjects; BD was responsible for formulating and preparing the test meals; SD and PL were responsible for measuring plasma lipids, lipoproteins, apoproteins, and CETP; BHSC was responsible for analyzing the fatty acyl chain composition of dietary fat and lipoprotein triacylglycerol; RAO was responsible for performing the statistical analysis; and B-HC and FF were responsible for drafting and revising the final version of the manuscript. None of the authors had any financial interest in this work or in the sponsors of this work

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