Decreased postprandial high density lipoprotein cholesterol and apolipoproteins A-I and E in normolipidemic smoking men: relations with lipid transfer proteins and LCAT activities

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Abstract **We have previously reported that normolipidemic smokers are lipid intolerant due to increased responses of triglyceride-rich lipoproteins (TRL) apolipoprotein B-48, triglyceride (TG), and retinyl esters to a mixed meal compared to non-smokers. To investigate whether postprandial high density lipoprotein (HDL), apolipoprotein A-I (apoA-I), apolipoprotein A-II (apoA-II), and apolipoprotein E (apoE) concentrations or lipid transfer protein activities are affected by cigarette smoking, we investigated 12 male smokers and 12 non-smokers with comparable fasting lipoprotein profile, BMI, and age. Plasma samples obtained after an overnight fast and postprandially were separated by density gradient ultracentrifugation. Postprandial apoA-I, lipoprotein AI-particles (LpA-I), HDL-cholesterol, and HDL apoE concentrations decreased in smokers, but remained unchanged in controls. Concomitantly, cholesterol and apoE concentrations increased significantly in TRL fractions in smokers. Fasting lecithin:cholesterol acyltransferase (LCAT) and phospholipid transfer protein (PLTP) activity levels, as well as esterification rates (EST) and phospholipid transfer rates were comparable between the groups. Cholesteryl ester transfer protein (CETP) activity levels were lower in the smokers. Postprandially EST increased, but CETP and PLTP activities deceased in smokers as compared to controls. We conclude, that even healthy, normolipidemic smokers have altered postprandial high density lipoprotein (HDL) cholesterol and apolipoprotein composition, as well as lipid transfer protein activities. The shift of cholesterol and apoE from HDL to the triglyceride-rich lipoprotein (TRL) fraction, together with decreased plasma apoA-I and LpA-I concentrations during alimentary lipemia may indicate impaired reverse cholesterol transport. Both the postprandial increase in TRL and the lowering of HDL may promote atherogenesis in smokers.—**Mero, N., A. Van Tol, L. M. Scheek, T. Van Gent, C. Labeur, M. Rosseneu, and M-R. Taskinen. **Decreased postprandial high density lipoprotein cholesterol and apolipoproteins A-I and E in normolipidemic smoking men: relationship with lipid transfer proteins and LCAT activities. *J. Lipid Res.* 1998.** 39: **1493–1502.**

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Although smoking is well recognized as a risk factor for coronary artery disease (CAD) (1–3), the factors responsible for increased atherosclerosis induced by smoking are largely unknown. Fasting plasma lipoprotein changes may at best explain only 8–10% of the increased CAD risk in heavy smokers (4). Smokers exhibit dose-dependent elevations of triglycerides (TG), total serum, and LDL cholesterol, as well as decreases of HDL cholesterol and apolipoprotein A-I (apoA-I) as compared with non-smokers (4, 5).

Recently, smoking was linked to postprandial lipid intolerance, which has been implicated as a risk factor for progression of CAD (6). An early report of acute smoking and postprandial cholesterol metabolism showed a rise in the HDL_2/HDL_3 cholesterol ratio in non-smokers, but not in smokers who consumed one cigarette per hour (7). Axelsen et al. (8) reported an abnormally large postprandial plasma TG response to a mixed meal, indicating fat intolerance in smokers. Recently, we have studied postprandial triglyceride-rich lipoprotein (TRL) metabolism in the present population and were able to demonstrate aggravated TG, apolipoprotein B-48 (apoB-48), and retinyl

Abbreviations: TRL, triglyceride-rich lipoproteins; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; apo, apolipoprotein; LpA-I, lipoprotein A-I particle; LCAT, lecithin: cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; EST, esterification rate; CAD, coronary artery disease; S_f, Svedberg flotation unit; AUC, area under curve; AUIC, area under incremental curve.

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palmitate responses, i.e., delayed clearance of exogenous particles in TRL fractions in smokers (9).

Postprandial TRL metabolism strongly influences HDL cholesterol and apolipoprotein composition, and thus may modify reverse cholesterol transport. Cholesteryl esters are formed in HDL by the action of lecithin:cholesterol acyltransferase (LCAT). The net transfer and exchange of lipids, i.e., TG, cholesteryl esters, and phospholipids, between donor/acceptor lipoprotein particles are mediated by lipid transfer protein activities, i.e., by cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP). Thus, lipid transfer activity is an important determinant for compositional changes in HDL and TRL particles (10–12). Previous studies in non-smokers during alimentary lipemia have shown enhanced CETP-mediated transfer of cholesteryl esters from HDL to less dense lipoproteins, especially to chylomicron remnants and very low density lipoproteins (VLDL) (11). A study with smokers and non-smokers matched for age and body mass index showed that, in the fasting state, smokers have increased plasma CETP and PLTP activity levels (13), but controversial data also exist (5, 14, 15). The reported changes in fasting lipid transfer protein activities and HDL concentrations in smokers raise the question whether these variables are changed postprandially, and if so, whether the changes are pro-atherogenic.

The effect of habitual smoking on postprandial cholesterol metabolism, apolipoprotein E (apoE) concentrations, lecithin:cholesterol acyltransferase, or lipid transfer protein activities is unknown. We have previously shown enhanced postprandial TG, apoB-48, and retinyl ester response in smokers (9). As these smokers and controls were matched for fasting TG and HDL cholesterol, which are known to have a major influence on postprandial lipemia (16), we wanted to study postprandial cholesterol and apolipoprotein metabolism after an oral fat load in this population. Our specific objectives were to identify differences in postprandial HDL, plasma cholesterol esterification, and lipid transfer protein activities, which might explain increased risk of CAD associated with smoking.

MATERIALS AND METHODS

Subjects

Twelve male habitual cigarette smokers and twelve nonsmoking men participated in the study (**Table 1**). All were healthy and lean men who were not on any medication. The groups were matched for fasting TG and HDL-cholesterol, but in addition age, body mass index, waist-hip-ratio, lifestyle, and both fasting glucose and insulin were comparable. The recruitment of the subjects has been reported in detail elsewhere (9). The study protocol was approved by the Ethics Committee of Department of Medicine, Helsinki University Central Hospital.

Oral fat-load test

Blood samples were drawn from an indwelling catheter placed in a forearm vein at 7:30 am after the subjects had fasted and abstained from smoking for 12 h and from alcohol intake for at least 2 days. A fat-rich mixed meal contained 63 g fat, 490 mg cholesterol, with a P/S ratio of 0.08, 25 g carbohydrate, and 35 g protein; 150,000 IU of vitamin A was administered during the meal. Postprandial blood samples were drawn 3, 4, 6, and 8 h after the fat load. Venous blood was collected into tubes containing EDTA and plasmas were separated within 20 min by low-speed centrifugation. Samples were protected from light and kept at 4°C before and after centrifugation. After the test meal the participants were allowed to drink only water until the last sample. Smokers were prohibited from smoking until the 8-h sample had been drawn.

TABLE 1. Subject characteristics

Variables	Smokers (n = 12)	Control Subjects (n = 12)
Age, yr	40.3 ± 1.7	36.9 ± 2.6
BMI, kg/m^2	25.0 ± 0.5	23.7 ± 1.0
Waist-hip ratio	0.92 ± 0.01	0.90 ± 0.02
ApoE phenotype	3/3 n = 9 3/4 n = 3	3/3 n = 12
Physical activity, h/wk	1.8 ± 0.6	3.0 ± 0.6
Smoking, yr	24.8 ± 2.0	
Cigarettes/day	23.0 ± 2.9	
Alcohol intake, drinks/wk	6.9 ± 3.2	4.8 ± 1.1
Glucose, mmol/l	4.4 ± 0.15	4.3 ± 0.16
Insulin, mU/l	6.0 ± 0.8	5.5 ± 0.7
C-peptide, nmol/l	0.68 ± 0.06	0.57 ± 0.07
LPL, mU/ml	218 ± 20	266 ± 29
HL, mU/ml	349 ± 24	316 ± 38
Triglycerides, mmol/L	1.10 ± 0.08	1.11 ± 0.07
HDL-cholesterol,mmol/l	1.55 ± 0.13	1.61 ± 0.12
Total cholesterol,mmol/l	5.22 ± 0.25	5.60 ± 0.29

BMI indicates body mass index; n, number of subjects; and apo, apolipoprotein. Data are mean ± SEM or number of subjects. $P > 0.05$ for each variable.

Density gradient ultracentrifugation

The density of plasma samples used for density gradient ultracentrifugation (DGU) was adjusted to d 1.10 kg/l with saline and preservatives (aprotinin 50 KIE/ml and PMSF 1 mmol/l) were added. Plasma (4 ml) was placed in 13.4-ml tubes (Ultra-Clear, Beckman Inc., Palo Alto, CA) and overlayered carefully with 3.0 ml of d 1.065 and d 1.020 kg/l and 2.8 ml d 1.006 kg/l NaCl solutions. Ultracentrifugation was performed with a SW40 Ti swinging bucket rotor at 40,000 rpm and at 15°C in a Beckman Optima LC ultracentrifuge. The $S_f > 400$ fraction representing chylomicrons was isolated after a run of 32 min. The tube was refilled with d 1.006 kg/l NaCl solution and, thereafter, ultracentrifugation was continued as described before for separation of other lipoprotein fractions (9, 17). Aliquots of the isolated fractions were frozen immediately at −80°C for apoE determinations and whole plasma for PLTP, CETP, and LCAT measurements.

LDL or HDL used as substrates in CETP and PLTP assays were isolated by ultracentrifugation from plasma collected from healthy blood donors at densities of $1.019 < d < 1.063$ and $1.063 < d < 1.210$ kg/l, respectively. The isolated lipoproteins were reisolated at the same density and dialyzed extensively against 10 mmol/l Tris-HCl, pH 7.4, containing 150 mmol/l NaCl, 1 mmol/l EDTA, and 0.1 g/l NaN_3. LDL was labeled with cholesteryl [1-^{14}C]oleate (Amersham, Arlington Heights, IL) with the lipid dispersion technique of Morton and Zilversmit (18). The labeled LDL was reisolated by density gradient ultracentrifugation (19).

Assay of plasma CETP, PLTP, and LCAT activity levels using exogenous substrate assays

Plasma CETP activity levels, as estimates of CETP mass, were analyzed as described (20), after removal of endogenous VLDL + LDL. The CETP activity measured with this method is strongly

correlated with CETP mass (21). Plasma PLTP activity levels were assayed using a phospholipid vesicles–HDL system. This method is specific for PLTP activity and the phospholipid transfer-promoting properties of CETP do not interfere with the assay (20, 22, 23). LCAT activity levels were determined using excess exogenous substrate, consisting of heat-inactivated plasma containing [^{3}H]cholesterol (24). Incubation time was 6 h at 37°C. After extraction, free and esterified cholesterol were separated on silica columns and [^{3}H]cholesteryl esters were eluted with hexane (25). The assays of CETP, PLTP, and LCAT were carried out in duplicate and the samples were assayed in one run, using single batches of substrates. The activities are expressed in arbitrary units, relative to the activity in a human pool plasma (% reference plasma).

Assay of plasma cholesterol esterification and phospholipid transfer with endogenous lipoproteins

The esterification rates of cholesterol were measured in total plasma after addition of [^{3}H]cholesterol (26). Finally, lipids were extracted and free and esterified cholesterol were separated on silica columns and [^{3}H]cholesteryl esters were eluted with hexane and counted (25). Activities are expressed in nmol/ml per h.

Phospholipid transfer was measured according to Lagrost et al. (27) with the following modifications: [^{3}H]phosphatidylcholine-labeled liposomes were added to 15 μl of plasma and incubated for 10 min, in order to limit the transfer to values $< 10\%$ and measure initial rates. The incubation mixture was the following: 125 nmol phospholipid as liposome vesicles and 120 nmol iodoacetic acid (LCAT inhibitor) were mixed in 65 μl of Tris-buffered saline (10 mmol/l Tris-HCl, pH 7.4, 154 mmol/l NaCl, 1 mmol/l EDTA, 0.2 g/l Na-azide). After incubation at 37°C, the incubation mixture was diluted to 320 μl with 240 μl of cold Tris-buffered saline and kept on ice. Subsequently the liposomes and apoB-containing lipoproteins were precipitated by addition of 240 μl of a solution containing 500 mmol/l NaCl, 215 mmol/l $MnCl_2$, and 4.7×10^5 U/l heparin. The transfer of radioactive phospholipids to endogenous HDL was measured by counting the supernatant obtained after low-speed centrifugation. Activities are expressed as % transfer.

Analytical methods

Concentrations of cholesterol were analyzed in total plasma and in all lipoprotein fractions. Cholesterol concentrations were measured by automated enzymatic methods using the Cobas Mira analyser (Hoffman-La Roche, Basel, Switzerland). Concentrations of apoE were measured in plasma and in chylomicron, $VLDL_1$, $VLDL_2$, and HDL fractions by sandwich ELISA as previously described (28). Apolipoprotein B (apoB) concentrations were measured in serum samples by an immunoturbidimetric method (Orion Diagnostica, Espoo, Finland; kit No 67249). The concentrations of apoA-I and apolipoprotein A-II (apoA-II) were measured by an immunoturbidimetric method with commercial kits (Boehringer GmbH). Lipoprotein A-I particles (LpA-I particles) were quantitated using a differential electro immunoassay (Sebia, Issy-les-Moulineaux, France). The concentration of LpA-I:A-II-containing particles was calculated by subtracting the concentration of LpA-I particles from immunoturbidimetrically measured total concentration of apoA-I in serum (29). ApoE phenotyping was performed in serum using the method of Havekes et al. (30). Concentrations of glucose, free fatty acids (FFA), insulin, and C-peptide were analyzed in samples obtained during the fat tolerance test. Insulin and C-peptide were measured by radioimmunoassay (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden and Byk-Sangtec Diagnostica GmbH & Co. KG, Dietzenbach, Germany, kit No 323 161, respectively). Baseline serum nicotine and cotinine concentrations were measured in the smokers by using gas chromatography as described previously (31). Quality of laboratory measurements was controlled with commercial samples for cholesterol (CV = 2.1%), TG (CV = 2.2%), apoA-I (CV = 3.5%), apoA-II (CV = 3.7%), LpA-I-particles (CV = 8.9%), apoB (CV = 4.4%), apoC-II (CV = 1.9%), apoC-III (CV = 3.5%), C-peptide (CV = 4.1%), and insulin (CV = 4.7%). TG, apoB-48, and apoB-100 determinations are described previously (9). Lipolytic enzyme activities were measured at a separate visit at least 1 week apart from the fat tolerance test after an intravenous bolus injection of heparin (100 IU per kg of body weight) using the method of Huttunen et al. (32).

Statistical analyses

For multiple comparisons, repeated measures ANOVA was used. Postprandial cholesterol and apoE responses in plasma and DGU subfractions were also calculated as areas under the curve (AUC) and areas under the incremental curve (AUIC) as described by Matthews et al. (33). For each subject the parameter measured was plotted against time, and the area between zero and 8-h concentration curve was determined by the trapezoid rule. Incremental areas were obtained by subtracting the fasting value from each postprandial value before area calculation (33). Statistical differences between the two groups were calculated with the nonparametric Mann-Whitney *U* test. Univariate associations were determined with Pearson's correlation coefficients. To study the independence of between-group differences, analyses of covariance were performed. We used age, body mass index, physical activity (hours of exercise per week and sedentary vs. active classification), alcohol intake, fasting TG, and apoE phenotype as covariates. Logarithmic transformations were used where appropriate. All values are expressed as mean ± standard error of the mean.

RESULTS

All subjects had normal fasting glucose levels and there were no significant differences in postprandial glucose, insulin, or C-peptide levels between the smokers and the non-smokers (data not shown). The baseline values and postprandial AUC or AUIC of FFA did not differ between the two groups. Fasting LDL (2.81 ± 0.21 vs. 3.06 ± 0.23 mmol/l) and HDL cholesterol (1.55 ± 0.13 vs. 1.61 ± 0.12 mmol/l) levels were comparable in smokers and non-smokers. The fasting TG concentration was 1.12 ± 0.07 mmol/l (range 0.78 to 1.62 mmol/l) in control subjects and 1.10 ± 0.07 mmol/l (range 0.70 to 1.62 mmol/l) in smokers. As reported previously, pre- and postprandial TG, retinyl esters, apoB-48, and apoB-100 were measured in each lipoprotein fraction. Smokers showed significantly increased postprandial TG response in chylomicrons, $VLDL_1$, and $VLDL_2$. The areas under the incremental curve (AUIC) of apoB-48 in chylomicrons (2.83 ± 0.84 vs. 0.56 ± 0.17 mg/l·h, $P < 0.05$) and $VLDL_1$ (10.17 ± 1.96 vs. 2.95 ± 2.44 mg/l·h, $P < 0.01$) were markedly higher in smokers than in controls (9). Changes of RE responses of all TRL fractions paralleled those of apoB-48. The difference between smokers and controls persisted after covariate analysis. Postprandial apoB-100 in TRL fractions was similar between the two groups.

Postprandial responses of cholesterol and unesterified cholesterol

Postprandial plasma, chylomicron, $VLDL_1$ and HDL cholesterol concentrations are shown in **Fig. 1** and AUICs in **Table 2**. In the smokers, whole plasma showed a significant decrease in postprandial cholesterol concentration ($P = 0.038$, ANOVA), whereas the concentration remained unchanged in controls. In TRL fractions ($S_f > 400$ and S_f 60–400) the cholesterol concentration increased in smokers during the postprandial period and returned to the baseline at 8 h. In controls this concentration remained unchanged and was significantly lower than in smokers ($P = 0.031$ in $S_f > 400$ and $P < 0.001$ in S_f 60–400, smokers vs. controls). The AUIC values in these fractions gave similar results after covariate analysis, see Table 2. The reduction of plasma cholesterol was due to decreased postprandial HDL cholesterol ($P < 0.0001$) in smokers. The postprandial difference in HDL-cholesterol concentrations between smokers and controls was highly significant ($P = 0.006$). Despite similar fasting concentrations, the HDL cholesterol concentration at 4 h was 8.5% lower in smokers as compared to controls (1.42 ± 0.11 mmol/l in smokers vs. 1.62 ± 0.12 mmol/l in controls). Consequently, the postprandial HDL cholesterol AUIC was negative in smokers (−0.68 ± 0.15 mmol/l·h). Fasting plasma unesterified cholesterol concentration tended to be lower in smokers than in controls (1.54 ± 0.07 vs. 1.67 ± 0.08, P = n.s.); this finding was in line with fasting total cholesterol concentrations (Fig. 1). The correlation between plasma total and unesterified cholesterol was highly significant at each time point in smokers ($r = 0.895$–0.978, $P < 0.001$) and in controls ($r = 0.549$–0.782, $P < 0.064$). Plasma unesterified cholesterol clearly increased postprandially in smokers ($P = 0.001$) but not in controls. However, there were no statistically significant differences between the curves of the two groups.

TABLE 2. Postprandial cholesterol AUIC (mmol/l·h) and apolipoprotein E AUIC (apoE, mg/dl·h) responses in chylomicrons ($S_f > 400$), $VLDL_1$ (S_f 60–400), and HDL fractions

	Smokers	Controls	*P* (ANOVA)	*P* (ANCOVA)
Chylo-C	0.39 ± 0.12	0.13 ± 0.04	0.043	0.001
Chylo apoE	0.43 ± 0.11	0.12 ± 0.06	0.006	0.072
$VLDL_1$-C	0.72 ± 0.13	0.04 ± 0.07	0.002	0.04
$VLDL_1$ apoE	1.09 ± 0.24	0.08 ± 0.14	0.001	0.003
HDL-C	−0.68 ± 0.15	0.17 ± 0.15	0.001	0.001
HDL apoE	−3.06 ± 0.45	−0.43 ± 1.06	0.044	0.158

Data are incremental postprandial responses from baseline (AUIC, see Methods for definition), mean ± SEM. ANCOVA indicates analysis of covariance of logarithmically transformed data, using age, body mass index, apoE phenotype, fasting triglycerides, physical activity, and alcohol intake as covariates.

Postprandial cholesterol esterification rates and plasma LCAT activity levels

As shown in Fig. 2, smokers exhibited a significant increase in plasma cholesterol esterification rate (EST) postprandially ($P < 0.001$) as compared to fasting values. In contrast, in controls esterification rate remained unchanged after the fatty meal. The difference in postprandial esterification rate was significant between smokers and controls ($P = 0.026$). Maximum difference in postprandial esterification rate was observed at 6 h. Esterification rate was increased by 34% at 6 h in smokers, whereas the respective number was only 13% in controls. We observed correlations between esterification rate and total plasma TG ($r > 0.733$, $P < 0.01$ in smokers and $r > 0.576$, $P < 0.05$ in controls) as well as in $VLDL_1$ TG ($r > 0.583$, $P < 0.06$ in smokers and $r > 0.562$, $P < 0.057$ in controls) at 3, 4, and 6 h postprandially in both groups.

Baseline and postprandial plasma LCAT activity levels were comparable in both groups and LCAT levels were virtually identical between smokers and non-smokers (see Fig. 2). There was a clear correlation between LCAT activ-

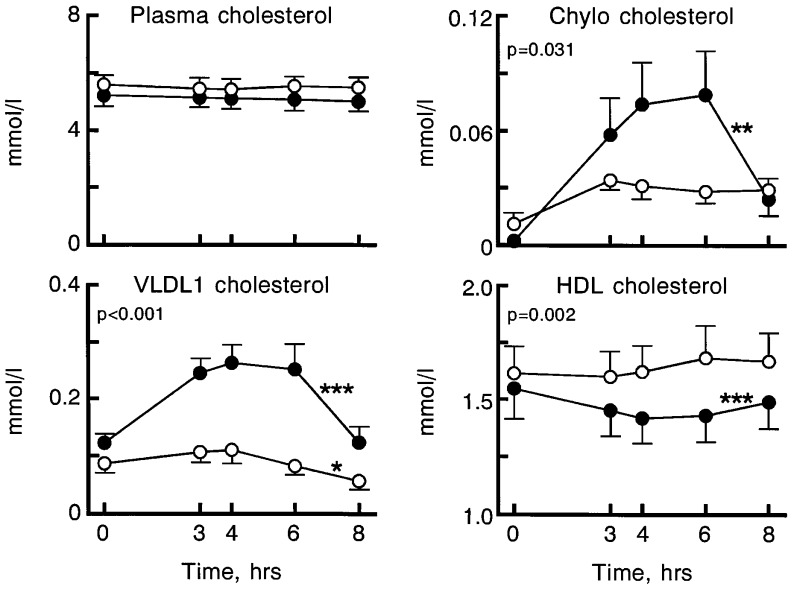

Fig. 1. Line plots show the postprandial responses of cholesterol in plasma, chylomicrons ($S_f > 400$), $VLDL_1$ (S_f 60–400), and in HDL in smokers (●) and in control men (○). Plotted on the y-axis is the concentration and on the x-axis time in hours after the fatty meal. Data points are mean values, (error bars indicate SEM). The difference between the two groups regarding postprandial concentration curves is indicated as *P*-value within each panel when significant. Significant postprandial change from fasting value for individual curves is indicated with symbols (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). All calculations are done by ANOVA for repeated measures.

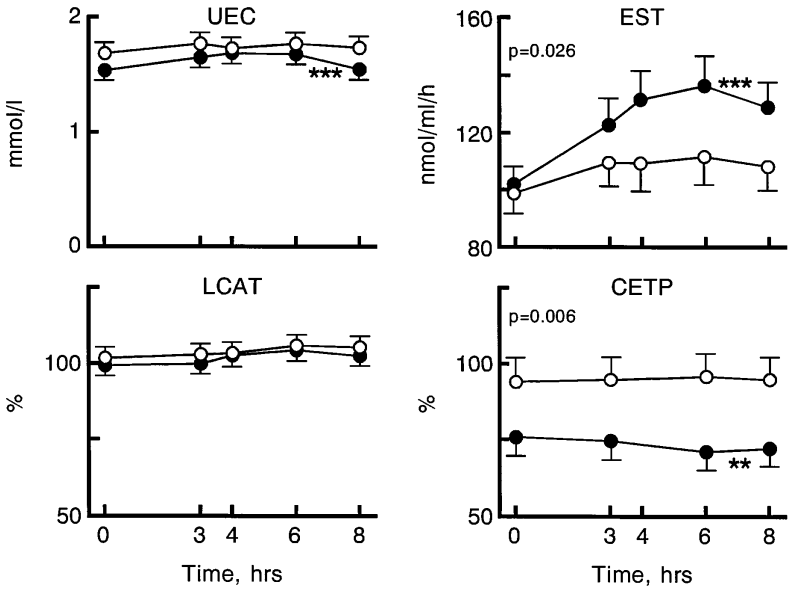

Fig. 2. Line plots show the postprandial responses of unesterified cholesterol (UEC), esterification rate (EST), lecithin:cholesterol acyltransferase (LCAT) activity levels, cholesteryl ester transfer protein (CETP) activity levels in smokers (●) and in control men (○). The difference between the two groups regarding postprandial concentration curves is indicated as *P*-value within each panel when significant. Significant postprandial change from fasting value for individual curves is indicated with symbols (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). All calculations are done by ANOVA for repeated measures.

ity levels and plasma cholesterol concentrations at baseline and after the fatty meal in both groups ($r > 0.558$, $P < 0.065$ for smokers and $r > 0.718$, $P < 0.001$ for controls at each time point). In contrast, no significant correlations were observed between LCAT levels and HDL cholesterol concentrations or HDL-TG (data not shown) in either group.

Postprandial PLTP activities and CETP activity levels

Both phospholipid transfer rates measured with endogenous HDL and plasma PLTP activity levels measured with excess exogenous HDL (% of reference plasma) decreased after the fatty meal in smokers ($P < 0.001$ and $P = 0.017$, respectively) compared to their baseline values, but not in controls ($P = 0.103$ and $P = 0.248$), see **Fig. 3**. The phospholipid transfer rate (% transfer) was significantly decreased postprandially in smokers as compared to controls ($P = 0.007$). Notably, fasting PLTP activity levels were not different between smokers and controls. Phospholipid transfer rates were related to HDL cholesterol in both groups ($r > 0.606$, $P < 0.048$ for smokers and $r > 0.688$, $P < 0.023$ for controls at each time), but not to postprandial total plasma TG, $VLDL_1$ TG, or HDL TG values (data not shown). PLTP activity levels measured with exogenous HDL did not correlate with any of the lipoprotein parameters in either group.

As shown in Fig. 2, CETP activity levels were unchanged postprandially in controls and decreased slightly postprandially in smokers. The CETP levels were significantly lower in smokers ($P = 0.006$) than in controls and strongly correlated with plasma cholesterol in both groups ($r > 0.586$, $P < 0.055$ in smokers and $r > 0.711$, $P < 0.012$ in controls at each time point). No correlation was observed between CETP levels and HDL cholesterol concentrations (data not shown).

Postprandial responses of apoE

Figure 4 shows the postprandial apoE responses in $S_f > 400$, S_f 60–400, and HDL fractions. At baseline, smokers had lower plasma ($P = 0.004$) and HDL ($P = 0.001$) apoE concentrations. In the fasting state, the majority of total plasma apoE was found in the HDL fraction (60% in smokers and 68% in controls, respectively). In smokers, the plasma apoE concentration decreased postprandially due to a marked decrease in the HDL fraction ($P < 0.001$), as compared with fasting levels. In contrast, in controls, both plasma and HDL apoE concentrations re-

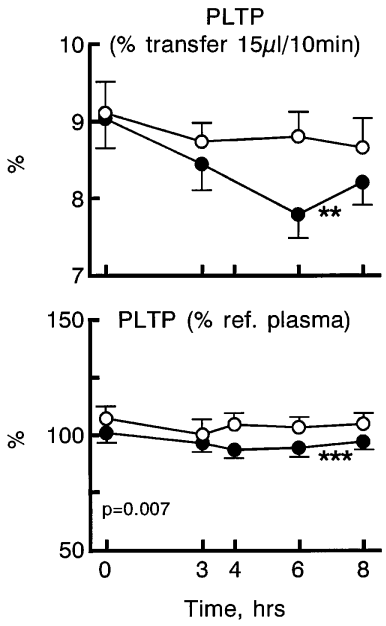

Fig. 3. Line plots show the postprandial responses of phospholipid transfer protein (PLTP) activity and PLTP activity levels as percentage of reference plasma in smokers (●) and in control men (○). The difference between the two groups regarding postprandial concentration curves is indicated as *P*-value within each panel when significant. Significant postprandial change from fasting value for individual curves is indicated with symbols (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). All calculations are done by ANOVA for repeated measures.

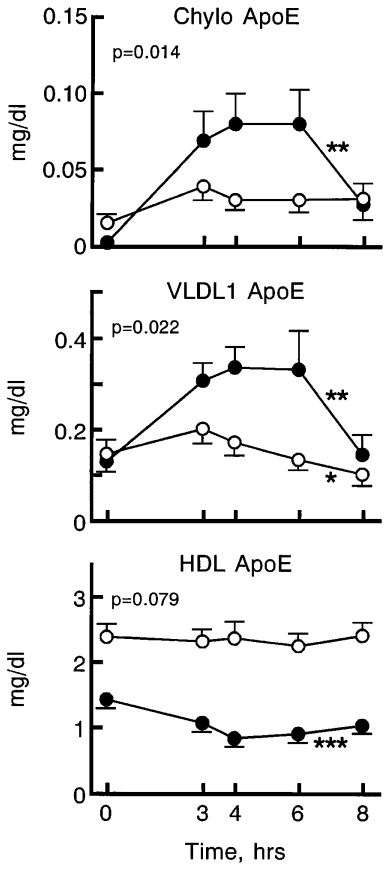

Fig. 4. Line plots show the postprandial responses of apolipoprotein E in chylomicrons ($S_f > 400$), $VLDL_1$ (S_f 60–400), and in HDL in smokers (●) and in control men (○).The difference between the two groups regarding postprandial concentration curves is indicated as *P*-value within each panel when significant. Significant postprandial change from fasting value for individual curves is indicated with symbols (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). All calculations are done by ANOVA for repeated measures.

mained unchanged after the fatty meal ($P = 0.061$ and $P = 0.726$, respectively). TRL apoE concentrations were measured in chylomicrons ($S_f > 400$) and in $VLDL_1$ (S_f 60–400) at the baseline and postprandially. Chylomicron and $VLDL_1$ apoE concentration increased postprandially, and the rise was more pronounced in smokers ($P = 0.006$ in chylomicrons and $P = 0.01$ in $VLDL_1$, smokers vs. controls). Likewise, the smokers had increased apoE AUIC in chylomicron (0.43 ± 0.11 vs. 0.12 ± 0.05 mg/dl·h, $P = 0.008$) and in $VLDL_1$ (1.08 ± 0.24 vs. 0.08 ± 0.12 mg/dl·h, $P = 0.001$). The changes in TRL and HDL apoE AUIC response persisted after adjustment for age, BMI, apoE phenotype, fasting TG, physical activity, or alcohol intake in covariate analysis as shown in Table 2. Correlations between apoE AUIC and cholesterol AUIC in chylomicron, $VLDL_1$, and HDL fractions are shown in **Table 3**. Responses of apoE and cholesterol were highly correlated in both groups in each fraction, except with the HDL fraction in smokers. Triglyceride AUIC correlated positively with apoE AUIC in chylomicrons and $VLDL_1$ in both groups (data not shown). In contrast, TG AUIC correlated negatively with apoE AUIC in the HDL fraction in smokers ($r = -0.613$, $P = 0.034$), but not in controls ($r = -0.067$, P = n.s.).

TABLE 3. Pearson's correlation coefficients regarding cholesterol area under the incremental curve (C AUIC, mmol/l·h) vs. apoE AUIC (mg/dl·h) in chylomicrons ($S_f > 400$), $VLDL_1$ (S_f 60–400), and HDL fractions, n = 12

	Smokers		Controls	
	r	*P*	*r*	*P*
Chylomicrons	0.931	0.001	0.717	0.009
$VLDL_1$	0.812	0.001	0.679	0.015
HDL	0.082	n.s.	0.739	0.006

Postprandial responses of apolipoproteins A-I, A-II, B, C-II and C-III and LpA-I and Lp A-I:A-II

In the fasting state plasma concentrations of apolipoproteins were similar between smokers and controls. **Figure 5** shows postprandial responses of apoA-I and LpA-I particles. In smokers, apoA-I and LpA-I concentrations decreased significantly postprandially from baseline levels ($P = 0.032$ and $P = 0.002$, respectively), but remained unchanged in controls. The changes in apoA-I and LpA-I concentrations correlated with concomitant changes in HDL cholesterol in both groups (for smokers $r > 0.603$ and $P < 0.035$ for each value, and for controls $r > 0.703$ and $P < 0.011$ for each value). LpA-I:A-II particle concentrations remained postprandially unchanged in both groups. Both fasting and postprandial plasma apoB concentrations were similar between smokers and controls (data not shown). Baseline apoC-II and apoC-III concentrations were also similar between the groups. No consistent postprandial changes were detected in total plasma apoC-II and apoC-III concentrations in either group (data not shown).

DISCUSSION

In the present study we found a significant postprandial decrease of HDL cholesterol in habitual smokers compared with non-smokers with identical fasting HDL levels. The decrease in HDL cholesterol was accompanied by a concomitant decrease in HDL-associated apolipoproteins, mainly apoA-I and apoE concentrations. Plasma LpA-I particles strongly decreased while LpA-I:A-II particles remained unchanged, suggesting significant compositional changes in the HDL subfraction. A concomitant increase in TRL apoE was found in smokers, but no such change in TRL apoE was seen in controls. We have previously confirmed the preliminary observation of Axelsen et al. (8) that normolipidemic smokers show fat intolerance and express accumulation of exogenous TRL in postprandial state. We observed higher TG, retinyl ester, and apoB-48 response in smokers than in controls, indicating accumulation of chylomicrons and their remnants as the main feature of fat intolerance (9). Although fasting TG and HDL cholesterol levels are important determinants of postprandial lipemia (16), our data imply that these fac-

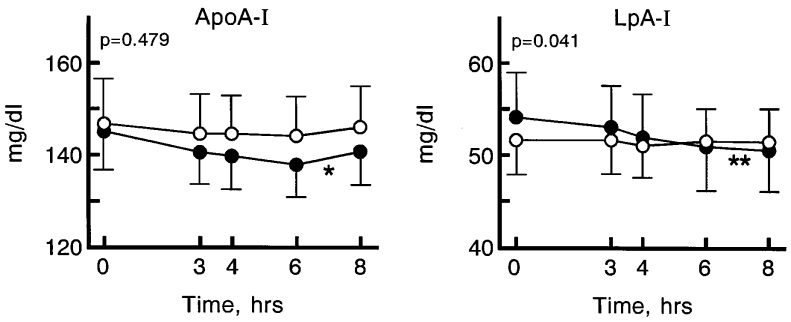

Fig. 5. Line plots show the postprandial responses of apolipoprotein A-I and lipoprotein A-I particles in plasma in smokers (●) and in control men (○).The difference between the two groups regarding postprandial concentration curves is indicated as *P*-value within each panel when significant. Significant postprandial change from fasting value for individual curves is indicated with symbols (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). All calculations are done by ANOVA for repeated measures.

tors cannot explain the exaggerated postprandial lipemia in smokers.

Low HDL and apoA-I concentrations are indicators of high cardiovascular risk (34, 35) and a well-recognized feature of dyslipidemia induced by smoking. There is also evidence of altered HDL composition, mainly a decrease of HDL_2-fraction in smokers (5, 15). A meta-analysis of 54 available studies by Craig, Palomaki, and Haddow (4) showed that heavy smokers have, on average, 9% lower HDL cholesterol and 6% lower apoA-I levels than matched non-smokers, but altogether changes in fasting lipoproteins accounted only for a minor proportion of the CAD risk associated to smoking. In this study we selected the smokers and controls to have identical fasting HDL cholesterol and TG. Likewise, fasting concentrations of apoA-I, apoA-II, LpA-I, and LpA-I:A-II were comparable between the two groups. However, we observed a postprandial decrease of HDL-associated apolipoproteins in smokers, the change being most pronounced for total plasma apoA-I and LpA-I. Karpe et al. (36) studied 32 postinfarction patients and 10 controls and found a decrease in postprandial HDL concentration in normo- and hypertriglyceridemic patients, but not in controls. Patsch et al. (37) have shown in normotriglyceridemic nonsmoking volunteers an inverse correlation between HDL_2 or apoA-I concentration and the ability to clear postprandial alimentary fat. We speculate that delayed clearance of exogenous TRL, i.e., postprandial hypertriglyceridemia, alters postprandial distribution of HDL subclasses in smokers to a more atherogenic picture as evidenced by decrease of mainly apoA-I-containing HDL particles.

Lipid transfer reactions play an important role in TRL and HDL metabolism during the postprandial state (38). In general, neutral lipid transfer is governed both by the concentration of the TRL and by the plasma levels of CETP. In the present study, CETP levels were not increased postprandially and correlated with total plasma cholesterol levels, but not with HDL-cholesterol changes in normotriglyceridemic smokers. Recently Föger et al. (39) reported that high-normal CETP activity may account for the low-HDL phenotype in moderately hypertriglycidemic patients. In their normotriglyceridemic subjects, CETP mass and activity levels correlated with postprandial incremental TG response, but no relationship existed between CETP and HDL cholesterol, HDL-associated apolipoproteins, and HDL composition independent of LPL activity. The smokers included in the present study had relatively low CETP levels, while their baseline HDL concentrations were not significantly different from the controls. The low CETP levels may be explained by the relatively lower total plasma cholesterol levels in the smokers compared to controls and the strong correlation between CETP activity and plasma cholesterol. The CETP activity levels did not correlate with HDL cholesterol. In a previous study (13), 21 smokers and controls were individually matched for age and body mass index and had comparable total plasma cholesterol values. HDL cholesterol concentrations were low and increased CETP activity levels were observed in the smokers (13). HDL cholesteryl ester concentrations were inversely related to CETP levels in smokers, but not in controls. The apparent discrepancy between these two case-control studies is probably due to differences in selection criteria and in the genetic background of the subjects. Recent studies on wine consumption with dinner, another condition with elevated postprandial lipemia, demonstrated increased net mass transfer of cholesteryl esters from HDL towards TRL without effects on CETP activity levels (40). It is possible, therefore, that in the present study net mass transfer is increased postprandially driven by high TRL concentrations in the smokers to a greater extent than in the controls, despite the lower CETP levels. The specific postprandial decrease in LpA-I in smokers, observed in the present study, could occur via accelerated CETP action driven by the high TRL concentrations.

PLTP activity levels (measured with excess exogenous HDL; expressed as % reference plasma) are likely to be related with PLTP mass, just as CETP activity levels are related with CETP mass (21). PLTP levels are clearly decreased postprandially in the smokers and not in the controls. Two possible functions of PLTP in lipoprotein metabolism have been proposed. First, PLTP could act in the transfer of surface fragments, formed during lipolysis of TRL, to HDL (41). Second, PLTP may act in HDL conversion (42, 43). Isolated HDL_3 is converted by purified PLTP to larger HDL_2-like HDL via a process of fusion (44), with the concomitant formation of small preβ-HDL (45). Also LpA-I particles are converted both to larger and smaller subspecies by PLTP in vitro (46). The postprandial decrease in LpA-I (an important component of HDL_2) in smokers, may by itself result in lower PLTP levels, as PLTP is carried in plasma on a large HDL particle subfraction (23). We also measured PLTP activity dependent on endogenous HDL (see Methods). This value also decreased

postprandially in smokers, but not in the controls, thus indicating that not only PLTP levels, but also phospholipid transfer to endogenous HDL is decreased in the postprandial phase in smokers.

LCAT is essential in maintaining the unesterified cholesterol concentration gradient between cell membranes and plasma HDL in reverse cholesterol transport (38). We found similar LCAT activity levels, both at baseline and in the postprandial state, in smokers and controls. This finding is in contradiction with in vitro findings of McCall et al. (47), who exposed human (non-smoker) plasma to filtered gas phase of cigarette smoke and found inhibition of LCAT by cigarette smoke. Our smokers abstained from smoking for at least 12 h before taking the fatty meal. Thus it seems that the effects of smoking on LCAT may be transient or absent in vivo. The plasma cholesterol esterification (taking place predominantly on HDL) increased postprandially in the smokers while the total concentration of HDL cholesterol (mostly consisting of cholesteryl esters) decreased. Therefore it may be speculated that net mass transfer of cholesteryl esters from HDL to TRL is very active in the smokers, e.g., due to the elevated postprandial levels of TRL. This active TG/cholesteryl ester exchange may initiate a remodelling of HDL, eventually resulting in formation of pre-β-HDL which are the initial acceptors of free cholesterol released from the cell surface (48).

Three common isoforms of apolipoprotein E (E2, E3, E4) can be distinguished in humans. ApoE plays an important role in lipoprotein metabolism through its interaction with the LDL receptor (49), and plasma lipoprotein levels are significantly affected by the apoE allele (50) in humans. Until now, no data were available on apoE status and metabolism in smokers. In this study the total plasma apoE, TRL, and HDL apoE concentrations in normal controls did not change as a result of fat feeding, in agreement with previous studies from others (51, 52) and from our laboratory (53). However, in smokers, the changes in the apoE content of TRL (both chylomicrons and $VLDL_1$) were striking. Upon fat feeding there was a clear shift of apoE to less dense lipoproteins, the increase being most pronounced in the $VLDL_1$ (S_f 60–400). Concomitantly there was a significant decrease in apoE associated with HDL, while in normal controls these decreases in HDL apoE did not reach statistical significance. The changes persisted after covariate analysis taking apoE phenotype and plasma TG into account. The apoE enrichment of VLDL during alimentary lipemia may have several implications for the metabolism of the VLDL particle. First, the apoE might enhance lipoprotein retention in the arterial wall due to its binding to heparan sulfate proteoglycans (54). Second, apoE and not apoB-100 is essential in the LDL-receptor-mediated uptake of large VLDL (55). Third, the LDL receptor-related protein (LRP) mediates uptake of TRL remnants when they carry an excess of apoE, and fourth, apoE has recently been proposed to have a role as a noncompetitive regulator of the lipolysis of TRL (56). ApoE might mediate the binding of LPL to its substrate thereby impairing its function. We have previously shown (9) that smokers have defective clearance of chylomicrons and their remnants, an observation that might be linked to the increases in apoE in the TRL.

Increased TRL apoE has been associated with risk of atherogenesis. Cohn et al. (51) observed higher concentrations of apoE in intermediate-sized remnant-like lipoprotein fraction in hyperlipidemic subjects compared to normolipidemic subjects. They also found a positive correlation with proatherogenic lipid parameters (plasma cholesterol, TG, and apoB) and apoE concentrations, and negative correlation with HDL cholesterol and apoE concentration, suggesting the importance of remnant-associated apoE predicting CAD. (57) A study by Syvänne et al. (52) found enrichment of apoE in S_f 12–60 in patients with NIDDM and CAD postprandially. In smokers, a fat-rich meal not only resulted in a significant shift of apoE to the TRL, but also induced a significant reduction of the apoE associated with HDL. As apoE also appears to play an important role in HDL metabolism and reverse cholesterol transport (RCT), the process by which excess cholesterol is removed from peripheral tissues and transported to the liver, these effects might partially explain the increased risk for the development of cardiovascular disease in smokers. (58, 59).

Fasting plasma apolipoproteins A-I and A-II associated with HDL were comparable between smokers and controls, as expected from the similar HDL cholesterol levels. Concentrations of apoA-I, apoA-II, and LpA-I decreased postprandially in smokers in parallel with the lowering of HDL cholesterol concentration. Alterations in lipase activities have been reported in smokers (15, 60); in the current study a tendency to lower LPL and higher HL was noted in smokers. Recently, LPL was also recognized to have a putative role in chylomicron remnant clearance. This action is independent of the catalytic activity of the enzyme (49, 61). If this LPL-mediated removal of exogenous TRL were disturbed in smokers, the result would be an elevation in the level of apoB-48-containing particles, as we observed before (9). Thus, enhanced postprandial TRL would lead to increased exchange of TG and cholesteryl ester via CETP between TRL and HDL. The decrease in HDL cholesterol would then be due to the increased level of circulating TRL and explain the poor correlation between CETP levels and HDL cholesterol.

We have shown that the constellation of lipid risk factors for CAD in smokers include postprandial lipemia and delayed clearance of TRL remnants (9). This study expands the picture of postprandial dyslipidemia by demonstrating striking compositional changes of both postprandial TRL and HDL particles in smokers. The observed shifts of apoE distribution between TRL and HDL particles in smokers are potentially atherogenic, impairing reverse cholesterol transport and inducing the formation of particles that might be more readily retained in the vessel wall. Concomitant lowering of postprandial HDL concentration, in particular LpA-I particles, is another atherogenic feature associated with postprandial lipemia in smokers. Finally, we speculate that increased transfer of cholesteryl esters to TRL particles with impaired delivery

of cholesterol to the liver may promote atherogesis in smokers.

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