Higher Plasma Lipid Transfer Protein Activities and Unfavorable Lipoprotein Changes in Cigarette-Smoking Men

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Abstract The mechanisms responsible for the atherogenic lipoprotein changes associated with cigarette smoking are largely unknown. Lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) are key factors in the esterification of plasma cholesterol and the transfer of cholesteryl ester from high-density lipoproteins (HDLs) toward very-low- and low-density lipoproteins (VLDLs+LDLs). Another transfer factor, phospholipid transfer protein (PLTP), recently has been shown to be involved in the interconversion of HDL particles in vitro, but its physiological function is not yet clear. We measured the activities of LCAT, CETP (as cholesteryl ester exchange activity), and PLTP using exogenous substrate assays as well as lipoprotein profiles in the plasma of 21 normolipidemic cigarette-smoking men (total plasma cholesterol below 6.5 mmol/L and triglyceride below 2.5 mmol/L) and 21 individually matched nonsmoking control subjects. HDL cholesterol, HDL cholesteryl ester, and plasma apolipoprotein A-I levels were lower in the smokers than in the control subjects (P≤.05 for all parameters). Median plasma CETP activity was 18%(61,296),(855,806)

Cigarette smoking is associated with higher levels of very-low- and low-density lipoprotein (VLDL+LDL) cholesterol and lower levels of high-density lipoprotein (HDL) cholesterol.1 Such unfavorable lipoprotein alterations can explain in part the increased risk of cardiovascular disease among smokers.2 Of other factors involved in lipoprotein metabolism, lecithin:cholesterol acyltransferase (LCAT, EC 2.3.1.43) and cholesteryl ester transfer protein (CETP) play key roles in the esterification of cholesterol in plasma and the subsequent transfer of cholesteryl ester (CE) toward apolipoprotein (apo) B-containing lipoproteins.3-6 High levels of CETP activity have been previously documented in primary and secondary hyperlipidemia.7-11 Furthermore, HDL cholesterol is extremely elevated in cases of familial CETP deficiency.12 Apart from CETP, another lipid transfer factor, phospholipid transfer protein (PLTP), specifically catalyzes the transfer of phospholipids in plasma.4-6,13 Although its physiological function is not well defined, PLTP is involved in regulating HDL particle size in vitro.14 The mechanisms responsible for the unfavorable lipoprotein levels in smokers still are not precisely understood. Previous studies did not demonstrate significant alterations in the plasma cholesterol esterification rate in smokers.15,16 CETP activity was found to be elevated in cigarette-smoking men with insulin-dependent diabetes mellitus,17 but it is unknown whether such abnormalities are present in smoking subjects without other cardiovascular risk factors.

We hypothesized that alterations in plasma lipid transfer protein activities could play a role in the development of unfavorable lipoprotein changes in smokers. In this case-control study we measured plasma CETP, PLTP, and LCAT activity in carefully matched healthy smokers and nonsmokers and related these factors to lipoprotein levels.

Methods

Subjects

Male subjects, aged 21 to 60 years, were recruited from hospital personnel and consented to the procedure after explanation of the purpose of the study. To be included in the study, subjects had to have fasting plasma total cholesterol (TC) and triglyceride (TG) levels less than 6.5 mmol/L and 2.5 mmol/L, respectively. The men were nonobese (body mass index [BMI] below 27 kg/m²), had a normal blood pressure (systolic blood pressure below 140 mm Hg and diastolic blood pressure below 90 mm Hg), and were free from other cardiovascular risk factors.
pressure below 95 mm Hg), and did not use any medication. Routine thyroid and liver function tests showed no abnormalities, and the subjects did not suffer from diabetes mellitus. Smokers were eligible if they smoked at least 5 cigarettes daily. Twenty-one smokers were individually matched for age and BMI with a similar number of nonsmokers. The smokers currently smoked a median of 15 (interquartile range [IR], 10 to 25) cigarettes per day and had been smoking for 11 (IR, 8 to 17) pack-years. Thirteen of the nonsmokers had never smoked, and 8 of them had stopped smoking for at least 2 years before the study (former smokers). Both groups were closely comparable with respect to age (33±8 versus 35±11 years for smokers and nonsmokers), BMI (23.1±1.7 versus 22.8±1.4 kg/m²), and waist-to-hip circumference ratio (0.86±0.09 versus 0.84±0.05, measured as the ratio of the smallest girth between the rib cage and iliac crest and the largest girth between the waist and thigh).

**Laboratory Measurements**

Venous blood was collected into tubes containing EDTA (final concentration, 1.5 mg/mL) and was placed on ice immediately. Plasma was separated within 30 minutes by centrifugation at 3000 rpm for 15 minutes at 4°C. Samples were frozen at −20°C until analyzed.

The plasma activities of LCAT, CETP, and PLTP were determined using excess exogenous substrates as previously described. CE exchange activity (designated CETP activity) was measured in the supernatant fraction of each plasma sample after removal of endogenous VLDL+LDL by phosphotungstate/MgCl₂ precipitation. The isotope assay detects the exchange of radioactive CE between exogenous ([1-³⁵S]oleate)-CE-labeled LDL and excess unlabeled pooled normal HDL. LCAT was completely inhibited with dithiothreitol (200 mmol/L). The CETP activity level obtained by this method is well correlated with CETP mass. PLTP activity was assayed in each plasma sample using a phospholipid vesicle–HDL system, exactly according to a recently described procedure. The method is specific for PLTP activity and is not influenced by the phospholipid transfer–promoting properties of CETP. The assays were each performed in duplicate using single batches of substrates. The intra-assay coefficients of variation were 4.5%, 2.7%, and 3.5% for LCAT, CETP, and PLTP, respectively.

**Results**

Plasma LCAT, CETP, and PLTP activities are shown in Fig 1. Plasma LCAT activity was not significantly
different between smokers and nonsmokers: 49 (IR, 41 to 52) versus 47 (IR, 45 to 55) nmol esterified cholesterol per milliliter plasma per hour, respectively; median difference -4% (95% CI, -15% to 10%) (Fig 1A).

Both plasma CETP and PLTP activities were higher in smokers versus nonsmokers: 100 (IR, 89 to 114) versus 85 (IR, 78 to 95) nmol CE per milliliter plasma per hour, respectively, P<.02, for CETP activity (Fig 1B) and 6.0 (IR, 5.5 to 6.4) versus 5.5 (IR, 5.0 to 6.0) μmol phosphatidylcholine per milliliter plasma per hour, respectively, P<.05 for PLTP activity (Fig 1C). The median differences in plasma CETP and PLTP activity between smokers and nonsmokers were 18% (95% CI, 4% to 31%) and 8% (95% CI, 0.1% to 18%), respectively. Among nonsmokers there were no differences in lipid transfer protein activities between the former smokers and men who never smoked (P>.10).

Mean HDL cholesterol and plasma apo A-I levels were lower, whereas the ratio of plasma TC to HDL cholesterol was higher in smokers versus nonsmokers (Table). Plasma TC, TG, and apo B levels were comparable between the groups. The distribution of plasma lipoprotein(a) was similar in smokers and nonsmokers. The reduction in HDL cholesterol in the smokers coincided with a decrease in HDL-CE concentration (0.83±0.12 versus 0.91±0.13 mmol/L in smokers versus nonsmokers, P=.05), as well as in HDL-CE content (33.4±3.2 versus 36.1±4.8 mol % of total lipids, P<.05). No significant differences in the HDL-FC, TG, and phospholipid content were present (data not shown).

Remarkable differences in the relations of HDL-CE to LCAT activity, CETP activity, and smoking. HDL-CE was inversely related to CETP activity (P<.005) and positively related to LCAT activity (P<.05, multiple r=.52). The contribution of smoking, introduced in the model either as a categorical covariate (smoking or nonsmoking, P=.44) or as the number of cigarettes smoked daily (P=.25), was not significant. Thus, there was no demonstrable lowering effect of smoking on the level of HDL-CE, independently of its influence on CETP activity. In addition, the relation of plasma TG with HDL-CE was not significant (P=.15). As shown in Fig 3A, VLDL+LDL-CE was positively related to CETP activity both in the smokers (r=.58, P<.01) and in the nonsmokers (r=.54, P<.02). VLDL+LDL-CE was also correlated with PLTP activity (smokers: r=.66, P<.01; nonsmokers: r=.51, P<.02) (Fig 3B). CETP and PLTP activities were interrelated in the smokers (r=.61, P<.01), but no further relations among LCAT, CETP, and PLTP activities were observed (all P>.10). Neither LCAT nor lipid transfer protein activities were significantly correlated with alcohol intake (all P>.10).

Discussion

The present study showed higher plasma activities of CETP and PLTP in healthy smokers compared with nonsmokers. Plasma HDL-CE was inversely related to the independent relations of HDL-CE to LCAT activity, CETP activity, and smoking. HDL-CE was inversely related to CETP activity (P<.005) and positively related to LCAT activity (P<.05, multiple r=.52). The contribution of smoking, introduced in the model either as a categorical covariate (smoking or nonsmoking, P=.44) or as the number of cigarettes smoked daily (P=.25), was not significant. Thus, there was no demonstrable lowering effect of smoking on the level of HDL-CE, independently of its influence on CETP activity. In addition, the relation of plasma TG with HDL-CE was not significant (P=.15). As shown in Fig 3A, VLDL+LDL-CE was positively related to CETP activity both in the smokers (r=.58, P<.01) and in the nonsmokers (r=.54, P<.02). VLDL+LDL-CE was also correlated with PLTP activity (smokers: r=.66, P<.01; nonsmokers: r=.51, P<.02) (Fig 3B). CETP and PLTP activities were interrelated in the smokers (r=.61, P<.01), but no further relations among LCAT, CETP, and PLTP activities were observed (all P>.10). Neither LCAT nor lipid transfer protein activities were significantly correlated with alcohol intake (all P>.10).
CETP activity in smokers, whereas VLDL+LDL-CE levels were positively related to the activities of both CETP and PLTP. Moreover, we could not demonstrate a lowering effect of smoking on HDL-CE independently of its influence on CETP activity. These findings suggest that alterations in plasma lipid transfer protein activities may contribute to unfavorable lipoprotein changes associated with smoking.

To avoid confounding influences of hyperlipidemia and adiposity on the activities of LCAT and lipid transfer proteins,6,9,10,20,23 only subjects with normal weight and without hyperlipidemia participated. Despite this selection, HDL cholesterol and plasma apo A-I levels were lower and the ratio of plasma TC to HDL cholesterol was higher in smokers than in nonsmokers. A recent meta-analysis has demonstrated accordingly that HDL cholesterol is about 6% lower in smokers.1 Other studies have shown that HDL cholesterol is decreased.16,24 The diminished HDL-CE content also points to an abnormal HDL composition. There was considerable overlap in individual HDL-CE as well as in plasma CETP activity levels between smokers and nonsmokers. Thus, in otherwise normolipidemic men the effects of smoking on both HDL-CE and CETP activity are modest. The higher plasma CETP activity level in healthy cigarette-smoking subjects is in accordance with observations in insulin-dependent diabetic patients determined using similar analytical methods.17

The present findings disagree with a recent study that showed slightly lower plasma CETP activity levels in smokers determined by assay with radiolabeled HDL3 and a mixture of VLDL and LDL.24 In contrast to our study, men and women as well as obese subjects were included in that report, and smokers were not individually matched for BMI and sex with nonsmokers.

Experimental evidence underscores the possibility that higher levels of CETP activity can contribute to lower HDL-CE concentrations in smokers. Administration of human CETP decreases HDL-CE in the rat,25 whereas immunologic inhibition of CETP increases HDL-CE and HDL particle size in the rabbit.26,27 HDL cholesterol is lowered in CETP-producing transgenic mice, and this effect appears to be dependent on the plasma level of CETP activity.28,29 Although not consistently observed,24 an inverse relation between HDL cholesterol and CETP activity has also been found in humans.30-32 The lack of a correlation between LCAT activity and HDL-CE in smokers, as opposed to the positive correlation in nonsmokers, would suggest that this enzyme-product relation is obscured by an enhanced CETP-mediated transfer of CE from HDL toward lipoproteins of lower density. Of note, the process of CE transfer in vitro is dependent on both the activity of CETP as such and the composition and concentration of CE donor and acceptor lipoproteins.4-6,9,31 For instance, elevations in VLDL-TG may enhance the net mass transfer of CE out of HDL.31 It is unlikely that this effect was operating in our study subjects because TG levels were similar in smokers and nonsmokers.

PLTP is presumed to facilitate the transfer of phospholipids from TG-rich lipoproteins toward HDL during lipolysis.4 Since phospholipid-enriched HDLs appear to be better substrates for CETP, it is possible that CETP and PLTP act synergistically.6 As expected, a positive correlation of VLDL+LDL-CE with CETP activity was found,7-11,30 but such a correlation with PLTP activity has not been demonstrated before. The extent to which these relations of lipid transfer protein activities to lipoprotein CE levels coincide with an altered CE mass transfer awaits further study.

Several mechanisms can be responsible for the higher lipid transfer activities in smokers. High levels of CETP activity are likely to be due to an increase in its mass concentration in plasma, since plasma CETP activity has been found to be closely correlated with CETP mass.6,10,21 Alternatively, a decrease in putative inhibitor activity could increase CETP activity.32 The interrelation between the activities of CETP and PLTP in smokers raises the possibility of a shared disturbance in the regulation of these proteins. In smokers the higher levels of VLDL+LDL-CE are thought to be caused partly by an increase in hepatic lipoprotein synthesis.33 In addition, differences in dietary habits in smokers, such as higher intake of total fat34 and lower intake of polyunsaturated fat,35 may contribute to their higher levels of VLDL+LDL cholesterol. Diet-induced changes in plasma CETP are in part genetically determined and are well related to changes in VLDL+LDL cholesterol.30,36 As dietary habits were not analyzed in our study, the higher levels of CETP activity in smokers could be directly caused by an effect of smoking but also by associated changes in dietary factors.

In conclusion, lipid transfer proteins could play a role in causing or aggravating unfavorable lipoprotein changes. Even in otherwise normolipidemic men, there
is an association between lower levels of HDL cholesterol, higher lipid transfer protein activities, and cigarette smoking.

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References

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