Effects of smoking on serum lipid and lipoprotein concentrations and lecithin : cholesterol acyltransferase activity

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Abstract : Cigarette smoking is one of the major risk factors for cardiovascular disease. The mechanism responsible for this association is still unknown. We measured the activity of lecithin : cholesterol acyltransferase (LCAT), a key factor in the esterification of plasma cholesterol and reverse cholesterol transport, and the levels of lipids and apolipoproteins in the serum of 27 cigarette smoking and 31 non-smoking (control) men. We could not find any significant difference among these parameters between the groups. Serum LCAT activity was lower in smokers, but the difference was statistically nonsignificant. We also classified the two groups in respect to their serum lipid levels as hyper- and normolipidemic, we observed that normolipidemic-smokers had lower ($p<0.05$) high density lipoprotein-cholesterol (HDL-C) and HDL-ester cholesterol levels compared to the normolipidemic-nonsmokers. While there were no any significant differences between hyperlipidemic-smokers and nonsmokers with respect to any of the parameters.

In the end we have got the idea that smoking seems to affect HDL-C and HDL-ester cholesterol levels in the normolipidemic-smokers group, only, Also, LCAT activity tended to be lower in smokers compared to nonsmokers. J. Med. Invest. 46 : 169-172, 1999

Key words **:** *Lecithin : cholesterol acyltransferase, smoking, lipids*

INTRODUCTION

Cigarette smoking is accepted as a major risk factor for ischemic heart disease (IHD) as well as hypertension and hyperlipidemia (1). Certain components of cigarette smoke, such as nicotine and carbon monoxide, have been reporterd to be responsible for the development of IHD by increasing plasma catecholamine levels and producing hypoxia (2). On the other hand, cigarette smoking alters plasma lipoprotein levels (3-5) and increases the susceptibility of low density lipoprotein (LDL) to oxidation (6). However, the exact mechanism responsible for the harmful effect of smoking on IHD is not fully understood (7).

High density lipoprotein-cholesterol (HDL-C) is known to be an important protective factor for IHD. Several epidemiological studies have indicated that low serum levels of HDL-C are associated with an increased risk of IHD (8-10). Reverse cholesterol transport is also an antiatherogenic process in which excess cholesterol is transported from peripheral tissues back to the liver or to other peripheral tissues that in need cholesterol. The excess cholersterol is taken by HDL and esterified by lecithin : cholesterol acyltransferase (LCAT, EC 2. 3. 1. 43) and cholesteryl esters are transfered from HDL towards very-low and low-density lipoproteins (VLDL and LDL) by cholesterol ester transfer protein (CETP) (11).

Conflicting data on the influence of smoking on LCAT activity have been published In this respect we investigated the effects of smoking on the levels of plasma lipids, lipoproteins, apolipoproteins and LCAT activity in fasting blood from healthy smokers and nonsmokers, matched for age and body mass index (BMI).

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MATERIALS AND METHODS

Twenty-seven cigarette smokers and thirty-one non-smokers were investigated. All subjects were men. The smokers smoked at least 10 cigarettes per day (mean, 18 ± 6 cigarettes ; range 10 to 30) for at least 7 years (mean, 15 ± 6 years, range 7 to 28). The non-smokers had never smoked in the past. The ages of the subjects were 29 to 53. Subjects with hepatic, renal or cardiovascular disease, and diabetes mellitus were excluded. None of them were taking any drugs, such as beta-blockers, diuretics or lipid-lowering agents. Informed consent was obtained from each participant in the study.

Blood was drawn from an antecubital vein into vacutainer tubes after 10-12 hours of fasting. Blood was centrifuged at 1500 g for 15 minutes. Sera were stored at -20°C and analyzed within 30 days. Serum triglyceride (TG) level was determined enzymatically (Biosystems, Spain). Total cholesterol (TC) was measured with the Lieberman-Burchard method. Free cholesterol (FC) was determined in serum and HDL fraction with the Lieberman-Burchard method after digitonin precipitation (12). HDL-C was assayed after the precipitation of apolipoprotein (apo) B-containing lipoproteins with dextran sulphate-magnesium chloride (13). LDL-C was determined according to Friedewald *et al* (14). Esterified cholesterol was deduced from the difference between total and free cholesterol. Apo A-I and apo B were measured by nephelometry (Sanofi Pasteur, France). Lipoprotein (a) was analyzed by ELISA (Chromogenix, Sweden). Serum LCAT activity was determined as a function of the decrease of free cholesterol which is esterified during incubation at 37°C, as described by Hitz *et al* (15).

Results are expressed as mean±SD. The Students' t-test was used to compare the smoker and non-smoker subjects. A p value of <0.05 was considered to be significant.

RESULTS

The study groups comprised of 27 male smokers (mean age ± SD, 41.9 ± 6.7 years) and 31 male non-smokers (41.5 ± 6.9 years). The body mass indexes were 25.6 ± 2.8 and 26.4 ± 3.5 kg/m^2 in smokers and non-smokers, respectively (Table 1). There were no significant differences in serum TC, TG, lipoprotein (a), apo A-I and B, LDL-C and HDL- and serum-free and- ester cholesterol levels between smokers and non-smokers. Serum LCAT activity was lower in smokers than in non-smokers, although this difference was statistically nonsignificant (Table 2 and 3).

The smoker and non-smoker subjects were grouped

Table 1. Characteristics of healthy smokers and non-smokers

	Smokers	Nonsmokers
n	27	31
Age, years	41.9±6.7	41.5±6.9
Body mass index, kg/m^2	25.6±2.8	26.4±3.5
Exposure to cigarette smoke*	278±160	-

* Brinkmann Index (cigarettes per day for X years)

Table 2. Serum lipid, lipoprotein and apolipoprotein concentrations (mg/dL) and LCAT activity (μmol.L^{-1}.h^{-1}) in smokers and non-smokers*

Analytes	Non-smokers	Smokers
TC	218±61	231±67
TG	200±136	201±103
HDL-C	48.6±7.2	51.7±9.9
LDL-C	127±47	141±64
TC/HDL-C	4.6±1.6	4.7±1.8
HDL-FC	14.5±7.0	13.0±5.3
HDL-EC	34.8±8.2	38.7±9.8
HDL-EC/HDL-FC	3.2±2.1	3.5±1.7
S. FC	81.5±36.4	84.4±30.6
S. EC	140.5±39.6	147.4±42.0
S. EC/S. FC	2.0±0.9	1.9±0.5
Apo A-I	155±28	149±25
Apo B	143±40	148±42
Lp (a)	36.6±37.7	37.4±40.2
LCAT	82.3±54.9	69.0±48.8

*All values are given as mean±SD
TC : Total cholesterol, TG : Triglyceride, HDL-C : High density lipoprotein-cholesterol
LDL-C : Low density lipoprotein-cholesterol, HDL-FC : HDL-Free cholesterol
HDL-EC : HDL-Ester cholesterol, S. FC : Serum free cholesterol, S. EC : Serum ester cholesterol, Apo-A-I and Apo B : Apolipoprotein A-I and B, Lp (a) : Lipoprotein (a),
LCAT : Lecithin : cholesterol acyltransferase
(Note : The statistical significance in all the parameters compared between the two groups were not significant, p>0.05)

Table 3. Serum LCAT activity, based on exposure to cigarette smoke.

	Brinkmann index<200 n : 12	Brinkmann index ≥ 200 n : 15	p
LCAT activity (μmol.L^{-1}.h^{-1})	81.4±50.8	59.2±45.1	>0.05

Table 4. Serum lipid, lipoprotein and apolipoprotein levels (mg/dL) and LCAT activity ($\mu mol.L^{-1}.h^{-1}$) in hyperlipidemic - and normolipidemic -smokers and nonsmokers*

Analytes	Normolipidemics		Hyperlipidemics	
	Smokers (n : 12)	Non-smokers (n : 18)	Smokers (n : 15)	Non-Smokers (n : 13)
TC	178±34	175±32	274±55	278±35
TG	137±40	128±55	253±110	300±153
HDL-C	50.1±6.9#	57.0±7.9	47.5±9.6	46.9±7.5
LDL-C	100±33	92±29	184±55	177±18
TC/HDL-C	3.5±0.8	3.1±0.6	5.9±1.4	6.1±1.2
HDL-FC	15.7±6.8	13.3±5.4	12.7±5.4	13.0±7.4
HDL-EC	35.4±8.6#	43.7±10.7	34.7±6.9	33.9±7.9
HDL-EC/FC	2.9±2.0	3.9±1.9	3.2±1.5	3.6±2.1
S.FC	60±9	61±22	104±27	108±35
S.EC	120±27	115±26	169±39	171±34
S.EC/FC	2.0±0.5	1.9±1.0	1.7±0.5	1.8±0.8
Apo A-I	150±22	157±15	150±27	154±38
Apo B	111±19	115±29	171±35	172±27
Lp (a)	39.6±52.8	33.7±31.4	36.1±32.5	39.5±44.3
LCAT	64.8±47.3	78.3±51.8	72.7±51.6	88.0±60.7

*X±SD
See Table 2 for abbreviations
#Significantly different from the non-smokers using Student's t-test, $p<0.05$

into normolipidemics (n : 30) and hyperlipidemics [n : 28, isolated hypercholesterolemia (n : 8) and mixed hypertriglyceridemia (n : 20)] HDL-C and HDL-esterified cholesterol were lower in normolipidemic-smokers than in normolipidemic-nonsmokers (Table 4).

DISCUSSION

Smoking is a significant risk factor for IHD. There are several studies showing associations between cigarette smoking and altered serum lipid and lipoprotein concentrations. These alterations, which are associated with smoking, are higher serum levels of TC, TG, VLDL-C, LDL-C and lower serum levels of HDL-C and Apo A-I. Many of these variables have been associated with an increased risk of IHD (5).

Several studies have reported that serum HDL-C level is found to be lower in smokers than in non-smokers (16-18). However Siekmeier *et al*. (19) reported that smoker and non-smoker subjects had similar HDL-C levels ; this result agrees with our result. In this study, concentrations of TC, TG, LDL-C, HDL-C, Lp (a), apo A-I and B did not differ between smokers and non-smokers.

There are several studies showing lower serum LCAT concentrations or activities in smokers compared to non-smokers (20, 21). Haffner *et al*. reported a negative correlation between smoking and LCAT mass (21). The positive correlation between LCAT concentration and TC and LDL-C was also reported in these studies (20-22). These findings are in accordance with the suggestion that LCAT plays an important role in the reverse transport of cholesterol. In addition to in *vivo* studies, in *vitro* studies have shown that cigarette smoke inhibited LCAT activity (23). In another study, it was reported that rats given nicotine had lower LCAT activity (24). However, in several studies, LCAT activity was found not to be different in smokers and non-smokers (7, 17, 22, 25). In our study, serum LCAT activity was found to be 15.8% lower in smokers than in non-smokers, but this difference was not statistically significant.

The data indicate that cigarette smoking does not affect lipid, lipoprotein and apolipoprotein levels. LCAT activity tended to be lower in smokers compared to non-smokers. So, it may be considered that the reverse cholesterol transport can be diminished and cholesterol can accumulate in the peripheral tissues, more in the smokers than non-smokers.

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