Cigarette Smoking, High-Density Lipoprotein Cholesterol Subfractions, and Lecithin:Cholesterol Acyltransferase in Young Women

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Much of the published data on the relationship of cigarette smoking (CS) with serum lipids and lipoproteins is based on studies of middle-aged individuals. Data on young women are scarce. This study examined the relationship of CS with high-density lipoprotein cholesterol (HDL-C) subfractions and lecithin:cholesterol acyltransferase (LCAT) activity in Japanese collegiate women. Twenty-three current smokers were individually matched for physical activity scores, age, and body mass index (BMI) with 23 nonsmokers. There were no significant differences between smokers and nonsmokers in the mean nutrient intakes. Smokers had significantly lower mean HDL-C, HDL_2-C, total cholesterol, and LCAT activity than nonsmokers. In univariate analyses, BMI significantly negatively correlated with HDL-C and HDL_2-C. LCAT activity significantly positively correlated with HDL_3-C, LDL-C, total cholesterol (TC) and triglycerides (TG). In multiple regression analyses, the number of CS was positively related to TG. BMI was negatively related to TC. LCAT activity was positively related to LDL-C, TC, and TG. These results suggest that the known associations in older adults of CS with HDL-C subfractions and LCAT activity are already apparent in young women.

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CIGARETTE SMOKING (CS) is one of the major risk factors for coronary heart disease.[1] It has been shown that plasma high-density lipoprotein cholesterol (HDL-C) concentrations are inversely correlated with coronary heart disease risk factors.[2] CS is associated with a lower concentration of HDL-C,[3] which can explain in part the increased risk of coronary heart disease in smokers. The lower HDL-C concentrations in smokers compared with nonsmokers has been attributed to lowered HDL_2-C,[4-6] HDL_3-C,[7] or to both subfractions.[8] However, these data are based on studies of middle-aged or older individuals. Data on young women are scarce. One study found that CS was significantly negatively related to HDL-C in white girls. However, this study did not measure HDL subfractions.[9]

Of the plasma enzymes known to affect HDL metabolims, lecithin:cholesterol acyltransferase (LCAT), which esterifies cholesterol on HDL,[10] has been compared in smokers and nonsmokers. Some studies[7,11] reported that smokers had significantly lower plasma LCAT concentrations than nonsmokers, whereas others[4,8,12,13] did not find significant differences in serum or plasma LCAT activity. The purpose of this study was to examine the relationship of CS with HDL subfractions and LCAT activity in Japanese collegiate women.

MATERIALS AND METHODS

Subjects and Self-Administered Questionnaire

Collegiate women were recruited from one university and consented to the procedure after explanation of the purpose of the study. To be included in the study, they had to meet the following criteria: (1) they were menstruating at normal intervals, ranging from 26 to 31 days, which fell within the normally accepted range[14]; (2) they drank alcohol less than once a week and even then only had a small amount; (3) they were not on any medication at the time of their participation in the study; and (4) they had never taken birth control pills. Smokers were eligible if they smoked at least for 1 year. Twenty-three current smokers volunteered, who were individually matched for physical activity scores, age, and body mass index (BMI) with 23 nonsmokers who had never smoked. The study protocol was approved by the Ethics Committee of the Nakamura Gakuen University and informed consent was obtained from each subject.

Information on smoking, physical activity habits, and coffee consumption was obtained via a self-administered questionnaire. Accuracy of the questionnaire was checked through individual interviews. Smokers were asked to indicate how many cigarettes a day (number of CS) and how many years did they smoke (duration of CS). The frequency, duration, and mode of physical activity were questioned, and scores ranging from 1 to 5 were given according to Young and Steinhardt.[15] Coffee consumption—how many cups of coffee a day, week, or month—was questioned.

Measurements

Body weight and height were measured with the subjects in underwear to the nearest 0.1 kg and 0.1 cm, respectively. The BMI was expressed as weight/height^2 (kg/m^2). Maximal oxygen uptake was measured with a continuous multistage exercise test to volitional exhaustion on a bicycle ergometer (Monark Ab., Varberg, Sweden). The test was conducted in air-conditioned facilities with a temperature set at 25°C. Ventilatory measurements were made by standard open-circuit calorimetry (Wyvern Software Physiologic Exercise Testing System; P.K. Morgan Instruments, Andover, MA) with 30-second sampling intervals. Details of the methods have been presented elsewhere.[16]

Dietary information was collected using a 3-weekday diet record. Each diet was analyzed by means of a computer program. Each food item was coded according to the Tables of the Japanese Foodstuff Composition.[17]

Blood Analysis

Physical exercise was not allowed 48 hours, and beverages other than water and CS were not allowed 24 hours prior to the blood sampling. Subjects arrived at the laboratory by 8 AM. The temperature of the laboratory was set at 25°C. Fasting (12-hour) blood samples were

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drawn from the antecubital vein after each subject had been seated quietly for at least 20 minutes. All blood samples were taken between days 6 and 12 of the menstrual cycle when estrogen levels were relatively low.[14] The samples were immediately stored on ice, and kept on ice until centrifuged within 10 minutes in a refrigerated centrifuge at 4°C. Samples were analyzed within 10 days, and all measurements were duplicated. Total cholesterol (TC) and triglycerides (TG) were analyzed by enzymatic methods. HDL-C was analyzed by direct assay with selective inhibition method. HDL_2-C and HDL_3-C were analyzed by ultracentrifugation method. Low-density lipoprotein cholesterol (LDL-C) was analyzed by hepalin and citrate precipitation method. Details of these methods have been presented elsewhere.[16] LCAT activity (Nescauto LCAT kit-S; Azwell, Osaka, Japan)[18] was analyzed by a dipalmitoyl lecithin substrate method using a Hitachi autoanalyser (model 7170; Hitachi, Tokyo, Japan). The intra-assay and interassay coefficients of variation were 0.54% and 2.71% for TC, 4.16% and 7.34% for TG, 0.91% and 1.73% for HDL-C, 7.18% and 8.71% for HDL_2-C, 7.27% and 7.33% for HDL_3-C, 1.37% and 3.70% for LDL-C, and 5.53% and 8.62% for LCAT activity, respectively.

Statistical Analysis

The results are expressed as means ± SD. The *t* test was used to compare the mean values between smokers and nonsmokers. Pearson correlation coefficients were used to examine simple correlations between 2 variables in 23 smokers. Multiple regression analyses were performed with HDL-C, HDL_2-C, HDL_3-C, LDL-C, TC, and TG as the dependent variables. Because of the small sample size (n = 23), the number of independent variables included in each model was limited to number of CS, duration of CS, LCAT activity, and other variables that showed significant correlation ($P < .05$) in the univariate analyses to serum lipids and lipoproteins.

RESULTS

The smokers currently smoked 12.4 ± 6.8 (range, 2 to 20) cigarettes per day and had been smoking for 2.7 ± 0.9 (range, 1.1 to 4.2) years. For each physical activity score from 1 to 5, there were 14, 1, 1, 4, and 3 smokers and nonsmokers, respectively. There were no significant differences between smokers and nonsmokers in the mean nutrient intakes (energy, protein, fat, vitamins A, B_1, B_2, and C, calcium, phosphorus, iron, sodium, potassium, salt, fiber, cholesterol, total fat, saturated fat, polyunsaturated fat and coffee) (data not shown).

Table 1. Characteristics and Biochemical Values

	Smokers (n = 23)	Nonsmokers (n = 23)
Age (yr)	20.7 ± 0.7	20.8 ± 0.7
Height (cm)	160.1 ± 5.9	158.5 ± 5.1
Weight (kg)	53.9 ± 7.0	52.3 ± 6.1
BMI (kg/m^2)	21.0 ± 2.4	20.8 ± 2.2
$\dot{V}o_2max$ ($mL \cdot kg \cdot min^{-1}$)	39.3 ± 7.7	39.2 ± 8.6
HDL-C (mmol/L)	1.56 ± 0.29*	1.80 ± 0.47
HDL2-C (mmol/L)	1.02 ± 0.26†	1.30 ± 0.43
HDL3-C (mmol/L)	0.47 ± 0.06	0.46 ± 0.07
LDL-C (mmol/L)	2.50 ± 0.57	2.60 ± 0.59
TC (mmol/L)	4.43 ± 0.63†	5.03 ± 0.72
TG (mmol/L)	0.91 ± 0.44	0.76 ± 0.29
LCAT (nmol/ml/h)	63.7 ± 13.8*	75.5 ± 17.5

NOTE. Values are the mean ± SD.

Abbreviations: BMI, body mass index; $\dot{V}o_2max$, maximal oxygen uptake; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; LCAT, lecitin:cholesterol acyltransferase.

*$P < .05$.

†$P < .01$.

Table 2. Pearson Correlation Coefficients Between Selected Variables in Smokers

	HDL-C	HDL_2-C	HDL_3-C	LDL-C	TC	TG
NCS	−0.27	−0.23	0.15	0.22	0.03	0.29
DCS	−0.05	−0.10	0.32	−0.11	−0.08	0.23
BMI	−0.50*	−0.44*	−0.14	0.01	−0.15	0.29
LCAT	0.08	0.08	0.46*	0.76†	0.83†	0.60†
TG	−0.08	−0.19	0.16	0.40	0.52*	

Abbreviations: NCS, number of cigarettes smoked/day; DCS, duration of cigarette smoking.

*$P < .05$.

†$P < .01$.

The mean characteristics and biochemical values are listed in Table 1. The smokers had significantly lower mean HDL-C, HDL_2-C, TC, and LCAT activity than nonsmokers.

The Pearson correlation coefficients between selected variables in smokers are shown in Table 2. BMI significantly negatively correlated with HDL-C and HDL_2-C. The LCAT activity significantly positively correlated with HDL_3-C, LDL-C, TC, and TG. The number of CS, duration of CS, BMI, and LCAT activity did not significantly correlate each other. Furthermore, the age, BMI, dietary habits such as intakes of cholesterol, total fat, saturated fat, polyunsaturated fat, and coffee consumption, as well as maximal oxygen uptake in $mL \cdot kg^{-1} \cdot min^{-1}$ and physical activity scores, did not significantly correlate with any of serum lipids and lipoproteins (data not shown).

Thus, we entered the number of CS, duration of CS, LCAT activity, BMI, and TG into the multiple regression analyses as independent variables. The number of CS was positively related to TG. BMI was negatively related to TC. LCAT activity was positively related to LDL-C, TC, and TG (Table 3).

DISCUSSION

Smokers tend to be less active than nonsmokers,[19] and less active people in comparison to active people tend to show higher TC and TG and lower HDL-C and LCAT activity.[20,21]

Table 3. Standaedized Partial Regression Coefficients of Serum Lipids and Lipoproteins With Selected Independent Variables in Smokers

	HDL-C	HDL_2-C	HDL_3-C	LDL-C	TC	TG
NCS	−0.25	−0.35	0.11	0.24	0.11	0.45†
DCS	0.05	0.04	0.41	−0.10	−0.08	0.19
BMI	−0.41	−0.28	−0.27	−0.23	−0.37†	0.30
LCAT	0.33	0.47	0.61	0.69†	0.74‡	0.61‡
TG	−0.24	−0.51	−0.19	0.14	0.23	
R^2	0.32	0.33	0.44	0.64†	0.80‡	0.63‡

*$P < .05$.

†$P < .01$.

‡$P < .001$.

Smokers are often leaner than nonsmokers.[22] Lean subjects tend to show lower TG and higher HDL-C than their lean counterparts.[20] Smokers show higher intakes of energy, total fat, saturated fat, cholesterol, and alcohol.[23] Saturated fatty acids, cholesterol, and excess caloric intake raise serum LDL.[24] In the present study, smokers were individually matched for physical activity scores, age, and BMI with nonsmokers. In addition, maximal oxygen uptake and dietary habits such as intakes of cholesterol, total fat, saturated fat, polyunsaturated fat, and coffee consumption did not differ significantly between smokers and nonsmokers. Also, these variables did not correlate with any of the serum lipids and lipoproteins. Furthermore, to avoid confounding influence of the acute effects of CS, menstrual cycle phase and alcohol intake on these parameters,[14,25-28] the smokers were asked to refrain from CS for 24 hours before blood samples were obtained between days 6 and 12 of the menstrual cycle. In addition, only collegiate women who drank alcohol less than once a week were admitted to the study. Despite this selection, smokers showed significantly lower mean HDL-C, HDL_2-C, TC, and LCAT activity than nonsmokers. The lower mean TC found in smokers could be due mainly to the relatively large decrease in HDL_2-C observed in smokers than in nonsmokers.

Some studies[7,11] reported that smokers had significantly lower plasma LCAT concentrations than nonsmokers, whereas others[4,8,12,13] did not find significant differences in serum or plasma LCAT activity. Haffner et al[7] reported a significant negative correlation between CS and LCAT mass. In addition to in vivo studies, a in vitro study has shown that CS inhibited LCAT activity.[29] In the present study, although LCAT activity did not correlate with the number and duration of CS, smokers showed significantly lower LCAT activity than nonsmokers. Albers et al[11] reported that LCAT concentrations were significantly positively correlated with TC and LDL-C but not with HDL-C. The results of the present study are consistent with this study.

Furthermore, LCAT activity was significantly positively correlated with HDL_3-C in univariate analysis. These results are consistent with the concept that LCAT plays an essential role in cholesterol removal. LCAT esterifies cholesterol on HDL, which are transferred to apolipoprotein B–containing lipoproteins.[10] Therefore, LCAT activity would be expected to be associated with HDL_3-C and LDL-C, as observed in the present study.

The lower HDL-C concentrations in smokers compared with nonsmokers has been attributed to lowered HDL_2-C,[4-6] HDL_3-C,[7] or to both.[8] In the present study, smokers showed significantly lower HDL_2-C. The magnitude of difference in HDL_2-C was 27.2%, which was slightly larger than the differences reported by others, ranging from 16.7% to 26.2%.[4-6,8] The divergent results obtained in these studies could be due to the differences in age, gender, physical activity, obesity, and/or other confounding factors as mentioned previously.

Although the mechanisms whereby CS decrease HDL_2-C observed in the present study using young women is not known, it is possible that they could be indirectly and/or directly caused by the gas phase of cigarette smoke, which possesses a variety of components (eg, free radicals and aldehydes) capable of damaging lipids and proteins,[29-31] and causing endothelial dysfunction.[32] It is also possible that they could be caused by an increase in hepatic lipase activity.[4,10] Hepatic lipase converts the large, TG-rich HDL_2 back into small TG- and cholesteryl ester–poor HDL_3-C.[10] Thus, the increase in hepatic lipase activity would be expected to increase HDL_3-C and decrease HDL_2-C. It has been reported that plasma hepatic lipase activity was significantly higher in smokers than in nonsmokers.[4] However, this study used acute myocardial infarction patients. Thus, the effects of smoking on plasma hepatic lipase activity need to be investigated in young normolipidemic women.

In conclusion, the present study revealed that young collegiate female smokers had significantly lower HDL-C, HDL_2-C, TC, and LCAT activity than similar nonsmokers. These results suggest that the known associations in older adults of CS with HDL-C subfractions and LCAT activity are already apparent in young women.

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