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The Effects of Exercise and Smoking on Serum Lecithin: Cholesterol Acyltransferase Activity in Young Men

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Abstract: Lecithin: cholesterol acyltransferase (LCAT) is an enzyme responsible for the formation of most cholesteryl esters (CE) in human serum. In this study investigating the effects of exercise and smoking on LCAT acitvity, serum triglyceride (Tg), total cholesterol (C), HDL-C, HDL3-C and LCAT activity were measured in the sera of 29 nonsmoking and 36 smoking (at least one packet/day) young men before and after their 6-week military training period. LCAT activity was determined by measuring the amount of cholesterol esters produced in the incubation of serum and reconstituted HDL including [^{14}C]-cholesterol. Tg and C were measured with enzymatic-colorimetric methods. The measuring of HDL-C and HDL_3-C was performed by dextran sulphate-$MgCl_2$ precipitation.

Before exercise, serum LCAT activity in the smoking group was found to be significantly lower than that of the nonsmoking group ($p<0.05$). There were no significant differences between the Tg, C, CDL-C, HDL-C, HDL_2-C and HDL_3-C levels of both groups. After exercise, LCAT, HDL-C and HDL_2-C levels in the nonsmoking group group were significantly higher than those of the smoking group ($p<0.001$, $p<0.05$ and $p<0.05$ respectively) and LDL-C was singificantly lower in the nonsmoking group ($p<0.05$), although Tg, C and HDL_3-C did not exhibit any significant difference between the groups. When the data was compared before and after exercise within each group, LCAT, HDL-C and HDL_2-C levels showed significant increases ($p<0.001$, $p<0.05$ and $p<0.05$ respectively), while LDL-C showed a significant decrease ($p<0.05$) only in the nonsmoking group. There were no significant differences in the smoking group. The results indicate that regular exercise has some positive effects on LCAT, HDL_2-C and LDL, and that heavy cigarette smoking may obscure these effects.

Key Words: Exercise, Smoking, LCAT, HDL-C, HDL_2-C, HDL_3-C

Introduction

Many epidemiological studies have demonstrated that plasma levels of high-density lipoprotein (HDL) correlate inversely with the incidence of coronary heart disease (CHD). It is postulated that HDL may facilitate cholesterol transport from peripheral cells to the liver and other tissues requiring cholesterol in a process that has been named 'reverse cholesterol transport' (RCT) (2).

An important enzyme in modulating plasma HDL levels is lecithin: cholesterol acyltransferase (LCAT) (EC.2.3.1.43), which is responsible for the formation of most of the cholesterol esters (CE) present in human plasma. This enzyme catalyzes the transfer of fatty acid from the sn-2 position of lecithin to the free hydroxyl group of cholesterol. The substrates for this reaction, lecithin and cholesterol, and the activator, apo A-I, are provided by HDL (1).

Physical activity and cigarette smoking are two factors closely related with the development of CHD (2-12). The great majority of adults in the Turkish population smoke cigarettes and are sedentary (13). Exercise has wellknown beneficial effects on the cardiovascular system. These effects correlate with the intensity of the regimen. The benefits may explain this association, including changes in blood-clotting factors, reduction of sympathetic nervous system activity, release of adrenaline and the improved transport of blood lipids (3, 4). Smoking is, in contrast, positively associated with CHD. Several mechanisms may explain this association, including changes in blood coagulation status, impaired integrity of arterial walls, and changes in blood lipids and lipoproteins (2, 5).

Some studies (6-8) have suggested that the exercise-induced increase in serum HDL-C levels may be related to the concomitant increase in LCAT activity, although

contradictory findings have been reported (9). It is known that smoking causes a reduction in serum HDL-C levels (2, 10). In addition, some compounds have been found in cigarettes that can inhibit plasma LCAT activities (14, 15).

Accordingly, we investigated the effects of exercise and smoking on serum LCAT activity *in vivo,* comparing data obtained from smoking and nonsmoking young men before and after a specific exercise program.

Material and Methods

Subjects: First, 75 healthy male volunteers, 20-23 years old, were selected from a group of military recruits prior to starting a basic military training program. Eligibility requirements for participation in the study were as follows: (a), no participation in any exercise program within the last year; (b), if employed prior to recruitment, the job required no heavy physical activity; (c), no heavy consumption of alcohol (recruits either not drinking at all or engaged only in social drinking); (d) no use of medication; (e) a body weight less than or equal to the ideal weight value according to the Metropolitan table; (f) blood tests revealed normolipidaemia; (g) no acute or chronic disease was on clinical and laboratory examination; and (h) the nonsmoking group consisted of recruits who never smoked cigarettes, while the smoking group consisted of recruits smoking at least one packet cigarette/day.

Data from 36 subjects in the smoking group and 26 subjects in the nonsmoking group were evaluated. These subjects had participitated in every exercise session and completed the exercise program without any health problems. A total of ten subjects were withdrawn for various reasons.

Exercise Program: After completion of the pretraining measurements, the subjects trained for 75 min/day and 6 day/week according to the standard military exercise program for a period of 6 weeks. Each session in this program consisted of a warm-up period (5 min); a 2500-3000 m run (15-20 min); a 300-500 m walk (5-8 min); endurance training, including push-ups, sit-ups, and pull-ups (30-40 min); and a cool-down period (5 min).

Diet: All subjects received the same diet intended to have a stabilizing effect during the exercise program. This diet consisted of 40-60% carbohydrate, 30-35% fat (P/S: 2), 15-20% protein and 3-4 g salt. The daily calorie intake of the subjects was about 3000 cal.

Physiological Measurements: To evaluate body composition, body weight and height were measured before and after the exercise program, and the Body Mass Index (BMI) was calculated using the following equation: BMI=weight (kg)/height^2 (m). The resting heart rate (RHR) of all subjects was measured before and after the exercise program.

Analytical Procedures: Venous blood samples were taken between 7:00 and 9:00 a.m. after a 12-hour fast and a period of no smoking. Post training samples were collected 2 days after the last day of exercise. The samples were centrifuged to obtain sera within two hours, which were stored at -40°C until analysis. All the chemicals used were the best grade available from commercial sources.

LCAT activity in the serum was determined using a modification of the method proposed by Albers et al. (11). In short, 16 ml aliquots of sera were incubated with a proteoliposome subtrate (reconstituted HDL) containing apo A-I, [^{14}C]-cholesterol, and egg lecithin at a molar ratio of 2.4:13.6:243 for 60 min at 37°C. The addition of a chloroform/methanol (2:1, v/v) mixture stopped the reaction. The lipids were extracted with chloroform. The free cholesterol and CE were separated on silica gel TLC plates using a solvent system of petroleum ether-ethyl acetate (85:15). The TLC plates were autoradiographed with high-sensitivity film (Amersham) for 2 days. After development of the films, spots of free cholesterol and CE on the film were evaluated using a densitometer (Helena Laboratories, EDC). The LCAT activity was expressed in terms of molar activity of nanomoles cholesterol esterified per hour per milliliter.

Triglyceride (Tg) and total cholesterol (C) were measured using standard enzymatic-colorimetric methods on a Technicon Dax 48 autoanalyzer. HDL-C and HDL_3-C were determined by the selective precipitation method with dextran sulphate-$MgCl_2$, at various concentrations and pH values (12). HDL_2-C was calculated by subtracting HDL_3-C from HDL-C. LDL-C was assessed according to Friedwald's formula, using data on C, HDL-C and Tg.

Statistical methods: The data obtained are presented as means±SD and the significance of the differences between both groups before and after exercise was assessed by Student's t test. Simple correlation coefficients between different parameters were calculated by the method of least squares. In all cases, $p<0.05$ was considered significant.

Results

Table 1 summarize the means of body weight, BMI and RHR, measured in the smoking and the nonsmoking

Table 1. Physiological findings in the nonsmoking and smoking groups before and after the exercise program.

	Nonsmoking		Smoking	
	Before	After	Before	After
Body weight (kg)	71.5±6.3	70.9±6.9	69.1±8.2	68.3±7.8
BMI (kg/cm^2)	23.2±1.8	23.7±1.2	23.2±1.7	23.3±1.9
RHR (pulse/min)	77.4±1.2	70.1±0.8*	78.4±1.1	70.6±0.7*

*: p<0.001

groups before and after the exercise program. After exercise, only RHR in both groups was significantly lower than before (p<0.001).

Figure 1 shows the LCAT activities measured in both groups before and after the exercise program. The difference of the mean LCAT activity before and after exercise in the nonsmoking group was significant (p<0.001).

Table 2 shows the means and standard deviations of LCAT, HDL-C, HDL_2-C, HDL_3-C, LDL-C, C and Tg, and statistical comparisons in both groups before and after the exercise program.

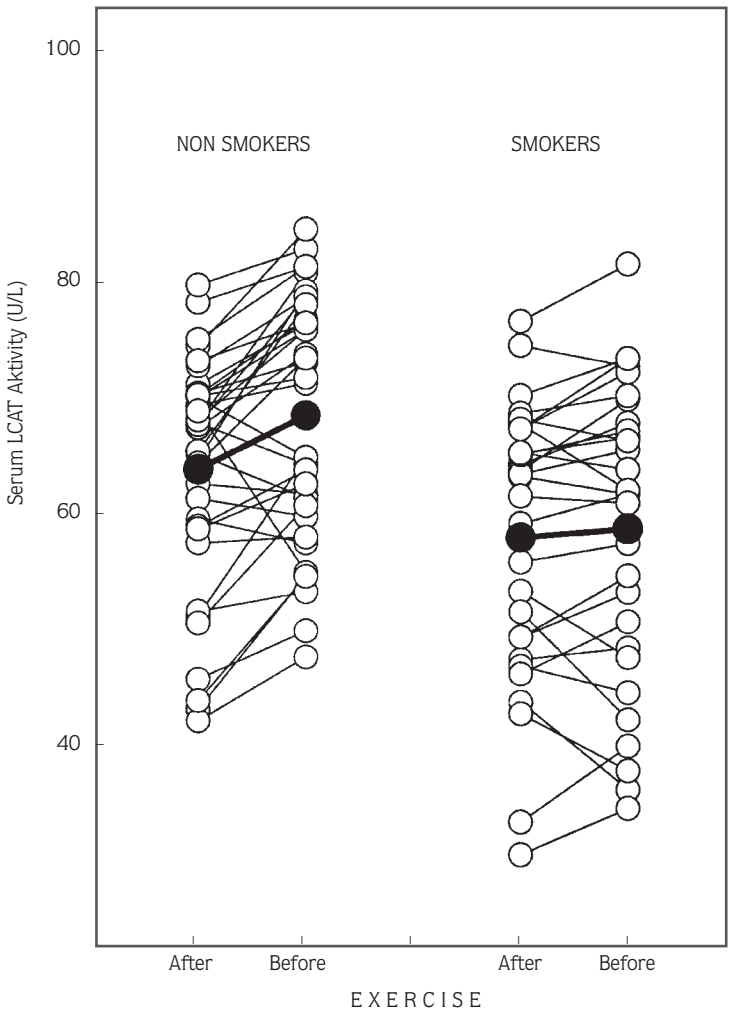

Figure 1. LCAT activities of 29 subjects from the nonsmoking group and 36 subjects from the smoking group before and after the exercise program. Open circles indicate individual LCAT activity. Solid circles indicate the mean of the group. * p<0.001.

Before exercise, serum LCAT activity in the smoking group was significantly lower than that in the nonsmoking group ($p<0.05$). There were no significant differences between the means of Tg, C, LDL-C, HDL-C, HDL_2-C and HDL_3-C in both groups. After exercise, the means of LCAT, HDL-C and HDL_2-C in the nonsmoking group were significantly higher than those in the smoking group ($p<0.001$, $p<0.05$ and $p<0.05$ respectively) and the mean of LDL-C was significantly lower in the nonsmoking group ($p<0.05$), although the means of Tg, C and HDL_3-C did not exhibit any significant differences between the two groups. When the data was compared in terms of val-ves before and after exercise within each group, LCAT, HDL-C and HDL_2-C showed significant increases ($p<0.001$, $p<0.05$ and $p<0.05$ respectively), while LDL-C showed a significant decrease ($p<0.05$) only in the nonsmoking group. There were no significant differences in the smoking group for values before and after exercise. Moreover, the correlations between LCAT activity and the other parameters measured were not significant in either group before and after the exercise program.

Discussion

It is currently understood that regular exercise leads to alterations in lipoprotein metabolism, such that it exerts a positive effect on the cardiovascular system. In contrast, cigarette smoking is associated with CHD, although the nature of its activity has not been fully elucidated (2-5). The goal of the present study was to examine *in vivo* the effects of exercise and smoking on LCAT activity, which is an important enzyme in RCT supposed to have certain anti-atherogenic effects, and which is responsible for forming most cholesteryl esters in human serum (1).

Our study groups were composed of young men newly inducted into military service, and who had not worked prior to this period in a job that may have increased their aerobic capacity. During the program, they shared the same living conditions, diet, physical activity, stress, housing, etc., a variable which may have an effect on the cardiovascular system. The diet was similar to that recommended by the American Heart Association for the general public in terms of caloric intake to maintain ideal body weight (16). This exercise program was composed of over 90% dynamic action. The hearth can normally adapt to a dynamic state, decreasing its RHR as a chronic response, which indicates an increase in aerobic capacity (17). There were no significant changes in body weight or BMI in our study subjects before and after participation in the exercise program. The data shows that the mean values of RHR in the nonsmoking and the smoking groups significantly decreased after the exercise program (Table 1). These results indicate that this exercise program was highly effective at increasing aerobic capacity in both groups.

Several studies have analyzed the effects of exercise on LCAT, although many reports have been published about the relationship between lipoprotein particles and exercise (2-4). In general, results from studies using any exercise program that can increase aerobic capacity have consistently reported significant and favorable changes in plasma lipoprotein levels, such as increases in HDL, and especially HDL_2 (3, 8, 18-21). Higher LCAT activity has been reported: for endurance-trained sportsmen, after endurance training in young and middle-aged men, and in athletes who have completed 10 hours per week of aerobic activity. This higher LCAT activity is thought to contribute to increased synthesis of HDL_2-C (6, 22, 23), although there is also data showing otherwise (9). The reason for this discrepancy between studies is not readily apparent, but the inconsistency may be related to the extent of training completed and/or the technological differences used to measure serum LCAT activity (22). According to the data in the present study (Table 2 and Figure 1), serum levels of HDL-C, HDL_2-C and LCAT activity increased, and LDL-C decreased significantly after the exercise program in the nonsmoking group. In the smoking group, there were no significant differences for any of the parameters measured. These data indicate that smoking can obscure the positive effects of exercise upon the lipid profile and LCAT activity. LCAT may play a key role in this process, as a report recently published (24) has shown, in which smokers exhibited significantly reduced plasma LCAT activity. Although the mechanism by which this occurs has not been established, it is known that tobacco contains reactive aldehydes which can inhibit plasma LCAT activities in vitro (14, 15). The effect of cigarette smoke extract (CSE) on the activity of LCAT in human plasma studied by Chen et al. (15) showed that when plasma is incubated with CSE, there is both a concentration-and time-dependent loss in LCAT activity. Incubation of plasma with some reactive aldehyde known to be present in cigarette smoke also inhibited LCAT activity. Of five aldehydes (acetaldehyde, acrolein, hexanal, formaldehyde, and malondialdehyde) tested, acrolein was the strongest inhibitor of LCAT, and acetaldehyde) was the weakest inhibitor of LCAT, with 85% enzyme inhibition at 50 mM. They concluded that reactive aldehydes are at least partially responsible for the reduction in LCAT activity in plasma treated with CSE

Table 2. Mean values and comparisons of LCAT, Tg, C, LDL-C, HDL-C, HDL_2-C and HDL_3-C in both groups before and after the exercise program.

	Nonsmoking		Smoking	
	Before	After	Before	After
LCAT (U/mL)	68.7±10.8 ¥	74.1±11.0 †	62.6±11.3	63.7±13.0 §
HDL_2-C (mg/dL)	21.9±2.53	23.1±3.07 ‡	21.0±3.01	21.1±3.38 *ƒ*
HDL_3-C (mg/dL)	17.7±3.16	18.7±2.51	18.4±3.10	18.4±2.81
HDL-C (mg/dL)	39.7±3.91	41.8±3.54 ‡	39.4±4.45	39.6±4.09 *ƒ*
LDL-C (mg/dL)	125±27.0	111±20.3 ‡	126±26.5	121±20.8 *ƒ*
TC (mg/dL)	185±27.9	173±23.3	186±26.5	181±21.6
Tg (mg/dL)	102±36.5	103±28.5	105±29.5	102±27.4

Significant differences between values before and after exercise within the group: †, $p<0.001$; ‡, $p<0.05$.
Significant difference between values in the nonsmoking and smoking groups before exercise: ¥, $p<0.05$.
Significant differences between values in the nonsmoking and smoking groups after exercise: §, $p<0.001$; *ƒ*, $p<0.05$.

(15). Bielicki et al. (14) investigated the mechanism (s) by which cigarette smoking inhibited LCAT activity and modified HDL. When dialyzed human plasma (12 ml) was exposed to the gas-phase of an equivalent of 1/8 of a cigarette (one 'puff') at 15 min intervals for 3 h, LCAT activity was reduced by 76 +/, 1% compared to the controls. Supplementation of plasma with glutathione produced dose-dependent protection of LCAT activity, where, at the highest concentration (1 mM), 78% protection was observed.

According to our data (Table 2), the nonsmoking group showed a significant increase in LCAT, HDL-C and HDL_2-C, and a decrease in LDL-C, when a comparison was made between the beginning and the end of the exercise program. In contrast, smokers did not show any significant changes in these parameters. These data suggest that exercise causes an increases in serum LCAT activity and has beneficial effects upon the lipid profile, and that smoking may obscure the positive effects of exercise.

In conclusion, increased serum LCAT activity, which has a key role in the reverse cholesterol transport system and is understood to be anti-atherogenic, may play a part in the positive effects that are observed with regular exercise, effects that may be cancelled by smoking.

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