Influence of Worksite Environmental Tobacco Smoke on Serum Lipoprotein Profiles of Female Nonsmokers

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The purpose of this study was to examine the influence of environmental tobacco smoke (ETS) in the workplace on high-density lipoprotein cholesterol (HDL-C), HDL-C subfractions, and apolipoprotein (apo) A-I and apo B in female workers. Premenopausal women free from factors known to influence HDL-C (cigarette smoking, vigorous physical exercise, etc) who were not taking oral contraceptives, were moderate consumers of alcohol, caffeine, and dietary fat, and were between the ages of 21 and 50 years participated in one of two groups: (1) nonsmokers who had never smoked cigarettes and were generally free from ETS exposure (nonsmokers), and (2) nonsmokers who had never smoked but were subjected to concentrated doses of ETS at least 6 hours per day, 4 days per week, for at least the past 6 consecutive months (ETS-exposed). A third group consisting of current cigarette smokers who smoked a minimum of 20 cigarettes per day for at least the past 5 consecutive years served as smoking controls (smokers). Subjects were matched by group as closely as possible with regard to criteria that can influence blood lipoprotein levels. Participants were solicited from taverns and restaurants where they were employed. It was hypothesized that individuals chronically exposed to ETS would demonstrate unfavorable lipoprotein profiles. Results showed that HDL-C, HDL_2, and apo A-I were significantly ($P < .05$) depressed for ETS-exposed and smokers as compared with nonsmokers. Values for ETS-exposed were not different from those for smokers. Total cholesterol, triglycerides, HDL_3, and apo B did not differ among the three groups. It was concluded that excessive exposure to ETS in female workers can have deleterious effects on HDL-C, HDL_2, and apo A-I in nonsmokers that are similar to effects observed in cigarette smokers. It is possible that these effects increase coronary artery disease (CAD) risk.

CIGARETTE SMOKING is known to contribute to unfavorable changes in blood lipids, that may increase coronary artery disease (CAD) risk through acceleration of the atherosclerotic process.[1,2] Cigarette smoking reduces high-density lipoprotein cholesterol (HDL-C) and its antiatherogenic subfraction, HDL_2.[3-10] Several epidemiological studies suggest that environmental tobacco smoke (ETS) also increases CAD risk.[11-13] Previous studies suggest that ETS in the home may exert a negative impact on blood lipids in children[14] and adolescents[15] similar to that which occurs from cigarette smoking. There also is some indication that a similar effect of ETS in the home may occur in adults.[16,17] The present study was undertaken to examine the possible effects of workplace ETS on blood lipids in women.

SUBJECTS AND METHODS

Subjects

Subjects were matched across groups with regard to criteria that can influence blood lipid and lipoprotein levels. Subjects were recruited from establishments that serve alcoholic beverages as a specialty and where smoking is permitted. Establishments were screened regarding their smoking policies and the probability that there would be smokers present throughout the day. Within those establishments that permitted smoking and were sufficiently popular to attract a consistent stream of smokers, participation of employees (cocktail waitresses) was solicited first by face-to-face interview followed by completion of a detailed questionnaire. Questions included the descriptive characteristics, age, height, weight, employment status and history, socioeconomic standing (wages and benefits), smoking history, smoking history of spouse or significant other, exposure to ETS (other than worksite), medications, disease status, physical activity patterns, and alcohol consumption, and an assessment of willingness of the respondent to allow blood samples to be taken, to keep accurate and detailed dietary records, and to come to the laboratory for testing.

Subjects for the nonsmokers and smokers groups were individuals with similar positions, socioeconomic standing, and job demands (physical, mental, and emotional), but free from ETS. Restaurants were screened, and those disallowing smoking were chosen with agreement of the owner. Waitresses were then interviewed and asked to complete standardized questionnaires.

All subjects taking part in the study shared the following characteristics: premenopausal and not taking oral contraceptives or any other type of medication, sedentary (meaning no exercise other than that associated with job tasks), aged 21 to 50 years, and moderate alcohol consumer ($\leq$150 kcal alcohol/d). Seven participants were nondrinkers: four from the ETS-exposed group, two from the nonsmokers group, and one from the smokers group. Subjects were moderate caffeine consumers ($\leq$400 mg/d) and moderate dietary-fat consumers ($<$40% of total calories from fat and $\leq$15% from saturated fat), and were asymptomatic and free of known heart or lung disease. Written informed consent was provided in accordance with the University-approved human subject protocol.

Beyond the shared characteristics, subjects were grouped in the following manner. ETS-exposed subjects (n = 19) had never smoked cigarettes and were exposed to concentrated doses of ETS at the worksite at least 6 hours per day for a minimum of 4 days per week for at least the past 6 consecutive months. Smokers (n = 12) smoked a minimum of 20 cigarettes per day for a minimum of the past 5 consecutive years, but worked in a smoke-free environment and generally were not exposed to concentrated doses of ETS. Nonsmokers (n = 9) had never smoked cigarettes, worked in a

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Table 1. Age and Anthropometric and Smoking Characteristics by Group (mean ± SD)

Group	Age (yr)	Height (cm)	Weight (kg)	Quetelet Index	Average No. of Cigarettes per Day	Years of Smoking
ETS-exposed (n = 19)	37.8 ± 5.8	164.8 ± 4.3	59.5 ± 5.7[a]	2.19 ± 0.11	0	0
Smokers (n = 12)	35.9 ± 3.5	166.1 ± 2.5	59.1 ± 5.1[b]	2.14 ± 0.08[a]	27.25 ± 6.38	15.75 ± 4.77
Nonsmokers (n = 9)	33.9 ± 6.5	164.8 ± 3.3	61.9 ± 4.8[ab]	2.28 ± 0.17[a]	0	0

NOTE. Column means that share a common superscript are significantly different ($P < .05$).

smoke-free environment, and generally were not exposed to concentrated doses of ETS.

Subjects were instructed to report to the laboratory for testing between 8 and 10 AM following a 12-hour overnight fast and a 12-hour abstinence from cigarette smoking or ETS exposure. Blood samples for determination of total cholesterol, HDL-C, HDL_2, HDL_3, low-density lipoprotein, triglycerides, and apolipoprotein (apo) A-I and apo B were drawn from an antecubital vein.

Repeated expired–carbon monoxide samples were taken on two occasions: (1) in the laboratory, and (2) at each individual subject's worksite. Worksite samples were taken after 2 hours on the job. Carbon monoxide levels of smokers were measured at the worksite after a minimum 2-hour abstinence from smoking. In addition, carbon monoxide level was measured a second and third time in smokers in the laboratory, after 12 hours without smoking and immediately after smoking one cigarette of their personal brand. Carbon monoxide determinations in exhaled air were made using the CMD/CO carbon monoxide monitor (Spirometrics, Auburn, ME). Calibration gases were applied regularly to ensure valid measurements (within ±2% over a range of 0 to 200 ppm CO). Previous analyses in our laboratory showed that readings are highly reproducible ($r = .93$).

Plasma Lipid, Lipoprotein, and Apolipoprotein Assays

Total cholesterol was determined by the method reported by Allain et al.[18] HDL-C level was measured by precipitating all other cholesterol fractions with phosphotungstate-magnesium,[19] leaving a supernatant that was assayed for cholesterol content. Subfractions of HDL-C, HDL_2 and HDL_3, were determined using the precipitation method reported by Gidez et al.[20] Radioimmunoassay procedures were used for measurement of apo A-I and apo B levels.[21,22] Triglycerides were quantitatively assayed by an enzymatic hydranalysis technique described by Buccolo and David.[23] Low-density lipoprotein was estimated using the formula reported by Friedewald et al.[24] Quality-control samples were also assayed to determine precision. The coefficient of variation was determined to be less than 2.2% for HDL-C, HDL subfractions, and total cholesterol and less than 3.7% for apo A-I and apo B. The coefficient of variation was 4.1% for triglycerides.

Dietary Intake Procedures

Dietary data were obtained from 3-day food diaries. Subjects were instructed on how to keep accurate records and were provided with written instructional aids. All food items were recorded on standard forms for 3 successive days including 1 weekend day. Records were checked for completeness and accuracy. Dietary records were analyzed for nutrient and caloric composition using a computerized nutrient database provided from an expanded version of Agriculture Handbook No. 8.[25]

Statistical Analysis

The formula of Cohen[26] was used to determine the required sample size (≥ 8 subjects per group) for a desired power of .80 and effect size of .70. Since it was anticipated that body weight may be different between groups, an analysis of covariance adjusted for the Quetelet Index (weight/height^2) was used with a Scheffé post hoc technique to determine significant differences between groups for blood lipid and lipoprotein parameters. A multifactorial ANOVA was used with a univariate F test to determine significant group differences for anthropometric and smoking characteristics, diet, and respiratory carbon monoxide levels. The criterion for acceptance of statistical significance was established at P less than .05.

RESULTS

Descriptive characteristics of the subjects are presented in Table 1. Groups did not differ according to age or height; however, ETS-exposed and smokers weighed significantly less than nonsmokers. Additionally, the Quetelet Index was significantly higher for nonsmokers as compared with smokers.

Serum cholesterol, triglycerides, and low-density lipoprotein were not different among groups (Table 2). Analysis of covariance adjusted for Quetelet Index showed that HDL-C and HDL_2 were significantly lower for ETS-exposed (14% and 31%, respectively) and for smokers (18% and 33%, respectively) as compared with nonsmokers. No differences were observed among groups for HDL_3. The ratio HDL_2/HDL_3 was significantly lower for both ETS-exposed and smokers.

Although blood apo B levels were not different among groups, levels of apo A-I for nonsmokers were 14% higher than for ETS-exposed and 20% higher than for smokers (Table 2). The apo A-I/apo B ratio was lower in smokers as compared with nonsmokers.

Respiratory carbon monoxide samples were taken in the laboratory after a 12-hour abstinence from smoking (Table

Table 2. Blood Lipid, Lipoprotein, and Apolipoprotein Profiles of ETS-exposed, Smokers, and Nonsmokes (mean ± SD)

Parameter	ETS-Exposed	Smokers	Nonsmokers
TC (mmol/L)	4.92 ± 0.47	5.14 ± 0.77	4.84 ± 0.43
Triglycerides (mmol/L)	2.06 ± 0.59	2.23 ± 0.25	2.17 ± 0.32
HDL-C (mmol/L)	1.25 ± 0.09[a]	1.18 ± 0.12[b]	1.44 ± 0.11[ab]
TC/HDL-C	3.93 ± 0.75	4.35 ± 0.88	3.36 ± 0.63
LDL (mmol/L)	3.23 ± 0.42	3.53 ± 0.69	2.96 ± 0.41
HDL-C/LDL	0.39 ± 0.05	0.33 ± 0.08	0.49 ± 0.07
HDL_2 (mmol/L)	0.38 ± 0.08[a]	0.37 ± 0.15[b]	0.55 ± 0.08[ab]
HDL_3 (mmol/L)	0.87 ± 0.20	0.81 ± 0.14	0.89 ± 0.13
HDL_2/HDL_3	0.44 ± 0.04[a]	0.45 ± 0.11[b]	0.62 ± 0.05[ab]
Apo A-I (mmol/L)	2.85 ± 0.69[a]	2.66 ± 0.81[b]	3.32 ± 0.48[ab]
Apo B (mmol/L)	2.32 ± 0.69	2.29 ± 0.53	2.29 ± 0.40
Apo A-I/apo B	1.23 ± 0.23	1.15 ± 0.18[a]	1.45 ± 0.17[a]

NOTE. Row means that share a common superscript are significantly different ($P < .05$).

Abbreviations: TC, total cholesterol; LDL, low-density lipoprotein.

Table 3. Carbon Monoxide Levels at the Worksite and in the Laboratory

Group	Carbon Monoxide (ppm)	
	Worksite	Laboratory
ETS-exposed	15.2 ± 4.9[abd]	3.1 ± 1.0[de]
Nonsmokers	2.9 ± 0.6[ac]	2.2 ± 0.4[f]
Smokers	7.8 ± 2.6[bc]	8.1 ± 1.7[ef]

NOTE. Means that share a common superscript are significantly different ($P < .05$).

3). Values were not different between ETS-exposed (3.1 ± 1.0 ppm) and nonsmokers (2.2 ± 0.4 ppm); however, values for smokers (8.1 ± 1.7 ppm) were significantly greater than those for the other two groups. Fifteen minutes after smoking one cigarette, values in smokers increased to 23.8 ± 6.8 ppm. Samples collected at each subject's respective worksite showed no differences in carbon monoxide when compared with samples collected at the laboratory for smokers and nonsmokers. The ETS-exposed group demonstrated a nearly 500% increase in respiratory carbon monoxide levels (15.2 ± 4.9 ppm) above laboratory levels after 2 hours exposure to ETS on the job.

Daily energy intake, the proportion of carbohydrate, protein, and fat, and the polyunsaturated to saturated fat ratio are presented in Table 4. Smokers consumed significantly more daily calories than nonsmokers, and the proportion of carbohydrates was greater among nonsmokers.

DISCUSSION

Every effort was made to ensure that participants across groups were as homogeneous as possible, including daily physical activity and energy expenditure. Exceptions included a significantly greater body weight and Quetelet Index for nonsmokers as compared with the other two groups. Nonsmokers were heavier despite consuming significantly fewer calories (150 kcal) per day than smokers. The fact that smokers may consume more calories and yet be lighter and leaner has been cited in other reports,[4,6,10] and most likely reflects the acute thermogenic effects of cigarette smoking.[27]

Intake of dietary fat, saturated fat, and alcohol (as a percent of total daily calories) did not differ among groups. Total serum cholesterol, triglycerides, and low-density lipoprotein also did not differ among groups. As expected, smokers demonstrated a lower HDL-C, HDL_2, HDL_2/HDL_3 ratio, apo A-I, and apo A-I/apo B ratio when compared with nonsmokers. The fact that cigarette smoke suppresses blood levels of HDL-C and HDL_2 is well documented.[3-10] The present findings suggest that those who are exposed to chronic high doses of ETS may possess characteristics similar to those of smokers with respect to the lipoprotein profile.

These important effects have been observed previously in children,[14] adolescents,[15] and adults[16,17] of both sexes. In each case, ETS exposure was in the home. Compared with circumstances in those studies, our subjects probably were exposed to higher concentrations of ETS but for shorter periods of time. In addition, in the other studies,[14-17] a larger number of subjects were studied, but the larger number prevented detailed attention to diet, exercise, and other factors that may influence blood lipid concentrations. Regardless of dissimilarities, the sum of our results and those of others strongly support an ETS-induced negative change in blood lipids in ETS-exposed persons regardless of age or sex.

Carbon monoxide levels were impacted substantially by ETS at the worksite. ETS-exposed demonstrated carbon monoxide levels similar to those of nonsmokers when samples were collected in the laboratory, reflecting absence from worksite pollution for a minimum of 12 hours. However, after 2 hours at the worksite, there was a nearly 500% increase in carbon monoxide in ETS-exposed. This elevation in carbon monoxide in ETS-exposed represents 64% of the observed elevation in carbon monoxide among smokers in the present study after smoking one cigarette in the laboratory. The apparent negative impact of ETS on the lipoprotein profile plus the substantial increase in carbon monoxide observed among ETS-exposed during work hours suggest that the influence of tobacco smoke to increase CAD risk may be exerted effectively through ETS, as well as through smoking.

When comparing the relative risks of cigarette smoking versus ETS, it is important to point out that the smoker has the option to quit. This decision would seem to remove the negative impact on the blood lipid profile and carbon monoxide levels, because these are acute and transient effects. The victim of ETS, unfortunately, may not have the choice not to be exposed.

There are a number of limitations imposed on the findings of the present study. The number of participants was small, the data were limited to premenopausal women, and the degree of ETS exposure was extreme when considering everyday circumstances common in the United States. Even so, controls imposed in the present study appear to warrant the conclusion that chronic concentrated doses of ETS may decrease blood levels of HDL-C, HDL_2, and apo A-I and expired carbon monoxide levels in nonsmokers. Based on these findings, it is reasonable that these combined effects may increase the risk of CAD in ETS-exposed, nonsmoking females.

Table 4. Energy Intake and Dietary Carbohydrate, Fat, and Protein as a Percent of Total Daily Energy Intake (mean ± SD)

Group	Energy Intake (kcal)	Carbohydrate (%)	Fat (%)	P:S	Protein (%)	Alcohol (%)
ETS-exposed	2,109 ± 84	46.5 ± 4.4	36.0 ± 5.2	1.6	15.7 ± 4.4	1.8 ± 0.4
Smokers	2,194 ± 101[a]	44.3 ± 3.8[a]	36.2 ± 5.7	2.1	16.2 ± 1.9	3.3 ± 1.6
Nonsmokers	2,044 ± 77[a]	47.2 ± 4.7[a]	35.8 ± 4.8	1.7	14.9 ± 2.4	2.1 ± 0.7

NOTE. Column means that share a common superscript are significantly different ($P < .05$).
Abbreviation: P:S, polyunsaturated to saturated fat ratio.

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