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EFFECT OF CIGARETTE SMOKING ON HIGH DENSITY LIPOPROTEIN PHOSPHOLIPIDS

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SUMMARY: The effect of acute inhalation of cigarette smoke on high density lipoprotein (HDL) phospholipid composition in White Carneau pigeons was examined. Four treatments included: 1) Shelf Control birds fed a chow diet and retained in their cages; 2) Sham pigeons fed a cholesterol-saturated fat diet and exposed to fresh air by a smoking machine; 3) Low nicotine-low carbon monoxide (LoLo) animals also fed the cholesterol diet and exposed to low concentrations of these cigarette smoke products; and 4) High nicotine-high carbon monoxide (HiHi) birds fed the cholesterol diet and subjected to high concentrations of these inhalants. The cholesterol-fat diet caused an increase in the concentration of most HDL phospholipid classes. Exposure to the HiHi regimen resulted in an increase in the HDL cholesterol/phospholipid ratio and a reduction in the concentration of HDL phosphatidyl ethanolamine, phosphatidyl serine/inositol, sphingomyelin and lysophosphatidyl choline. Cigarette smoking may thus attenuate HDL's anti-atherogenic properties by altering surface phospholipid components.

INTRODUCTION

A number of clinical studies have demonstrated an inverse relationship between the incidence of coronary heart disease and plasma high density lipoprotein (HDL) cholesterol concentration (1-3). Cigarette smoking as a major coronary risk factor causes a depression in HDL cholesterol and a reduction in HDL protein components, apoprotein A-I and A-II (4-6). While several investigations have examined the effect of smoking on total plasma phospholipid (7,8), no data is available concerning whether cigarette smoke alters the composition of the individual HDL phospholipid classes. This information is particularly important since lipoprotein phospholipids play a critical role in the normal functioning and integrity of the lipid transport system (9).

Abbreviations: HDL, high density lipoproteins; LoLo, low nicotine-low carbon monoxide; HiHi, high nicotine-high carbon monoxide; LCAT, lecithin cholesterol acyl transferase

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Circulating phospholipids through interaction with apoproteins serve to solubilize neutral lipids for transport in the blood (9), influence the clearance rate of lipoprotein particles (10), and in the case of HDL phosphatidyl choline, function as a substrate in the lecithin cholesterol acyl transferase (LCAT) reaction. This reaction is important in the removal of excess phospholipid and free cholesterol from lipoprotein remnants and serves to mobilize cholesterol from peripheral cell membranes (11). The relative concentration of lipoprotein cholesterol and phospholipid may also modulate the ratio of these components in cell membranes and thus influence membrane fluidity (12,13). In addition, HDL apoprotein-sphingomyelin complexes enhance the efflux of cholesteryl ester from aortic smooth muscle cells and may thus contribute to the molecular process by which HDL protects against ischemic heart disease (14).

We recently demonstrated that chronic exposure of White Carneau pigeons to cigarette smoke containing high levels of nicotine and carbon monoxide results in a significant depression in plasma LCAT activity (15). Although subsequent substrate efficiency experiments revealed no significant difference between smoke-exposed and sham-treated pigeons, we postulated that cigarette smoking might induce subtle alterations in HDL phospholipid which could be identified by quantitative measurement of the individual phospholipid classes. Results from the present study are unique in that they represent the first documentation that inhalation of cigarette smoke reduces the concentration of several different HDL phospholipid components.

MATERIALS AND METHODS

The present study was performed in collaboration with the Harvard University Tobacco and Health Research Program project (National Cancer Institute: ECI-SHP-RFP-75-111) entitled "Inhalation Bioassay of Cigarette Smoke in Pigeons: The Effects of Nicotine and Carbon Monoxide on Atherogenesis." Six month old male White Carneau pigeons purchased from Palmetto Pigeon Plant (Sumter, S.C.) were secured individually in an exposure rack by a Velcro cuff and a soft sponge rubber restraint and exposed to whole tobacco cigarette smoke three times a day, seven days/week, for six months, using the automated 30-port Lorillard smoking machine (Lorillard, Greensboro, N.C.). Only the pigeon's beak including

the nares was inserted into a rubber diaphragm leading to the smoke channel. This apparatus is equipped with a mechanism to automatically dilute cigarette smoke with prescribed levels of clean air or supplemental carbon monoxide. The amount of smoke delivered to the pigeons (exposure) as well as the amount of smoke retained by the birds (dose) was reproducible and adjusted to levels comparable to dosages in humans.

Four groups of pigeons consisting of six birds/group were designated as follows: 1) Controls fed ad libitum a low fat chow diet (Purina Pigeon Chow) and retained in their cages; 2) Sham-smoked birds (Sham) handled in the same manner as those exposed to tobacco smoke only with fresh air substituted in their exposure channels; 3) Low nicotine-low carbon monoxide (LoLo) animals exposed to low concentrations of these smoke products; and 4) High nicotine-high carbon monoxide (HiHi) exposed animals maintained on a smoking regimen consisting of high levels of these inhalants. The latter three groups were all fed Purina Pigeon Chow supplemented with 10% lard and 0.2% cholesterol by weight.

After six months of sham/smoke exposure or shelf control treatment, pigeons were fasted overnight, blood was collected from the alar vein into heparinized syringes, and plasma was harvested following low speed centrifugation at 4°C. Total plasma cholesterol was determined by the procedure of Wybenga et al (16). Following isolation by density gradient ultracentrifugation using an SW-41 swinging bucket rotor (Beckman Instruments, Inc., Palo Alto, Ca.) (17), HDL was collected by aspiration and dialyzed exhaustively as previously described (18). Lipoprotein purity was assessed by agarose gel electrophoresis (19). One aliquot of the HDL was then taken for total phospholipid phosphorous determination (20) and another portion was extracted with chloroform:methanol (21). Free and esterified cholesterol were separated by thin-layer chromatography (TLC) (22), scraped, and assayed colorimetrically (16).

Phospholipid standards (Sigma Chemical Co., St. Louis, Mo.) and aliquots of the HDL lipid extract were applied to 0.3 mm thick silica gel 60H thin layer plates (E. Merck, Darmstadt, Germany) impregnated with 1% ammonium sulfate. Phospholipid classes were separated by sequential use of two solvent systems (hexane-diethyl ether-acetic acid, 60:30:1 and chloroform-methanol-acetic acid-water, 50:30:8:4) (23). After visualization with iodine vapors, individual classes were scraped and phospholipid phosphorous was determined by the Bartlett procedure (20). Phospholipid mass was calculated from the following phosphorous weight percentages: phosphatidyl ethanolamine 4.16, phosphatidyl serine/inositol 3.78, lysophosphatidyl ethanolamine 6.70, phosphatidyl choline 3.93, sphingomyelin 4.13, and lysophosphatidyl choline 6.10.

Means and standard error of the mean were calculated for each measurement. Means of the treatment groups were analyzed for significant differences by one way analysis of variance and Duncan's multiple range test (24).

RESULTS

Carboxyhemoglobin levels were 10% and 26%, respectively, for LoLo and HiHi pigeons. Negligible amounts of carboxyhemoglobin were detected in the blood of Sham-exposed and Shelf-Control birds. Low and high nicotine cigarettes contained, respectively, 0.16±0.01 and 1.26±0.03 mg of nicotine/cigarette.

Table I

EFFECT OF CIGARETTE SMOKING ON HDL AND PLASMA CHOLESTEROL AND PHOSPHOLIPID

	Treatment Groups			
	Control	Sham	LoLo	HiHi
Plasma Cholesterol	260 ± 12[a,b]	450 ± 56[b]	409 ± 25[b]	458 ± 14[b]
HDL Cholesterol/ Phospholipid Ratio	0.47 ± 0.05[c]	0.55 ± 0.05[c]	0.56 ± 0.05[c]	0.81 ± 0.04[c]

[a]Values are means ± SEM from analysis of three separate plasma pools of 2 pigeons/pool expressed as mg/dl.

[b]Control mean significantly different ($P<0.05$) from means of other groups with superscript b.

[c]HiHi mean significantly different ($P<0.05$) from means of other groups with superscript c.

Plasma cholesterol levels ranging from 409-458 mg/dl were similar for Sham, LoLo, and HiHi treatments (Table I). Circulating cholesterol concentrations in these groups were, however, significantly different ($P<0.05$) from levels in the chow-fed Control animals (260±12 mg/dl). Data in Table I also shows that inhalation of smoke from high nicotine-high carbon monoxide cigarettes, independent of dietary cholesterol, caused a significant increase in the HDL cholesterol/phospholipid ratio.

Dietary saturated fat plus cholesterol produced a significant increase in the concentration of all HDL phospholipid classes except lysophosphatidyl ethanolamine (Table II). Smoke-related differences in the mass and relative percentage of the individual phospholipid classes were most notable between the Sham and HiHi groups (Table II). Specifically, cigarette smoking caused a depression in HDL phosphatidyl ethanolamine, phosphatidyl serine/inositol, sphingomyelin and lysophosphatidyl choline. Phosphatidyl choline was unaffected by cigarette smoke exposure.

Table II

EFFECT OF CIGARETTE SMOKING ON HDL PHOSPHOLIPID COMPOSITION

	Treatment Group			
	Control	Sham	LoLo	HiHi
Phospholipid				
Phosphatidyl-ethanolamine	23.8±3.1[b] (10.8±0.5)	47.2±3.1[b] (12.9±0.6)[b]	27.1±1.9[b] (9.2±1.1)[b]	30.0±0.5[b] (9.6±0.9)[b]
Phosphatidyl serine/inositol	17.3±1.1[b] (7.9±0.9)	28.7±0.4[b] (7.9±0.3)	22.8±3.7 (7.6±0.8)	21.0±2.2[b] (6.9±1.4)
Lysophosphatidyl ethanolamine	8.1±2.5 (4.0±1.7)	11.7±2.3 (3.2±0.6)	10.3±2.0 (3.6±1.0)	9.3±4.0 (2.8±0.9)
Phosphatidyl choline	140.7±20.3[b] (62.3±1.6)	226.5±15.5[b] (61.8±3.1)	191.7±20.7 (64.3±4.8)	215.0±36.7 (67.0±4.3)
Sphingomyelin	21.0±3.9[b] (9.2±0.8)	38.9±4.9[b] (10.6±1.2)	27.2±3.4 (9.1±0.7)	24.8±2.8[b] (8.2±1.8)
Lysophosphatidyl choline	13.7±2.6[b] (6.0±0.5)	22.6±0.9[b] (6.2±0.3)	18.0±1.4 (6.2±0.7)	17.1±1.1[b] (5.5±0.5)
Total phospholipid	224.6±28.4[b] (100.2)	375.6±19.0[b] (102.6)	297.1±23.9 (100.0)	317.2±36.0 (100.0)

[a]Values are means ± SEM from analysis of three separate plasma pools of 2 pigeons/pool expressed as mg/dl. Numbers in parentheses represent % of total phospholipid.

[b]Sham mean significantly different ($P<0.05$) from means of other groups with superscript b.

DISCUSSION

Several investigations have demonstrated associations between low HDL cholesterol levels and established coronary risk factors such as diabetes, obesity and cigarette smoking (3-5,25). Cigarette smoking also causes a reduction in HDL apoprotein components (6). The present study was designed to assess the effect of acute inhalation of cigarette smoke on HDL phospholipid composition. Previous studies involving the analysis of total plasma phospholipid in human smokers have produced equivocal results. Pozner and Billimoria (7) initially found no difference in plasma phospholipid concentration between nonsmokers, light smokers, and heavy smokers while a later study by these investigators (8) showed a moderate increase in male heavy smokers only. Chronic subcutaneous injection of nicotine in dogs produces a significant increase in total serum phospholipid (26), although similar

changes are not observed in rats following intraperitoneal administration of the alkaloid (27).

The present study demonstrates that exposure of pigeons to the high nicotine-high carbon monoxide treatment resulted in a significant reduction in phosphatidyl ethanolamine, phosphatidyl serine/inositol, sphingomyelin and lysophosphatidyl choline (Table II.) Smoke-induced depression in lysophosphatidyl choline, which is a product of the LCAT reaction, is consistent with our recent finding that HiHi pigeons have reduced plasma LCAT activity (15). While the exact function of lysophosphatidyl choline is uncertain, some evidence suggests that this lipid may increase the binding of phosphatidyl choline to apoprotein A-I by altering phospholipid bilayer fluidity (28). This action may in turn be essential for normal lipid-protein complex formation in HDL particles (10,29). The lysophospholipid also increases the α-helical structure of HDL apoproteins and may thus contribute to a protein configuration more resistant to denaturing agents (28).

Recent evidence points to an intravascular origin of HDL involving transfer of phospholipids from very low density lipoproteins (VLDL) and chylomicrons during their catabolism by lipoprotein lipase (30-32). Inhibition of LCAT activity has also been shown to impair the transfer of sphingomyelin from low density lipoprotein (LDL) to HDL without affecting the exchange of phosphatidyl choline between these lipoproteins (33). The latter finding is in agreement with the reduced LCAT activity reported for HiHi pigeons (15), the diminished levels of HiHi HDL sphingomyelin, and the comparable amounts of phosphatidyl choline measured in Sham treated and smoke-exposed animals (Table II). Moreover, reduction in HDL sphingomyelin may be important in light of the role this lipid plays, when complexed with apoproteins, in the removal of cholesteryl ester from aortic smooth muscle cells (14).

Several investigators have utilized the cholesterol/phospholipid ratio as an index of altered membrane function, abnormal lipoprotein

profile, and atherogenicity (13,34,35). Cooper and Shattil (13) emphasized the importance of controlling this ratio in plasma lipoproteins in order to prevent functional abnormalities in cell membranes which result from equilibrium redistribution and enrichment of membranes with excessive amounts of cholesterol. In this regard, it is interesting to note that the HDL cholesterol/phospholipid ratio in the HiHi group was significantly different from all other treatments (Table I). Blaton et al (35) earlier reported a similar increase in this index in HDL from baboons fed an atherogenic diet although our data suggests that smoking can independently modify this parameter.

Data in Tables I and II provide the first documentation that acute inhalation of smoke from high nicotine-high carbon monoxide cigarettes causes alterations in HDL surface phospholipids. The information is particularly significant since these polar lipids normally participate in exchange reactions with plasma membranes (10), serve as carriers for long chain polyunsaturated fatty acids (36), contribute to the structural integrity of HDL (28,29), and thus play an important role in the normal functioning of the lipid transport/delivery system (9). Smoke-induced reduction in lipoprotein phospholipids may thus impair the system and thereby attenuate HDL's anti-atherogenic properties. This alteration may in turn predispose smokers to the development of coronary heart disease.

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