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# ORAL NICOTINE IMPAIRS CLEARANCE OF PLASMA LOW DENSITY LIPOPROTEINS[1]

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Abstract. The effect of chronic oral nicotine intake on plasma low density lipoprotein (LDL) clearance, lipid transfer protein, and lecithin:cholesterol acyltransferase (LCAT) was examined in male atherosclerosis susceptible squirrel monkeys. Eighteen yearling primates were divided into two groups: 1) Controls fed isocaloric liquid diet; and 2) Nicotine monkeys given liquid diet supplemented with nicotine at 6 mg/kg body wt/day for a two-year period. Averaged over 24 months of treatment, animals in the Nicotine group had significantly higher levels of plasma and LDL cholesterol compared to Controls while plasma LCAT activity was similar for both groups. Following simultaneous injection of $^{3}H$ LDL and $^{14}C$ high density lipoprotein (HDL) cholesteryl ester (CE), removal of the latter was not altered by oral nicotine while plasma clearance of $^{3}H$ LDL was dramatically delayed in Nicotine monkeys. Transfer of $^{14}C$ HDL CE to very low density lipoprotein (VLDL)-LDL particles was greatly accelerated in the Nicotine group vs Controls while the reciprocal movement of $^{3}H$ LDL CE to HDL was only higher in experimental animals at two time points following injection of the isotopes. Results from this study provide evidence that one major detrimental effect of long-term oral nicotine use is an increase in the circulating pool of atherogenic LDL which is due to: 1) accelerated transfer of lipid from HDL; and 2) impaired clearance of LDL from the plasma compartment. Diminished removal of LDL is of particular importance because an extended residence time of these particles in circulation would increase the likelihood of their deposition in the arterial wall.



Our recent finding that chronic oral nicotine intake causes an elevation in atherogenic low density lipoproteins (LDL) (1) is significant because it indicates that a circulating insult to the arterial wall can be induced by a single tobacco component and may in part be responsible for the increased arteriopathy observed in cigarette smokers (2). It appears that accelerated synthesis of LDL through enhanced lipolytic conversion of precursor lipoproteins is one metabolic process responsible for creation of an atherogenic lipoprotein profile by nicotine (3). On the other hand, synthesis is but one mechanism which regulates plasma LDL concentrations. Imbalances in the rate of removal of these macromolecules from the plasma compartment (4) and transfer of cholesteryl ester (CE) from high density lipoproteins (HDL) to LDL following cholesterol esterification by lecithin:cholesterol acyltransferase (LCAT) (5), may also modify LDL levels. LDL clearance is in turn facilitated by both high-

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affinity receptor and receptor-independent pathways (4) while redistribution of CE among lipoproteins is promoted by lipid transfer protein (LTP) (6). Virtually nothing is known about whether nicotine alters these processes in vivo. Consequently, the purpose of this study was to determine whether long-term oral consumption of the alkaloid increases LDL levels by delaying removal of these particles from circulation and/or by accelerating transfer of lipid from HDL with resultant overloading of the LDL core.

Materials and Methods. Eighteen yearling male Bolivian squirrel monkeys were divided into two treatment groups consisting of 9 monkeys/ group: 1) Controls fed Primate Liquid Diet # 19 (Bioserv Inc., Frenchtown, NJ); and 2) Nicotine animals given liquid diet supplemented with nicotine (Eastman Kodak Co., Rochester, NY) at 6 mg/kg body wt/day. This concentration was chosen because a comparable oral level administered to rats has been shown to increase hepatic microsomal enzyme activity (7) while a somewhat higher intravenous dose (9.6 mg/kg/day) has been used to evaluate habituation in nonhuman primates (8). Diet composition, feeding protocol, and primate housing have been previously described (9).

On a monthly basis for 2 years, plasma cholesterol (10) and triglyceride (Sclavo Diagnostics, Wayne, NJ) were measured colorimetrically and HDL cholesterol was quantitated (10) following precipitation of very low density lipoprotein (VLDL)-LDL with heparin-manganese (11). VLDL-LDL cholesterol was calculated as the difference between plasma and HDL cholesterol, and LDL cholesterol was determined using the formula (VLDL+LDL cholesterol) - plasma triglyceride/5 (12). Plasma lecithin:cholesterol acyltransferase (LCAT) activity was also assayed each month as previously described (9).

LDL and HDL radiolabeled predominantly in the CE moiety were prepared separately as described by Portman et al (13) using plasma from non-study animals. Following isolation of LDL and HDL by density gradient ultracentrifugation (14), lipoprotein cholesterol mass was quantitated (10) and radioactivity was measured by liquid scintillation spectrometry. $^{3}H$ LDL and $^{14}C$ HDL with specific activities of $1.22 \times 10^{7}$ DPM/mg cholesterol and $1.25 \times 10^{6}$ DPM/mg, respectively, were then combined, injected into Control and Nicotine monkeys, and plasma samples were collected at 5, 10, 15, 30, 60, 90, and 120 min. Following separation of HDL from VLDL-LDL (11), aliquots from each fraction were removed for counting and cholesterol mass determination as described above. Specific activity measurements were corrected to a plasma volume calculated as 4% of body weight. All data was expressed as mean ± SEM and analyzed for significant differences ($P<0.05$) by Student's t-test.

Results and Discussion. Averaged over 24 months of treatment, Nicotine monkeys had significantly higher levels of both plasma (223 ± 6 mg/dl) and LDL (75 ± 6 mg/dl) cholesterol compared to Controls (210 ± 4, 62 ± 4). Plasma clearance of $^{14}C$ HDL cholesteryl ester was essentially the same for both groups. However, as seen in Fig. 1, removal of $^{3}H$ LDL cholesteryl ester from circulation was significantly

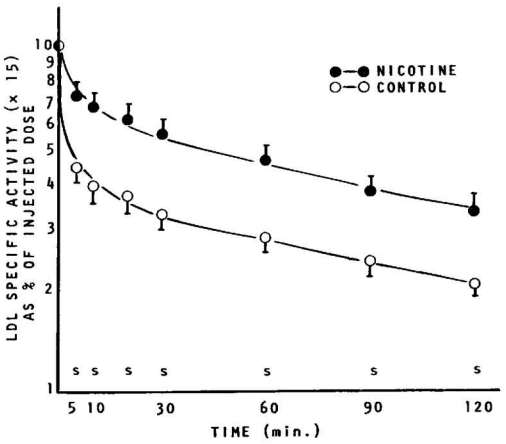


Fig. 1. In vivo clearance of $^{3}H$ LDL cholesteryl ester from the plasma. Each point represents the mean ± SEM for 5 monkeys/group. S indicates significant difference ($P<0.05$) between Control and Nicotine means.

delayed in nicotine treated primates. Moreover, while the transfer of $^{3}H$ LDL cholesteryl ester to HDL was significantly greater in Nicotine animals vs Controls at 60 and 120 min after injection of the isotope, reciprocal movement of $^{14}C$ HDL cholesteryl ester to VLDL-LDL was accentuated as a result of oral nicotine at 5, 30, 60, 90 min suggesting a more active transfer in the direction of HDL→VLDL-LDL (Fig. 2).

Defective LDL clearance in nicotine treated monkeys (Fig. 1) is consistent with an earlier study which showed that chronic subcutaneous injection of the alkaloid into dogs retards the removal of radiolabeled plasma cholesterol (15). Although LDL is degraded in both extrahepatic and hepatic tissues, the latter represents the major site for LDL catabolism via apo B, E receptors (4). Since smoking does not interfere with receptor mediated degradation of LDL by peripheral lymphocytes (16), the liver appears to be the likely site where tobacco components may act to delay lipoprotein uptake and degradation. Two experiments which support this hypothesis include one study with carbon monoxide exposed rabbits in which hepatic removal of lipoprotein remnants was inhibited (17) and our finding that liver slices from animals exposed to tobacco smoke containing high levels of nicotine have reduced capacity to degrade radiolabeled lipoproteins in vitro suggesting a permanent impairment in liver function (18).

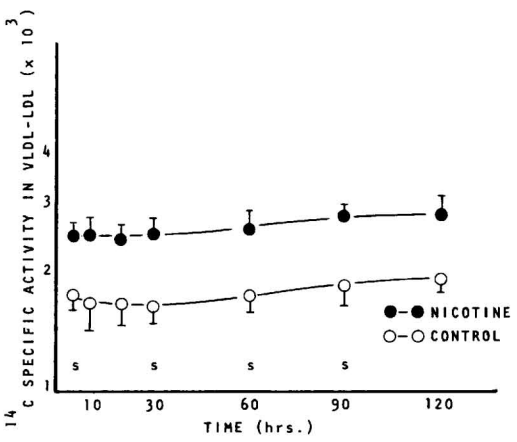


Fig. 2. $^{14}C$ cholesteryl ester appearing in the VLDL-LDL fraction following in vivo plasma transfer from radiolabeled HDL. Time points and statistical significance same as in Fig. 1 legend.

Although the exact mechanism responsible for delayed lipoprotein clearance is unknown, direct delivery of oral nicotine to the liver from the intestine and "first pass" metabolism to cotinine and other by-products (2,19) may in some way affect hepatic lipid and lipoprotein metabolism. For example, because the liver represents the major organ with microsomal enzymatic capabilities for nicotine degradation (2,15), active catabolism of the alkaloid could influence microsomal hepatic cholesterol synthesis since both processes require common cofactors (15). Moreover, chronic delivery of oral nicotine to liver compartments could conceivably alter apo B, E membrane receptors, hepatic synthesis of LCAT and lipid transfer protein (LTP) (5,7), or as we recently demonstrated, biliary cholesterol excretion (1).

Although human smokers have reduced LCAT mass compared to non-smokers (20) and animals exposed to tobacco smoke containing high levels of nicotine demonstrate lower plasma cholesterol esterification (21), chronic administration of oral nicotine had no effect on acyltransferase activity in the plasma of Nicotine (0.128 ± 0.005% esterification/min) vs Control (0.132 ± 0.004) monkeys. However, Fig. 2 shows that the alkaloid may alter LTP which is secreted by the liver and which is functionally related to LCAT because it is responsible for the uni- or bidirectional redistribution of CE among circulating lipoproteins (6). Accentuated activity of LTP may account for the active transfer of HDL cholesteryl ester to VLDL-LDL (Fig. 2) and resultant expansion of the LDL pool in Nicotine primates. Accelerated LTP activity may in turn be related to elevated lipoprotein lipase (LPL) observed in these monkeys (Mulligan, J. et al, unpublished data) since active lipolysis of triglyceride-rich lipoproteins stimulates transfer of HDL cholesteryl ester to lower density lipoproteins (22). This

data is particularly significant in light of the suggestion by Albers et al (6) that information about LTP may be important in elucidating the pathophysiological basis of lipoprotein abnormalities.

Considered together, our findings are unique because they indicate that besides stimulating synthesis from precursor lipoproteins (3), oral nicotine can also increase the LDL concentration by impairing clearance of these particles from the plasma compartment and by overloading LDL with lipid via enhanced transfer from HDL. Nicotine-induced alterations in liver function may play a central role in both of these defects in lipoprotein metabolism. Diminished removal of LDL would in turn enhance its atherogenic potential since an extended residence time in circulation would increase the likelihood of LDL deposition in the arterial wall (23).

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1. Hojnacki JL, Mulligan JJ, Cluette-Brown JE, Igoe FD. Nicotine induced changes in primate lipoproteins. Directory of On-Going Research in Smoking and Health. DHHS pp 348-349, 1985.

2. The Health Consequences of Smoking: Cardiovascular Disease. A Report of the Surgeon General. U.S. Department of Health and Human Services. DHHS Publication No. (PHS) 84-50204, pp 21-48, 212-213, 1983.

3. Augustin J, Beedgen B, Spohr U, Winkel F. The influence of smoking on plasma lipoproteins. Innere Medizin 9:104-108, 1982.

4. Grundy SM. Pathogenesis of hyperlipoproteinemia. J Lipid Res 25: 1611-1618, 1984.

5. DeParscau L, Fielding PE. Lecithin: cholesterol acyltransferase and cholesteryl ester transfer activity from the isolated perfused rabbit liver. J Lipid Res 25:721-728, 1984.

6. Albers JJ, Tollefson JH, Chen CH, Steinmetz A. Isolation and characterization of human plasma lipid transfer proteins. Arteriosclerosis 4:49-58, 1984.

7. Ruddon RW, Cohen AM. Alteration of enzyme activity in rat liver following the acute and chronic administration of nicotine. Toxicol Appl Pharmacol 16:613-625, 1970.

8. Deneau GA, Inoki R. Nicotine self-administration in monkeys. Ann NY Acad Sci 142:277-279, 1967.

9. Mulligan JJ, Cluette-Brown JE, Noring R, Igoe FD, Chong J, Hojnacki JL. Effect of ethanol on lecithin: cholesterol acyltransferase (LCAT) activity. Res Commun Chem Pathol Pharmacol 47:181-202, 1985.

10. Wybenga DR, Pilleggi UJ, Dirstine PH, DiGiorgio J. Direct manual determination of serum total cholesterol with a single stable reagent. Clin Chem 16:980, 1970.

11. Warnick GR, Albers JJ. A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholesterol. J Lipid Res 19:65-76, 1978.

12. Schaefer EJ, Levy RI. Pathogenesis and management of lipoprotein disorders. N Engl J Med 312:1300-1310, 1985.

13. Portman OW, Alexander M, O'Malley JP. Metabolism of free and esterified cholesterol and apolipoproteins of plasma low and high density lipoproteins. Biochim Biophys Acta 619: 545-558, 1980.

14. Redgrave TG, Roberts DCK, West CE. Separation of plasma lipoproteins by density-gradient ultracentrifugation. Anal Biochem 65:42-49, 1975.

15. Gudbjarnason S. Effect of chronic nicotine administration on cholesterol metabolism of liver, serum, heart and brain. J Pharmacol Exp Therap 161: 47-54, 1968.

16. Seidel D, Walli A. Influence of smoking on cellular lipid metabolism. Klinische Wochenschrift 62:28-31, 1984.

17. Topping DL. Effects of tobacco smoke and its constituents on lipid and carbohydrate metabolism. In: Clemens MJ, ed. Biochemistry of Cellular Regulation. CRC Press, Florida, Vol 2, pp 165-183, 1980.

18. Mulligan JJ, Cluette JE, Kew RR, Stack DJ, Hojnacki JL. Cigarette smoking impairs hepatic uptake of high density lipoproteins. Biochem Biophys Res Commun 112:843-850, 1983.

19. Russell MAH, Raw M, Jarvis MJ. Clinical use of nicotine chewing gum. Brit Med J 280:1599-1602, 1980.
20. Haffner SM, Applebaum-Bowden D, Wahl PW, Hoover J, Warnick GR, Albers JJ, Hazzard WR. Epidemiological correlates of high density lipoprotein subfractions, apolipoproteins A-I, A-II, and D, and lecithin cholesterol acyltransferase. Arteriosclerosis 5: 169-177, 1985.
21. Mulligan JJ, Hojnacki JL, Cluette JE, Kew RR, Stack DJ, Nicolosi, RJ, Flanagan S. Cigarette smoke-induced depression in LCAT activity. Exp Mol Pathol 35:25-35, 1981.
22. Tall AR, Sammett D, Vita GM, Deckelbaum R, Olivecrona T. Lipoprotein lipase enhances the cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from high density lipoproteins to very low density lipoproteins. J Biol Chem 259:9587-9594, 1984.
23. Steinberg D, Pittman RC, Carew TE. Mechanisms involved in the uptake and degradation of low density lipoprotein by the artery wall in vivo. Ann NY Acad Sci 454:195-206, 1985.

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