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RAPID COMMUNICATIONS

ORAL NICOTINE INDUCES AN ATHEROGENIC LIPOPROTEIN PROFILE[1]

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Abstract. Male squirrel monkeys were used to evaluate the effect of chronic oral nicotine intake on lipoprotein composition and metabolism. Eighteen yearling monkeys were divided into two groups: 1) Controls fed isocaloric liquid diet; and 2) Nicotine primates given liquid diet supplemented with nicotine at 6 mg/kg body wt/day. Animals were weighed biweekly, plasma lipid, glucose, and lipoprotein parameters were measured monthly, and detailed lipoprotein composition, along with postheparin plasma lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) activity, was assessed after 24 months of treatment. Although nicotine had no effect on plasma triglyceride or high density lipoproteins (HDL), the alkaloid caused a significant increase in plasma glucose, cholesterol, and low density lipoprotein (LDL) cholesterol plus protein while simultaneously reducing the HDL cholesterol/plasma cholesterol ratio and animal body weight. Levels of LDL precursors, very low density (VLDL) and intermediate density (IDL) lipoproteins, were also lower in nicotine-treated primates while total postheparin lipase (LPL + HTGL) activity was significantly elevated. Our data indicate that long-term consumption of oral nicotine induces an atherogenic lipoprotein profile (↑LDL, ↓HDL/total cholesterol ratio) by enhancing lipolytic conversion of VLDL to LDL. These results have important health implications for humans who use smokeless tobacco products or chew nicotine gum for prolonged periods.

Increased coronary heart disease (CHD) in cigarette smokers may be related to elevations in circulating atherogenic low density lipoproteins (LDL) and concurrent depressions in coronary protective high density lipoproteins (HDL) (1). However, because tobacco smoke contains approximately 4000 constituents, it has been difficult to determine which component is responsible for inducing an atherogenic lipoprotein profile (1). Consequently, recent interest has focused on the role of nicotine in lipoprotein alterations because it is one of the most pharmacologically active tobacco components with a wide range of cardiovascular effects (1). Short-term experiments with rabbits (2) and humans (3) have shown that oral administration of the alkaloid raises plasma and LDL cholesterol and lowers HDL. These observations are clinically important because of the widespread use of smokeless tobacco products (4) and the increased prescription of nicotine chewing gum in smoking cessation therapy (5). Since little is known about the underlying molecular mechanisms responsible for these changes, the objectives of our study were to examine the chronic effects of oral nicotine on lipoprotein levels and to identify metabolic

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alterations responsible for observed elevations in LDL.

Materials and Methods. Eighteen yearling male squirrel monkeys were assigned to 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. Diet composition, feeding protocol and primate housing have been previously described (6).

Plasma total cholesterol (7), triglyceride (Sclavo Diagnostics Inc., Wayne, NJ), and glucose (glucose oxidase method, Beckman Instruments Inc., Palo Alto, CA) were measured at monthly intervals. Following precipitation of lower density lipoproteins (8), HDL cholesterol was assayed colorimetrically (7).

After 24 months of treatment, VLDL, LDL and HDL were isolated by density gradient ultracentrifugation (9) and intermediate density lipoproteins (IDL) (d 1.006 - 1.019 g/ml) were separated by sequential ultracentrifugation (10). Purity of lipoproteins and apoprotein B was assessed by cellulose acetate and polyacrylamide gel electrophoresis (PAGE), respectively. Aliquots of each lipoprotein were analyzed for total cholesterol (nonesterified + esterified), protein, phospholipid, and triglyceride (11).

Postheparin plasma samples were collected from fasted primates 10 min after the intravenous injection of 100 IU/kg body wt heparin (Lipo-Hepin, Riker Labs, Inc., Northridge, CA). Triglyceride hydrolysis was assayed as described by Musliner et al (12) and free fatty acids were extracted by the Belfrage and Vaughn procedure (13). Lipoprotein lipase (LPL) activity was calculated as the difference between total postheparin lipolytic and hepatic triglyceride lipase (HTGL) activities (12). All data was expressed as the mean ± SEM and analyzed for significant differences ($P<0.05$) by Student's t-test.

Results and Discussion. Mean body weights of Control (690 ± 38g) and Nicotine (681 ± 32g) primates were initially similar but after 24 months of treatment, Controls weighed significantly more than animals fed oral nicotine (805 ± 10 vs 700 ± 6). This nicotine weight effect is consistent with similar observations reported for rats fed oral nicotine (14), and for human smokers compared to non-smokers (15). Differences in diet consumption patterns may be one explanation for the more modest weight gain in Nicotine (3%) vs Control (17%) primates since over a two year period the former consumed 87% of the liquid diet presented to them while the latter ate 96% of their food supply. However, alternative explanations for the group differences in body weight include elevated catecholamine release and enhanced caloric expenditure, increased gut motility, impaired nutrient absorption, or hyperactivity (15). Similar to human smokers (1), plasma cholesterol and glucose were significantly elevated in Nicotine monkeys (223 ± 6 mg/dl, 101 ± 3 mg/dl) compared to Controls (210 ± 4, 95 ± 3). Increased fasting glucose may be a response to greater percentages of plasma catecholamines which we recently reported in our nicotine-treated primates (16). In addition, the HDL cholesterol/plasma cholesterol ratio, a sensitive index of vascular disease risk, was significantly depressed in experimental (0.61 ± 0.02) vs control primates (0.64 ± 0.02). However, HDL mass (lipid + protein) was similar for Control (548 ± 27 mg/dl) and Nicotine (541 ± 27) monkeys which contrasts with observations of a small, transient decline in HDL cholesterol in humans who use nicotine gum (3).

Data in Table I provide the first documentation that chronic oral nicotine intake causes an elevation in both LDL protein and cholesterol suggesting that the alkaloid promotes an increase in the entire lipoprotein particle. Subsequent examination of LDL protein revealed a single band on PAGE indicative of apoprotein B. Previous short-term studies with rabbits given oral nicotine (2) and humans who use nicotine chewing gum (3) showed increases in only LDL cholesterol.

Diminished VLDL lipid and protein in Nicotine monkeys (Table I) contrasts

TABLE I

Effect of Oral Nicotine on Lipoprotein Composition

Lipoprotein Constituent	Treatment Groups Control	Nicotine
VLDL		
Cholesterol	11±3[a]	7±2
Phospholipid	6±1	4±0.3[b]
Triglyceride	34±3	27±2[b]
Total lipid	51±4	38±3[b]
Protein	13±1	11±1[b]
Total Lipoprotein	64±4	49±3[b]
LDL		
Cholesterol	73±2	86±5[b]
Phospholipid	40±2	46±4
Triglyceride	17±2	17±2
Total lipid	130±4	150±9[b]
Protein	61±4	71±4[b]
Total Lipoprotein	191±7	221±12[b]

[a]Values represent means ± SEM for 9 monkeys/group expressed as mg/dl.

[b]Nicotine group mean significantly different ($P<0.05$) from Control mean.

with what has been reported in human smokers (17), and in squirrel monkeys following intravenous infusion of nicotine (18). However, the VLDL decrease is in keeping with our observation of a non-significant reduction in plasma triglycerides in Nicotine (69 ± 2 mg/dl) vs Control (71 ± 4) monkeys and with a human study which showed that VLDL levels decline during acute smoking episodes (3). Since Nicotine animals also had significantly lower amounts of IDL cholesterol plus protein compared to Controls (13 ± 2 mg/dl vs 20 ± 2), our data indicate that oral nicotine intake reduces circulating levels of LDL precursors in the VLDL→IDL→LDL metabolic cascade (19). Diminished VLDL and elevated LDL in Nicotine primates (Table I) is also consistent with an earlier study which suggested that high concentrations of product LDL in squirrel monkeys may inhibit hepatic secretion of precursor VLDL (20).

Alternatively, lower VLDL and IDL levels and elevated LDL in nicotine treated monkeys may be the result of enhanced lipolysis by LPL and HTGL. The former is responsible for hydrolysis of the triglyceride core of VLDL and formation of IDL in extra-hepatic tissues (19) while HTGL may contribute to the final conversion of IDL to LDL (19). Table II shows that there was a trend toward higher levels of both LPL and HTGL in Nicotine monkeys and that total postheparin lipolytic activity was significantly greater in this group. Elevated LPL has previously been reported in smokers (21) and may account for the rapid lipolysis of VLDL and subsequent increase in plasma LDL in these individuals (3). Chronic oral nicotine intake in our primates may similarly lead to elevated LDL through enhanced catabolism of VLDL and remnant particles (Tables I and II).

Besides greater production from VLDL, two other mechanisms which may account for the LDL increase in Nicotine monkeys are accelerated direct hepatic secretion and impaired clearance from the plasma compartment. Direct hepatic production of LDL has been reported in humans with familial hypercholesterolemia (19) and in normolipemic squirrel monkeys (22). Since a substantial amount of oral nicotine is absorbed in the intestine and then metabolized by hepatic microsomes (1,23), the alkaloid could enhance LDL synthesis by modifying hepatocyte smooth endoplasmic reticulum where lipoproteins are

TABLE II

Effect of Oral Nicotine on Postheparin Plasma Lipolytic Enzymes

Enzyme	Treatment Groups Control	Nicotine
Lipoprotein Lipase	22±2[a]	26±1
Hepatic Triglyceride Lipase	4±1	6±1
Total Lipase	26±2	31±1[b]

[a]Values represent means ± SEM for 7 monkeys/group expressed as μmole free fatty acid released/ml/hr.

[b]Nicotine mean significantly different ($P<0.05$) from Control mean.

manufactured prior to secretion. In this regard, chronic oral nicotine intake by rats at a dose comparable to that given to our squirrel monkeys increases hepatic microsomal enzyme systems and stimulates protein synthesis (24).

Finally, preliminary results from our laboratory suggest that oral nicotine may also delay LDL clearance from circulation and accelerate transfer of cholesteryl ester from HDL→LDL, thereby overloading the LDL particle with lipid. Consequently, oral nicotine, independent of other tobacco components, causes elevations in atherogenic LDL by multiple molecular mechanisms. Since LDL acts as a major circulating insult to the arterial wall (1), nicotine induced pathophysiological alterations in plasma lipoproteins have important health implications for humans who use smokeless tobacco products or chew nicotine gum for prolonged periods.

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