

# Lack of effect of chronic nicotine administration on fatty acid distribution in the liver, testis, and adipose tissue of male Fischer-344 rats

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**Abstract** A comparison is made of the percentage compositions of major fatty acids in liver and testis phospholipids, liver and abdominal adipose tissue triglycerides, and liver sterol esters in male Fischer-344 rats administered a physiological saline control or a "smoking" dose of nicotine (1000  $\mu\text{g}$  base/kg/day, subcutaneously) for 2 or 22 months. Results indicate that there is no major trend, or significant difference, between nicotine- or saline-treated rats with respect to major fatty acid distribution. Some differences in fatty acid distribution in the various lipid fractions were found between young and old rats.

**Supplementary key words** aging · fatty acids · phospholipids · triglycerides · sterol esters

Epidemiological studies have shown that there is a significant association between cigarette smoking and cardiovascular disease (1). However, the mechanism(s) responsible for the reported deleterious effects of tobacco smoking is not known. It is well recognized that nicotine and tobacco smoke cause release of tissue catecholamines from various storage sites (2–5). Release of catecholamines in turn causes mobilization of free fatty acids from adipose tissue to the serum (6, 7), a response which may be an important contributory factor in the development of atherosclerosis (8). In 1966, Kershbaum, Pappajohn, and Bellet (9) suggested that nicotine and tobacco smoke may cause an increased utilization of FFA, an alteration in the ratio of FFA incorporation into neutral lipid and phospholipid in plasma, and possibly a change in the FFA tissue pool ratios.

We had the opportunity to examine the effects of subacute and chronic nicotine administration on fatty acid distribution in rats that had been subjected to the daily administration of the alkaloid for 2 or 22 months (10). In these studies we measured the percentage compositions of major fatty acids of liver and testis phospholipids, liver and adipose tissue triglycerides, and liver sterol esters.

## MATERIALS AND METHODS

### Animals and animal care

The rats used comprised animals in a chronic nicotine toxicity study (10). 60 male Fischer-344 rats (Charles River Breeding Laboratories Inc., Wilmington, Mass.) weighing  $142.0 \pm 2.7$  g were initially taken. This strain of rat was selected because it has a relatively slow rate of growth. At about 8 wk of age, the rats were randomly divided into four groups as follows: control group 1 ( $n = 6$ ), control group 2 ( $n = 10$ ), nicotine group 1 ( $n = 6$ ), and nicotine group 2 ( $n = 38$ ). Animals were housed individually in hanging rat cages,  $20 \times 20 \times 27.5$  cm (Acme Metal Products, Inc., Chicago, Ill.); they were given tap water and commercial rat chow (Ralston Purina Co., St. Louis, Mo.) ad lib. The rats were weighed daily for the first 6 months of study, and thereafter once weekly. Environmental conditions were: light, 5:00 a.m.–7:00 p.m.; temperature, 68–72°F; and humidity,  $40 \pm 5\%$  RH. No smoking or chemical spraying was permitted in the animal room.

Rats were injected subcutaneously with either nicotine or physiological saline (NaCl) every day for 2 months (group 1) or 22 months (group 2). Injections were given between 4:00 and 7:00 p.m. each day. Alternate injection sites were used on the dorsal surface of the animal. Animals were fed ad lib. up to the time they were killed. Details of growth, pathology, and tissue preparation other than described here have been presented previously (10). Animals given nicotine gained significantly less weight than the corresponding control rats.

### Drugs

The solution of nicotine (1000  $\mu\text{g}$  of nicotine base/ml of 6% gelatin [w/v]) was prepared as follows. 120 g of gela-

Abbreviations: FFA, free fatty acids.

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TABLE 1. Percentages of major fatty acids in testis and liver phospholipids in control and nicotine-treated rats<sup>a</sup>

Fatty Acid	Liver				Testis			
	2-month Injection		22-month Injection		2-month Injection		22-month Injection	
	Control (5) <sup>b</sup>	Nicotine (6)	Control (5)	Nicotine (6)	Control (7)	Nicotine (9)	Control (5)	Nicotine (19)
16:0 <sup>c</sup>	19.5 ± 3.0 <sup>d</sup>	17.9 ± 3.7	17.6 ± 2.6	20.3 ± 2.0	32.6 ± 7.8	28.6 ± 4.2	18.0 ± 3.1 <sup>††</sup>	23.0 ± 7.0*
16:1	2.6 ± 0.7	3.1 ± 0.9	3.6 ± 0.5	3.5 ± 2.2	5.3 ± 2.0	3.4 ± 2.0	3.2 ± 1.1	3.0 ± 1.4
18:0	18.1 ± 4.6	23.0 ± 3.8	31.3 ± 5.2 <sup>††</sup>	24.7 ± 3.5	10.6 ± 2.0	10.3 ± 1.7	14.3 ± 2.4 <sup>**</sup>	13.4 ± 4.0*
18:1	13.2 ± 4.1	12.7 ± 3.3	11.6 ± 1.7	13.0 ± 2.1	16.3 ± 7.2	14.1 ± 2.0	16.0 ± 0.7	17.1 ± 3.5*
18:2	15.3 ± 2.7	15.2 ± 2.5	10.1 ± 1.9 <sup>†</sup>	11.9 ± 2.3*	4.5 ± 3.1	5.1 ± 2.0	8.7 ± 3.6	9.9 ± 3.0 <sup>†</sup>
20:4	29.3 ± 5.6	27.0 ± 5.4	25.8 ± 6.6	26.6 ± 1.9	16.2 ± 4.6	19.9 ± 1.8	30.3 ± 5.6 <sup>†</sup>	23.5 ± 6.5
22:5					12.2 ± 6.6	15.6 ± 3.5	4.9 ± 4.8	8.5 ± 10.9

<sup>a</sup> There were no significant differences between control and nicotine-treated groups at either 2 or 22 months. Differences between 2- and 22-month control or nicotine-treated rats, respectively, for either tissue are indicated: \* < 0.05; \*\* < 0.02; † < 0.01; †† < 0.005; ††† < 0.001.

<sup>b</sup> Number of rats per group.

<sup>c</sup> Number of carbon atoms: number of double bonds.

<sup>d</sup> Values are means ± SD.

tin (lot no. 267-17, Ruger Chemical Co., Inc., Irvington-on-Hudson, New York, N.Y.) was dissolved in approximately 1500 ml of 0.85% NaCl (w/v); a boiling water bath was used to aid solution. To this solution was added 6.148 g of nicotine hydrogen tartrate (British Drug Houses Ltd., B.D.H. Laboratory Chemicals, Poole, England). The volume was brought to 2 l with the 0.85% NaCl, and the solution was stored at 36°F in brown, stoppered bottles, each containing a 1-day supply. The pH and osmolality of the final solution were 4.40 (73°F) and 2743 mosmol/kg of water, respectively.

The physiological saline (0.85% NaCl [w/v] in 6% gelatin [w/v]) was made and stored as above. The solution was adjusted to pH 4.40 (73°F) with 5 M tartaric acid (Ruger Chemical Co.), and the osmolality was 2865 mosmol/kg of water.

### Chemical analyses

Lipids from liver slices and adipose tissue were initially extracted with chloroform-methanol 2:1 (v/v) by the method of Folch et al. (11). Subsequently, the liver extract was fractionated by thin-layer chromatography; the developing solvent was petroleum ether-anhydrous ether-acetic acid 85:15:1 (v/v/v). Methyl esters of fatty acids derived from sterol esters, phospholipids, and triglycerides of the rat liver chloroform extract and the triglycerides from the rat adipose tissue chloroform extract were prepared by methanolysis with methanol-benzene-concentrated hydrochloric acid 15:4:1 (v/v/v) (12). Methanolysis was shown to be complete by subsequent purification of the methyl esters by thin-layer chromatography.

Lipids from testis were extracted with chloroform-methanol 2:1 (v/v) by the method of Folch et al. (11). Phospholipids were then separated from neutral lipids on a silicic acid microcolumn as follows. Neutral lipids were eluted from the column with pentane-ether 1:1 (v/v). The column was then washed with ether, and the phospholipids were then eluted with methanol. Methyl esters were prepared by methanolysis as described above.

The methyl esters derived from liver and adipose tissue

triglycerides, liver and testicular phospholipids, and liver sterol esters were analyzed by gas-liquid chromatography, using a Varian Aerograph model 1200 equipped with a hydrogen flame ionization detector. A 6-ft copper column containing 14.5% Hi-Eff 2BP (Applied Science Laboratories, State College, Pa.) on 60-80 mesh Gas-Chrom G (Applied Science Laboratories) was utilized. The analyses were run at 195°C with nitrogen as the carrier gas at a flow rate of 33.0 ml/min. Reagent grade anhydrous ether (Mallinckrodt) was used in the chromatography procedures. Peaks on the chromatograms were identified by comparison of retention times with those of known standards obtained from Applied Science Laboratories. Triangulation of peak areas was used to calculate the percentage distribution of the methyl esters. The gas-liquid chromatograph was calibrated using mixtures of known percentages of fatty acid methyl esters (nos. K108 and L209) supplied by Applied Science Laboratories.

Data are presented as mean values ± 1 SD. Significance of difference between groups was determined using Student's *t* test.

## RESULTS

### Testis and liver phospholipid fatty acids

The major fatty acid compositions in testis and liver phospholipids are indicated in Table 1. There were no significant differences between control and nicotine-treated rats at either time period. These results suggest that the FFA tissue pool ratios of testis and liver phospholipids are unaltered by the administration of nicotine.

Statistical analyses of the differences in fatty acid distribution between the young and the old groups are significant in some cases. In rat liver total phospholipids, stearic acid increased and linoleic acid decreased significantly in old compared with young control animals. On the other hand, stearic acid remained unchanged and linoleic acid decreased significantly in the old compared with the young nicotine-treated animals. In rat testis total phospholipids,

TABLE 2. Percentages of major fatty acids in triglycerides in liver and adipose tissue of control and nicotine-treated rats<sup>a</sup>

Fatty Acid	Liver				Adipose Tissue			
	2-month Injection		22-month Injection		2-month Injection		22-month Injection	
	Control (6) <sup>b</sup>	Nicotine (6)	Control (5)	Nicotine (6)	Control (5)	Nicotine (6)	Control (6)	Nicotine (27)
14:0	1.4 ± 1.3 <sup>c</sup>	0.3 ± 0.5	0.7 ± 0.3	0.8 ± 0.3	1.8 ± 0.1	1.9 ± 0.5	1.2 ± 0.6	0.7 ± 0.3
16:0	31.7 ± 3.6	28.7 ± 4.3	33.0 ± 3.4	30.7 ± 4.6	29.2 ± 3.6	26.3 ± 1.0	23.8 ± 1.0†	22.8 ± 2.0†
16:1	5.4 ± 3.6	3.9 ± 1.4	3.9 ± 1.3	4.1 ± 1.3	5.2 ± 1.9	6.9 ± 1.9	4.9 ± 1.8	4.7 ± 1.5
18:0	5.9 ± 0.3	6.6 ± 0.8	6.1 ± 1.6	4.8 ± 1.8	1.4 ± 1.2	2.1 ± 1.4	2.0 ± 0.7	2.5 ± 1.0
18:1	26.8 ± 2.8	32.7 ± 6.5	31.6 ± 2.6*	30.5 ± 2.0	34.8 ± 2.0	36.1 ± 2.5	39.3 ± 1.7††	39.5 ± 2.2††
18:2	22.7 ± 3.7	21.4 ± 3.2	21.5 ± 1.6	24.7 ± 3.5	27.8 ± 1.6	26.7 ± 0.4	28.9 ± 1.4	29.9 ± 1.3‡
20:4	6.1 ± 1.7	6.3 ± 1.6	3.2 ± 1.8*	4.5 ± 2.5				

<sup>a</sup> There were no significant differences between control and nicotine-treated groups at either 2 or 22 months. Differences between 2- and 22-month control or nicotine-treated rats, respectively, for either tissue are indicated: \* < 0.025; † < 0.01; †† < 0.005; ‡ < 0.001.

<sup>b</sup> Number of rats per group.

<sup>c</sup> Values are means ± SD.

palmitic acid decreased while stearic and arachidonic acids increased significantly in old compared with young control animals. Concurrently, palmitic acid decreased and stearic, oleic, and linoleic acids increased significantly in old compared with young nicotine-treated rats.

### Liver and adipose tissue triglyceride fatty acids

The major fatty acid compositions in liver and adipose tissue are indicated in Table 2. There were no significant differences between control and nicotine-treated groups at either time period. The lack of an altered fatty acid distribution pattern suggests that the mobilization of FFA from adipose tissue after nicotine administration is uniform, so that the percentage composition of individual fatty acids is not altered.

Differences in the fatty acid distribution between the two age groups are significant. In rat liver triglycerides, oleic acid increased and arachidonic decreased in old compared with young control rats, while no significant differences in fatty acid distribution were found between the young and old nicotine-treated groups. Adipose tissue triglycerides exhibited a decrease in palmitic acid and an increase in oleic acid in the old compared with the young control groups, while palmitic acid decreased and oleic and linoleic acids increased in the old compared with the young nicotine-treated rats.

TABLE 3. Percentages of major fatty acids in liver cholesterol esters in control and nicotine-treated rats<sup>a</sup>

Fatty Acid	2-month Injection		22-month Injection	
	Control (5) <sup>b</sup>	Nicotine (5)	Control (5)	Nicotine (5)
14:0	7.8 ± 4.1 <sup>c</sup>	6.3 ± 3.0	7.2 ± 2.8	10.2 ± 2.6
16:0	31.3 ± 8.5	30.8 ± 7.5	33.5 ± 3.3	37.4 ± 10.1
16:1	5.4 ± 1.6	7.1 ± 2.1	8.7 ± 4.2	6.0 ± 1.9
18:0	10.6 ± 4.5	14.4 ± 4.5	11.2 ± 2.3	9.5 ± 2.5
18:1	34.4 ± 12.5	30.7 ± 9.7	28.1 ± 7.3	24.4 ± 10.2
18:2	10.6 ± 3.9	8.7 ± 2.2	10.3 ± 4.0	12.3 ± 5.0
20:4	0.0 ± 0	2.1 ± 2.9	1.0 ± 2.3	0.2 ± 0.5

<sup>a</sup> There were no significant differences between control and nicotine-treated groups at either 2 or 22 months, or between the two control or two nicotine-treated groups, respectively.

<sup>b</sup> Number of rats per group.

<sup>c</sup> Values are means ± SD.

### Liver cholesterol ester fatty acids

The major fatty acids present in hepatic cholesterol esters are indicated in Table 3. There were no significant differences between control and nicotine-treated rats at either time period. No significant differences in the fatty acid distribution were found in liver cholesterol esters between the young and old control or nicotine-treated groups, respectively.

### DISCUSSION

The results reported here show that subacute (2-month) or chronic (22-month) nicotine treatment in rats does not significantly alter the fatty acid distribution in testis, adipose tissue, or liver.

The absence of an alteration in the distribution of fatty acids due to nicotine is of interest, since this alkaloid has been shown to liberate FFA from adipose tissue to serum (6, 7). Thus, it seems clear that if fatty acids were liberated by nicotine in our rats, they were released in a uniform fashion. This does not agree with the hypothesis of Kershbaum et al. (9), who suggested that nicotine might produce a change in the FFA tissue pool ratios.

Hansson (13) reported that the liver, kidney, and lung could metabolize nicotine. However, the testis was not examined in Hansson's investigations, leaving doubt as to whether the testis can metabolize the alkaloid. Since the degradation of nicotine requires NADPH and also utilizes the  $\beta$ -oxidation system of the cell, fatty acid metabolism might also be altered (14, 15). An alteration in fatty acid metabolism might effect a change in the fatty acid distribution in various tissues. However, such was not found to be true.

In the sterol esters of human serum, Schrade, Biegler, and Böhle (16) demonstrated that the percentage of dienoic and tetraenoic fatty acids is somewhat lower with increased age, while the percentage of monosaturated fatty acids is slightly higher. Schrade et al. (16) found little effect on the fatty acid distribution in human serum phos-

pholipids with aging, while serum triglycerides showed a slight decrease in linoleic and arachidonic acids with age.

In the present experiment, little effect on the fatty acid distribution in rat liver cholesterol esters in young and old control or nicotine-treated groups, respectively, was found (Table 3). In rat liver triglycerides, however, oleic acid increased while arachidonic acid decreased in old compared with young control rats (Table 2) in similar fashion as in human plasma triglycerides (16). Young and old nicotine-treated rats showed no differences in oleic and arachidonic acid distribution in liver triglycerides. The reason for this discrepancy between the control and nicotine-treated rats is not known.

The finding of decreased palmitic and increased oleic acids in old compared with young rats was also noted in both control and nicotine-treated groups in adipose tissue triglycerides. However, the increase in linoleic acid in the old compared with the young nicotine-treated groups was not found between the two control groups (Table 2).

The finding (Table 1) of an increased percentage of stearic acid in liver phospholipids in old compared with young control rats was not seen in the nicotine-treated groups. The reason for this difference is not known.

Testis phospholipids (Table 1) demonstrate a more complex pattern between young and old animals. Nicotine seems to inhibit the rise in the percentage of arachidonic acid that was present between the old and young control groups. On the other hand, nicotine seems to increase the percentages of both linoleic and oleic acids in the old compared with the young rats, effects not seen between the control groups. These seemingly complex and contradictory phenomena in testis phospholipids may reflect the presence of Leydig-cell testicular tumors (found in 66% of the 22-month control rats and 89% of the 22-month nicotine-treated rats [10]) or the still maturing testis of the 4-month-old rat. **66**

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