Alterations of lipolysis and lipoprotein lipase in chronically nicotine-treated rats

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Division of Endocrinology, Gerontology, and Metabolism, Department of Medicine, Stanford University School of Medicine, Stanford 94305; Department of Veterans Affairs Medical Center, Palo Alto 94304; and Departments of Neurobiology, Physiology, and Behavior and of Nutrition, University of California, Davis, California 95616-8519

Sztalryd, Carole, Jock Hamilton, Barbara A. Horwitz, Patricia Johnson, and Fredric B. Kraemer. Alterations of lipolysis and lipoprotein lipase in chronically nicotine-treated rats. Am. J. Physiol. 270 (Endocrinol. Metab. 33): E215-E223, 1996.—These studies examined the cellular mechanisms for lower adiposity seen with nicotine ingestion. Rats were infused with nicotine or saline for 1 wk and adipocytes isolated from epididymal fat pads. Nicotine-infused rats gained 37% less weight and had 21% smaller fat pads. Basal lipolysis was 78% higher, whereas the maximal lipolytic response to isoproterenol was blunted in adipocytes from nicotine-infused rats. The antilipolytic actions of adipocytes were not associated with any changes in hormone-sensitive lipase. Nicotine caused a 30% decrease in lipoprotein lipase (LPL) activity, without any changes in LPL mass or mRNA levels, in epididymal fat in the fed state. In contrast, LPL activity, mass, and mRNA levels in heart were increased by nicotine whether animals were fed or fasted. These studies provide evidence for multiple mechanistic events underlying nicotine-induced alterations in weight and suggest that nicotine diverts fat storage away from adipose tissue and toward utilization by muscle.

hormone-sensitive lipase; adipose tissue; heart; nicotine; immunoblot; messenger ribonucleic acid

THE MEDICAL COMPLICATIONS of smoking are well known to health care professionals and to the general public at large, with over 350,000 deaths from cardiovascular disease, cancer, and chronic obstructive lung disease each year in the United States attributable to the consequences of smoking (29). Despite knowledge of the health consequences of cigarette smoking, 26% of the adults in the United States continue to smoke, and a significant percentage of adolescents begin to smoke (29). One of the immediate consequences upon cessation of smoking is weight gain. Both cross-sectional and longitudinal studies have shown that smokers weigh, on the average, 3 kg less than comparably aged nonsmokers and that individuals gain ~3 kg, and up to 8 kg or more, of weight after they stop smoking (1, 29). Indeed, the fear of gaining weight is often seen as a major obstacle for individuals who attempt to stop smoking; however, the mechanisms underlying the alterations in weight with smoking and smoking cessation are incompletely understood.

In both human studies and experimental animal models, the sustained reduction in weight observed among smokers is directly proportional to a decrease in body fat content (16, 31). On the basis of experimental animal models, this decrease in adiposity with smoking appears to be due to the effects of nicotine rather than other constituents or behaviors associated with smoking (22, 31). Analysis of the reasons for the reduction in weight with smoking or, conversely, for the increase in weight with cessation of smoking in humans has yielded somewhat conflicting and inconclusive results when alterations in energy consumption or energy expenditure have been examined. Some studies have suggested that there are increases in total energy consumption or in dietary constituents (more simple carbohydrates and fats) upon cessation of smoking (21, 29), whereas others report that energy consumption is similar or actually increased in smokers compared with nonsmokers (29). The amount of physical activity has been reported either to be no different in smokers and nonsmokers (29) or to be decreased in smokers (16, 29). Smoking or nicotine ingestion acutely increases the basal metabolic rate (14, 18, 24), which could potentially explain an increased energy expenditure in smokers. Although some studies have been able to detect increases in basal metabolic rate during long term evaluation (29), others have not (14, 25); however, a decrease in resting metabolic rate has been noted upon smoking cessation (21). Nevertheless, it has been suggested that there is an enhancement of the metabolic rate when nicotine is combined with exercise (23). This observation would explain the reduction in weight that occurs with smoking through a recurrent, albeit temporary, exaggerated increase in energy expenditure whenever a smoker exercises. Thus, although there is some controversy, the consensus among published reports suggests that the sustained reduction in weight observed among smokers or the increase in weight with cessation of smoking cannot be explained exclusively by differences between smokers and nonsmokers in caloric intake or in physical activity but is probably due, in some degree, to alterations in metabolic rate, either at rest, acutely while smoking, or with exercise.

The weight loss associated with smoking or with nicotine is due to a decrease in adiposity, which is presumably related to changes in fat cell size. Fat cell size is regulated by the balance between the synthesis and the degradation of triglycerides within adipocytes. The rate of de novo fatty acid synthesis by adipocytes is generally low (17); thus the fatty acid precursors for triglyceride synthesis are derived from cellular uptake. The synthesis of triglycerides by adipocytes is favored by high activity of lipoprotein lipase (LPL), the enzyme
that hydrolyzes circulating triglyceride-rich lipoproteins to fatty acids and thus serves as the “gatekeeper” for triglyceride uptake by adipocytes. The degradation of triglycerides within adipocytes is catalyzed by the enzyme hormone-sensitive lipase (HSL), the name of which reflects the ability of hormones such as catecholamines and adrenocorticotrophic hormone to stimulate the activity of this intracellular neutral lipase. The current studies were undertaken to explore the cellular mechanisms responsible for the lower adiposity induced by nicotine by examining the regulation of adipocyte lipolysis and the expression of LPL in rats infused with nicotine.

MATERIALS AND METHODS

Chemicals. Reagents were obtained from the following sources: collagenase (Worthington Biochemicals, Freehold, NJ); Triton X-100, l-o-phosphatidylcholine, cholesterol oleate, leupentin, aprotinin, isoproterenol, adenosine deaminase, nicotine, cotinine (Sigma Chemical, St. Louis, MO); (-)-N8- (phenylisopropyl)adenosine (PIA; Boehringer Mannheim Diagnostics, Houston, TX); heparin (Upjohn, Kalamazoo, MI); bovine serum albumin (Intergen, Purchase, NY); [9,10-3H(N)]triolein, cholesterol [14C]oleate (Du Pont de Nemours, Boston, MA); ECL Western blotting detection reagents, horseradish peroxidase linked whole antibody anti-rabbit immunoglobulin G (IgG; Amersham Life Sciences Products, Arlington Heights, IL); nitrocellulose paper (Schleicher and Schuell, Keene, NH); and oligolabeling kit (Pharmacia LKB Biotechnology, Piscataway, NJ). All other chemicals were obtained from standard commercial sources.

Animals. Male Sprague-Dawley rats weighing 180–200 g were obtained from B and K (Fremont, CA) and were maintained according to Stanford University guidelines on a 12:12-h (0600–1800) light-dark cycle. Alzet miniosmotic pumps (Alza, Palo Alto, CA) were implanted subcutaneously in animals after anesthesia with Brevital Sodium (5 mg/animal). Rats (8–10/group) were infused daily with nicotine tartrate (12 mg/kg body wt) or sodium tartrate for 1 wk. Rats were fed Purina Laboratory Chow (St. Louis, MO) and had access to water ad libitum. Some animals (8/group) were maintained individually in metabolic cages, and the amount of powdered chow consumed was daily assessed. A subset of control rats was fasted overnight before being killed. Animals were killed between 0900 and 1000 by decapitation, and aliquots for neutral cholesterol esterase activity.

Preparation of isolated adipocytes. Adipocytes were prepared from epididymal fat pads as described previously (26, 27). The fat pads were separately minced with scissors and were placed in plastic flasks in KRBH [Krebs-Ringer-bicarbonate-N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)] buffer [in (mM) 120 NaCl, 4 KCl, PO4, 1 MgSO4, 7H2O, 1 CaCl2, 10 NaHCO3, 27 HEPES, pH 7.4] containing 3% bovine serum albumin (BSA) and 2 mg collagenase/ml. Collagenase digestion was carried out at 37°C in a gyratory water bath shaker for 60 min. Cells were washed three times in KRB with 3% BSA and 2.5 mM glucose and was allowed to separate from the infranatant by floatation. A 100-μl aliquot of diluted cells was fixed in a solution of 2% osmium tetroxide in collidine buffer and was counted in a Coulter counter (Coulter Electronics, Hialeah, FL) to determine the cell number.

Measurement of lipolysis. Basal and isoproterenol-stimulated lipolysis was assessed as described previously (27). For each experiment, adipocytes from one control and two nicotine-treated rats were diluted in KRB 3% BSA 2.5 mM glucose buffer (pH 7.4), and aliquots of diluted cells were placed into plastic vials (1 × 106 cells/ml). To determine basal lipolysis, cells were incubated with adenosine deaminase (1 U/ml). Stimulated lipolysis was measured in the presence of various concentrations of isoproterenol (0–10−5 M). Incubations were carried out for 1 h at 37°C in an atmosphere of 95% air-5% CO2. At the end of the incubation, an aliquot (0.25 ml) of infranatant was removed, and aliquots (50–100 μl) were assayed in duplicate for neutral cholesterol esterase activity.

Measurement of HSL activity. Measurement of HSL activity was performed on whole fat pads and heart tissue homogenates using a cholesteryl [14C]oleate emulsion as described previously (27). Adipose tissue (0.5 g) was homogenized with a poltron in 1 ml of 50 mM tris(hydroxymethyl)amino methane (Tris)-HCl, 1 mm EDTA, and 0.2% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (wt/vol) containing 1 U/ml leupeptin. After the homogenates were centrifuged at 14,000 g for 15 min, the infranatants under the fat cake were carefully removed, and aliquots (50–100 μl) were assayed in duplicate for neutral cholesterol esterase activity.

Measurement of LPL activity. LPL activity was measured in tissue extracts using a radioenzymatic assay with a glycerol-stabilized triolein substrate emulsion as described previously (28). A 50% homogenization solution of fat or heart was prepared in Tris-HCl (pH 8.3) containing 1% BSA, heparin (10 U/ml), 0.5% deoxycholate, and 0.02% nonidet P-40. After the homogenates were centrifuged at 14,000 g for 15 min, the infranatants under the fat cake were carefully removed, and aliquots were assayed for LPL activity and protein. Specific LPL activity was defined as the difference in activity measured in the presence and absence of serum during the incubation.

Immunoblotting. The quantity of HSL and LPL was determined by immunoblotting tissue extracts. Tissue fragments from epididymal fat pads or heart were homogenized briefly with a poltron in 1 ml ice-cold lysis buffer containing 0.15 M NaCl, 3% Triton X-100, 0.1% lauryl sarcosyl, and 1 U/ml leupeptin. All homogenates were centrifuged at 14,000 g for 15 min. The infranatant below the fat cake was removed, and samples were electrophoresed on 10% polyacrylamide gels under reducing conditions and transferred onto nitrocellulose membranes as described previously (27). To detect IISL, the
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was considered significant. For all analyses, a P value < 0.05 using StatView software (ABACUS Concepts, Berkeley, CA) was considered significant difference test or by unpaired Student’s t-test. The relative amounts of immunodetectable LPL and HSL contained in each lane were determined by scanning with an LKB Ultra scan XL enhancer laser densitometer and Gel scan XL software (Pharmacia Biotechnology) on a NEC computer.

RNA isolation and measurement. Total cellular RNA was extracted from freshly isolated adipocytes by CHCl₃-phenol extraction as described previously (27). RNA pellets were dissolved in sterile water and quantified by standard ultraviolet absorbance. After denaturation with 1 M glyoxal and 50% dimethyl sulfoxide, RNA was analyzed by Northern blot hybridization after electrophoresis on 1% agarose gels. A rat LPL cDNA (a kind gift of Dr. R. Eckel, University of Colorado) and a human glyceraldehyde-3-phosphate dehydrogenase cDNA (a kind gift of Dr. R. Rabkin, Stanford University) were labeled with [32P]dCTP to a specific activity of 1-2 X 10⁹ disintegrations/min/μg with an oligolabeling kit from Pharmacia LKB Biotechnology. Prehybridization and hybridization procedures were performed as previously described. Autoradiographs were obtained by exposure to Kodak X-AR film with an intensifying screen at -80°C for 1-2 days. The autoradiographs were analyzed by scanning as previously described.

Other assays. Glucose, triglycerides, cholesterol, and free fatty acids were measured as previously described (28). Cotinine levels were measured by gas chromatography in the Biochemistry laboratory of the Stanford Center for Research in Disease Prevention. Serum catecholamines were determined by high pressure liquid chromatography (19). Protein was measured with a bicinchoninic acid protein assay kit (Pierce Chemicals, Rockford, IL).

Statistical analysis. Data are expressed as means ± SE. Statistical analyses were performed by analysis of variance and comparisons among groups by Fisher’s protected least significant difference test or by unpaired Student’s t-test using StatView software (ABACUS Concepts, Berkeley, CA) on a Macintosh II computer. For all analyses, a P value < 0.05 was considered significant.

RESULTS

Body weight, food consumption, and serum values. Infusion of rats with nicotine (12 mg/kg) or sodium tartrate carrier via Alza miniosmotic pumps for 1 wk led to serum cotinine (the major metabolite of nicotine) levels of 790 ± 29 and 0 ± 0 ng/ml in nicotine-infused and control rats, respectively, values that are very similar to those achieved in humans during smoking (14). The control and nicotine-infused rats were of comparable weights at the time of implantation of the pumps; however, the nicotine-infused rats weighed significantly less than the control rats after 1 wk of nicotine infusion because they gained 31% less weight (39.8 ± 6.1 vs. 55.9 ± 6.4 g; P < 0.03) than the controls (Table 1), even though the two groups continued to consume similar quantities of food daily (30 ± 0.5 vs. 31 ± 1 g rat chow; P = not significant). This decreased weight gain was reflected by an -20% decrease in the wet weights of epididymal fat pads (1.25 ± 0.07 vs. 1.54 ± 0.08 g; P < 0.06) of the nicotine-infused rats (Table 1). The decrease in weight of the epididymal fat pads resulted from a 36% reduction in fat cell size (P < 0.005) without any change in adipose cell numbers (data not shown). Thus, despite a daily food consumption similar in both groups, the nicotine-infused rats have a reduction in adiposity and a reduced fat cell size. Although there was a weight differential between the nicotine-infused and control rats, there were no differences in the concentrations of serum glucose (7.8 ± 0.2 vs. 8.0 ± 0.2 mmol/l), triglyceride (1.59 ± 0.5 vs. 1.34 ± 0.15 mmol/l), cholesterol (1.55 ± 0.05 vs. 1.66 ± 0.05 mmol/l), or free fatty acid (568 ± 38 vs. 572 ± 32 μM/l) between the groups, respectively. In addition, there were no differences in serum norepinephrine (1.35 ± 0.16 vs. 1.34 ± 0.12 ng/ml) or epinephrine concentrations (2.75 ± 0.33 vs. 2.78 ± 0.36 ng/ml) between the control and nicotine-infused rats. Thus the lower weight gain in the nicotine infused rats was not due to elevated levels of catecholamines.

Measurement of oxygen consumption. Because the decrease in adiposity induced by nicotine treatment occurred without differences in food consumption, the question was raised whether an elevated resting metabolic rate might be responsible for the decrease in adiposity. To determine the effects of nicotine on oxygen consumption, a separate group of 10 rats (5 control and 5 nicotine-infused rats) was studied (Table 2). Total body mass, carcass mass (carcass mass is the body mass minus internal viscera and included all adipose tissue except mesenteric fat), and brown adipose tissue mass were significantly diminished in the nicotine-infused rats; however, there was no difference in oxygen consumption measured at either temperature studied or when adjusted for body mass. These results suggest either that nicotine infusion does not influence the overall basal metabolic rate in these rats or that any differences that might have existed were too small to be detected in this experimental setting.

Table 1. Effects of nicotine on weight and adiposity

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial Wt, g</th>
<th>Final Wt, g</th>
<th>Weight Gain, g</th>
<th>Daily Food Intake, g</th>
<th>Epididymal Fat Pads, g</th>
<th>Fat Cell Size, mg/10⁶ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>236 ± 6</td>
<td>283 ± 7</td>
<td>55.9 ± 6.4</td>
<td>31 ± 1</td>
<td>1.54 ± 0.08</td>
<td>0.59 ± 0.02</td>
</tr>
<tr>
<td>Nicotine</td>
<td>12</td>
<td>229 ± 6</td>
<td>262 ± 4</td>
<td>35.5 ± 6.1</td>
<td>30 ± 0.5</td>
<td>1.20 ± 0.07</td>
<td>0.38 ± 0.01</td>
</tr>
</tbody>
</table>

Results are means ± SE; n, no. of animals. Rats were infused with sodium tartrate (control) or 12 mg/kg nicotine tartrate via Alza miniosmotic pumps for 1 wk. Food was weighed each evening and morning to determine intake, after which animals were weighed, killed, and epididymal fat was removed for study. *P < 0.05 compared with control; †P < 0.005 compared with control.
Lipolysis. To examine the mechanisms responsible for the reduction of fat cell size with nicotine infusion, lipolysis was studied in isolated adipocytes from the experimental groups. When cells were incubated in the presence of adenosine deaminase to eliminate the influence of endogenously released adenosine, basal lipolysis was 78% higher (48.6 ± 9.0 vs. 27.3 ± 3.7 nmol glycerol released·h⁻¹·10⁸ cells⁻¹; P < 0.05) in adipocytes isolated from nicotine-infused rats compared with controls (Fig. 1). Thus a chronic infusion of nicotine into rats almost doubles basal lipolysis, substantiating a potentially important mechanism to explain any increases in basal metabolic rate observed with nicotine. Moreover, evidence for further derangement in lipolysis associated with nicotine infusion was observed when the ability of isoproterenol to stimulate glycerol release was examined in adipocytes isolated from nicotine-infused or control rats (Fig. 2). The response of adipocytes isolated from nicotine-infused rats was markedly different (P < 0.001) from that of adipocytes from control rats. Although increased glycerol release was seen basally and at 10⁻¹⁰ M isoproterenol (P < 0.05) in cells from nicotine-infused rats, the lipolytic responses to 10⁻⁷ (P < 0.001) and 10⁻⁵ M (P < 0.01) isoproterenol were markedly blunted (~30% less). Thus isoproterenol increased glycerol release ~16.5-fold above basal in control adipocytes, whereas cells from nicotine-infused rats were stimulated only ~5.5-fold. Even in view of this marked difference in maximal velocity (Vmax), the Michaelis constant (Km) for isoproterenol was similar in the two groups (~2 nM), suggesting that the sensitivity of the cells to isoproterenol, and thus the number of β-adrenergic receptors, was unaltered by nicotine and that a postreceptor change may likely be responsible for the decrease in Vmax. Although these data support a nicotine-induced derangement in basal lipolysis and the response to fast-acting lipolytic agents, the next experiments explored whether nicotine affected the response to antilipolytic agents. To investigate the response to antilipolytic agents, the ability of a nonmetabolizable analogue of adenosine, PIA, to inhibit lipolysis in adipocytes from control and nicotine-infused rats was compared. Adipocytes isolated from control and nicotine-infused rats were stimulated with 10⁻⁷ M isoproterenol in the presence of adenosine deaminase to eliminate endogenous adenosine and with increasing concentrations of PIA. Nicotine treatment did not have any effect on the ability of PIA to inhibit isoproterenol-stimulated lipolysis in the presence of adenosine deaminase (Fig. 3), suggesting that nicotine does not influence adenosine receptors or their signaling pathways in adipocytes.

**Table 2. Effects of nicotine on basal oxygen consumption**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Mass, g</th>
<th>Carcass Mass, g</th>
<th>BAT, g</th>
<th>Oxygen Consumption, ml O₂/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>225 ± 5</td>
<td>173 ± 4</td>
<td>0.23 ± 0.01</td>
<td>3.60 ± 0.19</td>
</tr>
<tr>
<td>Nicotine</td>
<td>203 ± 7*</td>
<td>156 ± 6*</td>
<td>0.17 ± 0.03*</td>
<td>3.21 ± 0.17</td>
</tr>
</tbody>
</table>

Results are means ± SE; n, no. of animals. BAT, brown adipose tissue. Rats were infused with sodium tartrate (control) or 12 mg/kg nicotine tartrate via Alza miniosmotic pumps for 1 wk. Rats were fasted overnight and placed into metabolic chambers at 21–23°C at 0700. Oxygen consumption was initiated 30 min later and was monitored continuously for 1 h, after which time temperature was increased to 25–27°C, and measurements of oxygen consumption were continued for an additional hour. *P < 0.05 compared with control.

Fig. 1. Effects of nicotine on basal lipolysis. Adipose cells were isolated from rats that were infused either with sodium tartrate (control) or with nicotine tartrate (12 mg/kg) for 7 days. Cells were incubated for 1 h in presence of adenosine deaminase (1 U/ml), and amount of glycerol released was assessed. Results are means ± SE of triplicate measurements and are representative of 6 separate experiments. *P < 0.05 compared with control.

Fig. 2. Effects of nicotine on isoproterenol-stimulated lipolysis. Adipose cells were isolated from rats that were infused either with sodium tartrate (control) or with nicotine tartrate (12 mg/kg) for 7 days. Cells were incubated for 1 h in presence of varying concentrations of isoproterenol, and amount of glycerol released was assessed. Results are means ± SE of triplicate measurements and are representative of 6 separate experiments. *P < 0.05 compared with control; †P < 0.001 compared with control; ‡P < 0.01 compared with control.
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Fig. 3. Effects of nicotine infusion on adenosine-mediated inhibition of lipolysis. Adipose cells were isolated from rats that were infused either with sodium tartrate (control) or with nicotine tartrate (12 mg/kg) for 7 days. Cells were stimulated with 10^-7 M isoproterenol in presence of adenosine deaminase to eliminate endogenous adenosine and with increasing concentrations of phenylisopropyl adenosine (PIA), and amount of glycerol released was assessed. Maximal lipolysis was set at 100%. Results are means ± SE of triplicate measurements and are representative of 3 separate experiments. 

Fig. 4. Effects of nicotine on human-sensitive lipase (HSL) activity (A) and mass (B) in adipose tissue. Adipose cells were isolated from rats that were infused either with sodium tartrate (control) or with nicotine tartrate (12 mg/kg) for 7 days. Cell extracts were assayed for neutral cholesterol esterase activity (A) or immunoblotted with anti-HSL/fusion protein immunoglobulin G (IgG; B), as described in MATERIALS AND METHODS. Con, control rats; Nic, nicotine-infused rats.

Fig. 5. Effects of nicotine on lipoprotein lipase (LPL) activity in adipose tissue. Adipose cells were isolated from rats that were infused either with sodium tartrate (control) or with nicotine tartrate (12 mg/kg) for 7 days. Fed rats were killed at 0800 h and fasted rats at 1600 h. Cells were assayed for LPL activity as described in MATERIALS AND METHODS. Results are means ± SE of septuplicate measurements and are representative of 3 separate experiments. *P < 0.05 compared with control.

Fig. 6. Effects of nicotine on LPL mass (A) and mRNA levels (B) in adipose tissue. Epididymal fat pads were obtained from fed rats that were infused either with sodium tartrate (control) or with nicotine tartrate (12 mg/kg) for 7 days. LPL mass (A) was determined by immunoblotting with chicken anti-bovine LPL IgG, as described in MATERIALS AND METHODS; LPL mRNA levels (B) were determined by Northern analysis as described in MATERIALS AND METHODS. LPL mRNA levels are expressed relative to glyceraldehyde-3-phosphate dehydrogenase mRNA. Results are means ± SE of septuplicate measurements and are representative of 3 separate experiments.

Adipose cells isolated from control and nicotine-infused animals. No changes in HSL activity could be detected (Fig. 4A; 24.8 ± 3.2 vs. 21.3 ± 1.9 nmol·h^-1·mg protein^-1 in nicotine-infused and control animals, respectively). In addition, no differences in the amount of HSL immunoreactive protein could be detected between the two groups (Fig. 4B). Thus it seems unlikely that the significant reduction in adiposity observed in the nicotine-infused rats could be completely explained by changes in HSL expression and lipolysis.

LPL expression in adipose tissue. Fat cell size is determined by the balance between the accretion of cellular triglyceride and the hydrolysis of stored triglyceride. Although our initial studies documented alterations in the hydrolysis of stored triglyceride in nicotine-infused rats, we next examined the effects of nicotine on the expression of LPL, the enzyme that serves as the gatekeeper for triglyceride uptake by adipocytes. When the animals were killed in the morning in the fed state (Fig. 5), LPL activity in epididymal fat was reduced by 10.2 ± 0.3 vs. 9.87 ± 0.84 U/mg protein; P < 0.03); however, when the animals were fasted overnight before death, LPL activity in epididymal fat was reduced equally in both the control and nicotine-infused rats. As an attempt to address the mechanism underlying the decrease in LPL activity in adipose tissue with nicotine infusion, the amount of LPL immunoreactive mass and the steady-state levels of LPL mRNA were assessed (Fig. 6). No differences were observed in LPL immunoreactive mass (Fig. 6A) or in the steady-state levels of LPL mRNA (Fig. 6B) between the two groups.

LPL expression in heart. LPL is produced not only by adipose tissue but also by heart in which LPL expression allows the uptake of triglycerides to be oxidized for energy (3). To explore whether nicotine infusion altered the expression of LPL in heart, LPL activity, mass, and mRNA levels were determined in the hearts of control and nicotine-infused rats. When animals were killed in...
the morning in the fed state (Fig. 7), LPL activity in heart was increased ~80% in the nicotine-infused rats (0.81 ± 0.10 vs. 0.44 ± 0.03 U/mg protein; P < 0.005). Although fasting increased LPL activity in both the control and nicotine infused animals (Fig. 7), LPL activity in nicotine-infused animals was still elevated ~25% above control (P < 0.05). As an attempt to address the mechanism underlying the increase in LPL activity in the heart with nicotine infusion, the amount of LPL immunoreactive mass and the steady-state levels of LPL mRNA were assessed (Fig. 8). Both LPL immunoreactive mass (Fig. 8A) and LPL mRNA levels (Fig. 8B) were increased in parallel to the elevation of LPL activity (P < 0.05).

DISCUSSION

The current studies were undertaken to understand the cellular mechanisms responsible for the lower adiposity observed with nicotine by examining the regulation of adipocyte lipolysis and the expression of LPL in rats infused with nicotine. Our results clearly show that a continuous infusion of nicotine into rats for 1 wk, which achieves serum cotinine levels (the major metabolite of nicotine) of approximately the same degree as observed with cigarette smoking in humans (14), significantly blunts the amount of weight gained by rats. Moreover, this lack of weight gain is due to a decrease in adiposity that occurs without any alterations in food consumption. These results confirm earlier reports in both human studies and experimental animal models that showed that the sustained reduction in weight observed among smokers is directly proportional to a decrease in body fat content (16, 29, 31). Because the reduction in weight and adiposity is seen without any changes in food consumption, our findings support the notion that changes in weight with nicotine are due to alterations in energy expenditure; however, we were unable to directly document any changes in basal metabolic rate due to nicotine treatment. Previous reports have been inconsistent in their ability to document long-term changes in energy expenditure in smokers, but most studies have observed increases in basal metabolic rates acutely during smoking or nicotine ingestion (14, 18, 24, 29). Furthermore, some investigators have suggested that any enhancement of the metabolic rate with nicotine occurs primarily when combined with exercise (23). Although we were unable to detect any differences in resting metabolic rate with nicotine infusion, it is possible that any nicotine-induced increases in basal metabolic rate might have been below the level of sensitivity of the method used or that nicotine-induced increases in metabolic rate occurred primarily during physical activity, which was not assessed in the current studies.

We found that the reduction in adiposity reflects a decrease in fat cell size. Because fat cell size is regulated by the balance between lipolytic and lipogenic pathways, we examined changes in these metabolic pathways. We observed a marked increase in the rate of basal lipolysis in adipocytes from nicotine-infused rats. These findings are similar to the recent observation that acute, but not chronic, smoking in humans increases lipolysis and reesterification of fat when analyzed by stable isotopes and indirect calorimetry; however, these changes in lipolysis could only account for a small (15%) portion of the increase in energy expenditure observed (14). In addition to an alteration in the rate of basal lipolysis with nicotine infusion, we found that the response of adipocytes from nicotine-infused rats to isoproterenol stimulation is also markedly attenuated. One potential explanation for these results could be that smoking or nicotine ingestion acutely increases plasma catecholamine levels (7). Thus nicotine-induced elevations in catecholamines could have increased basal lipolysis, whereas desensitization to chronic elevations of catecholamines (30) would result in a blunted response to acute isoproterenol stimulation. This, however, was not the case since plasma catecholamine levels were unchanged by nicotine infusion in the current studies, consistent with previous
observations in which catecholamine levels were unaffected by chronic nicotine administration in rats (13). Additionally, because the $K_m$ for isoproterenol stimulation of glycerol release is similar in control and nicotine-infused rats, it appears that the sensitivity of the cells to isoproterenol is unaltered by nicotine, suggesting that the number of $\beta$-adrenergic receptors was unchanged. To explore possible mechanisms underlying the changes in basal and isoproterenol-stimulated lipolysis with nicotine infusion, we measured the activity and quantity of HSL, the rate-limiting enzyme in lipolysis. We were unable to detect any changes in either HSL activity or the amount of immunoreactive HSL protein. The activity of HSL is regulated acutely via phosphorylation-dephosphorylation reactions, with adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (A-kinase) phosphorylating HSL and resulting in an increase in hydrolytic activity (34). In addition to A-kinase, HSL is phosphorylated by AMP-activated protein kinase at a secondary site that appears to impair phosphorylation and thus activation by A-kinase (34). Dephosphorylation of HSL appears to be mediated primarily by protein phosphatases-2A and -2C (33). No phosphatase inhibitors were present during homogenization of tissue in the current experiments since most phosphatase inhibitors interfere with the measurement of HSL activity (12). Thus these experimental conditions favor dephosphorylation and decreased HSL activity, making it possible that the measured HSL activity represents an underestimation of basal activity in situ that could have obscured differences in HSL activity. Nevertheless, the combination of the nicotine-induced increase in the rate of basal glycerol release with the blunted lipolytic response to isoproterenol and an invariant amount of immunoreactive HSL protein suggests that these alterations are due to posttranslational regulation of HSL via phosphorylation-dephosphorylation events. This conclusion is further supported by our observation that adenosine-mediated inhibition of isoproterenol-stimulated lipolysis, which occurs via regulation of cAMP production (15), was unaltered by nicotine; however, it is possible that nicotine might have affected the amount of adenosine released by adipocytes.

Although our results demonstrating effects of nicotine on lipolysis could potentially explain a portion of the decreased adiposity seen with smoking or nicotine administration, it was important to examine whether changes in lipogenesis also occurred that could contribute to the decrease in adiposity. Because the de novo synthesis of fatty acids is low in adipocytes (17), the expression of LPL is the primary means through which triglyceride uptake into adipocytes for storage is controlled (9). LPL is known to be regulated by multiple mechanisms. Evidence exists for the regulation of the rate of transcription or the processing or stability of LPL mRNA (2), the efficiency of or rate of translation of LPL, the half-life of the protein, or the posttranslational control of the active form of the enzyme (2, 8, 28). In the current studies, LPL activity was significantly diminished in adipocytes from nicotine-infused rats in the fed state, although LPL activity was equally low in control and nicotine-infused rats in the fasted state. Although this diminution of LPL activity expressed per milligram protein may be partially explained by a decrease in fat cell size, LPL activity expressed per milligram tissue was also diminished (results not shown). Interestingly, no changes in the amount of LPL protein or LPL mRNA levels could be detected, suggesting that LPL activity under these conditions is regulated posttranslationally. Previous reports on the effects of nicotine on the activity of LPL in adipose tissue have been conflicting and difficult to interpret. The first report examining LPL activity in smokers found an increase in the fasting activity of adipose tissue LPL in smokers compared with nonsmokers (4). Other investigators have been unable to detect differences in fasting LPL activity between smokers and nonsmokers whether the data were expressed per fat cell number or per weight of fat (5, 10). Later studies again noted fasting adipose tissue LPL activity to be similar in smokers and nonsmokers when expressed per fat cell number, but the expected rise of LPL activity after a glucose load was blunted in smokers compared with nonsmokers (6), suggesting that the glucose/insulin-mediated regulation of LPL is defective in smokers. These results are similar to our observation of alterations in adipose tissue LPL activity only in the fed state and are particularly intriguing when considered within the context of the recent observation that cigarette smoking is associated with insulin resistance (11). Thus these findings imply that alterations in the regulation of LPL activity (perhaps mediated through abnormal insulin action) might occur with smoking or nicotine, which could possibly explain part of the reduction in fat cell mass with smoking or nicotine.

LPL is produced not only by adipose tissue but also by muscles in which LPL expression allows the uptake of triglycerides to be oxidized for energy (3). Although LPL activity differs among various muscle types, with high activities observed in cardiac and slow-twitch skeletal muscles and low activities in fast-twitch skeletal muscles, LPL activity among these different muscle groups tends to be regulated in parallel and in an inverse relationship with adipose tissue (3). When the effects of nicotine on LPL in heart were examined, a large increase in LPL activity was observed in nicotine-infused rats, which was exaggerated in the fed state. As opposed to adipose tissue in which the decrease in LPL activity by nicotine administration appears to be mediated via posttranslational mechanisms, the increase in LPL activity in heart was paralleled by increases in the amount of LPL protein and LPL mRNA levels, suggesting pretranslational control. No other studies have examined the effects of nicotine on LPL expression in muscle; however, the recent observations of Hellerstein et al. (14) are pertinent to our results. These investigators reexamined the issue of energy expenditure in smokers using stable isotopes and indirect calorimetry to try to evaluate the mechanism for any changes. They observed an ~50% increase in whole body fat oxidation in smokers vs. individuals who were abstaining; however, this increase in whole body fat oxidation was not
affected acutely by smoking in these subjects while they were fasting (14). To the extent that changes in LPL activity in cardiac muscle reflect parallel changes in skeletal muscle (3), one can speculate that, in the fed state, the nicotine-induced decreases in adipose tissue LPL combined with the nicotine-induced increases in muscle LPL that we observe would tend to divert fat storage away from adipose tissue and toward utilization, i.e., oxidation, by muscle, thus possibly causing the decrease in adiposity and contributing to the increase in whole body fat oxidation. Because fasting causes substantial changes in the expression of LPL in both adipose tissue and muscle (8), the alterations in LPL expression induced by nicotine are much less pronounced during fasting and would not be expected to influence these processes further.

In conclusion, our current studies suggest that the chronic administration of nicotine has dramatic effects on lipolytic and lipogenic pathways and that these effects are exaggerated in the fed state. Thus nicotine infusion increases the rate of basal lipolysis while decreasing LPL activity in adipose tissue. Both of these changes favor the production of smaller fat cells by simultaneously stimulating the breakdown of stored triglyceride from the fat cell and inhibiting the uptake of triglyceride by the fat cell. At the same time, infusion of nicotine increases the expression of LPL by muscle (cardiac and, by inference, skeletal), allowing more triglyceride to be taken up by these tissues. Thus these studies provide evidence that there are multiple mecha-nistic events underlying nicotine-induced alterations in weight and suggest that nicotine diverts fat storage away from adipose tissue and toward utilization by muscle.

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