

# Effects of Low- and High-Nicotine Cigarette Smoking on Mood States and the HPA Axis in Men

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The acute effects of smoking a low- or high-nicotine cigarette on hypothalamic–pituitary–adrenal (HPA) hormones, subjective responses, and cardiovascular measures were studied in 20 healthy men who met American Psychiatric Association Diagnostic and Statistical Manual IV criteria for nicotine dependence. Within four puffs (or 2 min) after cigarette smoking began, plasma nicotine levels and heart rate increased significantly ( $P < 0.01$ ), and peak ratings of 'high' and 'rush' on a Visual Analogue Scale were reported. Reports of 'high,' 'rush,' and 'liking' and reduction of 'craving' were significantly greater after smoking a high-nicotine cigarette than a low-nicotine cigarette ( $P < 0.05$ ). Peak plasma nicotine levels after high-nicotine cigarette smoking ( $23.9 \pm 2.6$  ng/ml) were significantly greater than after low-nicotine cigarette smoking ( $3.63 \pm 0.59$  ng/ml) ( $P < 0.001$ ). After smoking a low-nicotine cigarette, adrenocorticotropin hormone (ACTH), cortisol, dehydroepiandrosterone (DHEA), and epinephrine did not change significantly from baseline. After high-nicotine cigarette smoking began, plasma ACTH levels increased significantly above baseline within 12 min and reached peak levels of  $21.88 \pm 5.34$  pmol/l within 20 min. ACTH increases were significantly correlated with increases in plasma nicotine ( $r = 0.85$ ;  $P < 0.0001$ ), DHEA ( $r = 0.66$ ;  $P = 0.002$ ), and epinephrine ( $r = 0.86$ ;  $P < 0.0001$ ). Cortisol and DHEA increased significantly within 20 min ( $P < 0.05$ ) and reached peak levels of  $424 \pm 48$  and  $21.13 \pm 2.55$  ng/ml within 60 and 30 min, respectively. Thus cigarette smoking produced nicotine dose-related effects on HPA hormones and subjective and cardiovascular measures. These data suggest that activation of the HPA axis may contribute to the abuse-related effects of cigarette smoking.

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## INTRODUCTION

Cigarette smoking remains a leading cause of death and disease and is associated with over 440 000 premature deaths each year (CDC, 2000). Despite increasing social and legal pressures to restrict tobacco use, an estimated 46 million adults smoke cigarettes in the United States (CDC, 2004). Evidence that implicates cigarette smoking in respiratory and cardiovascular disease continues to accumulate (USDHHS, 2004). The 2004 Surgeon General's Report on the health effects of smoking identifies additional cancers as well as several other diseases that are now thought to be causally related to cigarette smoking (USDHHS, 2004). Although awareness of these health risks has reduced the overall prevalence of cigarette smoking, relapse rates during and after treatment are very high (Jaffe, 1990).

Cigarette smoking is generally acknowledged to be an addictive disorder characterized by tolerance and physical dependence (Jaffe, 1990; APA, 1994; Henningfield *et al*, 1995; Benowitz, 1996; CDC, 2001). Nicotine appears to be the primary addictive agent in cigarette smoking, and i.v. nicotine is self-administered by humans and experimental animals under a number of conditions (Goldberg *et al*, 1981; Spealman and Goldberg, 1982; Henningfield and Goldberg, 1983; Corrigan, 1999; Donny *et al*, 1999; Harvey *et al*, 2004). With nicotine, as with many abused drugs, speed of the injection is an important determinant of relative reinforcing efficacy (Balster and Schuster, 1973; Wakasa *et al*, 1995; Abreu *et al*, 2001). The reinforcing effects of nicotine are mediated, in part, by stimulating nicotinic acetylcholine receptors on mesolimbic dopamine neurons to release dopamine (Fu *et al*, 2000; Gerasimov *et al*, 2000; Watkins *et al*, 2000). Nicotine also stimulates rapid release of adrenocorticotropin hormone (ACTH) in rodents following i.p. or i.c.v. administration and microinjection into catecholaminergic regions, and these effects are blocked by the nicotine antagonist mecamylamine (Sharp and Beyer, 1986; Matta *et al*, 1990, 1993b, 1998). Hypothalamic corticotropin-releasing hormone (CRH) cannot be measured in venous blood, but one index of CRH activation is the release of ACTH from the anterior pituitary, followed by

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an increase in cortisol in humans and corticosterone in rodents. There is also evidence from preclinical studies that nicotine activates CRH neurons in extrahypothalamic regions that mediate responses to stress (Matta *et al*, 1993a, 1997).

Although the interactions between the hypothalamic–pituitary–adrenal (HPA) axis and the abuse-related effects of drugs are poorly understood, HPA axis activation is thought to contribute to drug abuse at several phases of the addictive process (Koob and Le Moal, 2001; Sinha, 2001; Marinelli and Piazza, 2002; Contoreggi *et al*, 2003; Heinrichs and Koob, 2004). For example, it has been suggested that high CRH levels may contribute to the anxiety and irritability often associated with nicotine withdrawal (Watkins *et al*, 2000). It is generally agreed that CRH stimulation of ACTH is one component of an integrated physiological response to ‘stress’ (Nemeroff, 1996; Tsigos and Chrousos, 2002). Yet paradoxically, stimulation of ACTH is also a consistent effect of a highly reinforcing drug, cocaine. Increases in ACTH are temporally concordant with reports of positive subjective effects by cocaine abusers (Mendelson *et al*, 1992, 2002; Mello and Mendelson, 2002). Although these opposite subjective effects of HPA activation seem counterintuitive, it has been suggested that control of a predictable stressor may diminish its aversive effects (Goeders, 2002a,b). Cocaine, skydiving, roller coaster riding, and similar risk-taking behaviors may fall into this category (Mendelson *et al*, 2002). BF Skinner’s concept of a reinforcer as anything that maintains behavior leading to its self-administration provides a useful semantic description of these behaviors.

Several lines of evidence suggest that stimulation of CRH, as indicated by increases in ACTH and cortisol, may contribute to the reinforcing effects of cocaine (Goeders, 1997, 2002a,b; Mello and Mendelson, 2002; Mendelson *et al*, 2002). For example, the adrenal hormone corticosterone is critically important for the acquisition and maintenance of cocaine self-administration in rats (Goeders and Guerin, 1996; Mantsch *et al*, 2000). Consistent with the hypothesis that ACTH and corticosterone may contribute to cocaine’s reinforcing effects, administration of a CRH-1 antagonist decreased *i.v.* cocaine self-administration by rats, with minimal effects on food-maintained responding (Goeders and Guerin, 2000). CRH antagonists are also associated with alleviation of stress-induced anxiety (Habib *et al*, 2000; Ayala *et al*, 2004). Yet, in rhesus monkeys, with normal glucocorticoid levels, drug-induced increases in ACTH did not appear necessary to maintain cocaine self-administration (Broadbear *et al*, 1999a,b).

The relevance of these studies of cocaine to nicotine is suggested by the several similarities between the abuse-related and hormonal effects of these drugs. Both cocaine and nicotine produce rapid onset, positive subjective effects of relatively brief duration (Jones *et al*, 1999; Mendelson *et al*, 2003; Sobel *et al*, 2004). Both cocaine and nicotine activate the mesolimbic dopamine system and increase extracellular dopamine levels, although by different mechanisms (Kuhar *et al*, 1991; Watkins *et al*, 2000). In addition to similar subjective and cardiovascular effects, both *i.v.* cocaine and cigarette smoking produced rapid increases in luteinizing hormone (LH) that were significantly correlated with the rapid increases

in plasma levels of cocaine and nicotine (Mendelson *et al*, 2003).

The present study was designed to explore the interactions between HPA axis hormones and the abuse-related effects of cigarette smoking. There has been relatively little attention to the temporal covariance between the endocrine, cardiovascular, and subjective effects of cigarette smoking. Early clinical endocrine studies of the hormonal effects of cigarette smoking were limited by the lack of sensitive and precise radioimmunoassays (RIAs) for ACTH (Pomerleau *et al*, 1983). Moreover, samples for ACTH and/or cortisol analysis usually were collected at relatively infrequent intervals, or as single daily samples (Seyler *et al*, 1984, 1986; Baron *et al*, 1995; Pickworth and Fant, 1998; del Arbol *et al*, 2000), and plasma nicotine levels were seldom measured. Because ACTH is released from corticotropes in the anterior pituitary in rapid bursts of short duration (3–10 min) (Iranmanesh *et al*, 1990), frequent blood sample collection is important to measure accurately drug effects on hormone release patterns (Teoh *et al*, 1994; Sarnyai *et al*, 1995). Moreover, rapid sample collection is essential to assess the temporal relationship between anterior pituitary and adrenal hormones, cardiovascular measures, and the subjective reports during cigarette smoking.

One goal of this study was to assess the temporal covariance between reports of subjective effects, changes in anterior pituitary and adrenal hormones, plasma nicotine levels, and cardiovascular measures *during* as well as *after* cigarette smoking. A second goal was to determine if cigarette smoking under controlled conditions produces a rapid perturbation of the HPA axis in nicotine-dependent men, similar to the effects of nicotine in rodents (Sharp and Beyer, 1986; Matta *et al*, 1990, 1993b). A third goal was to determine if the subjective, hormonal, and cardiovascular effects of cigarette smoking were nicotine dose-related. The effects of smoking a low- and high-nicotine cigarette were compared under identical experimental conditions, and all dependent measures were sampled at 2 min intervals during and immediately following cigarette smoking.

## MATERIALS AND METHODS

### Subjects

A total of 20 healthy adult men were recruited through newspaper advertisements and provided written informed consent for prestudy screening procedures and for participation in this study. The study was approved by the Institutional Review Board of the McLean Hospital. All men fulfilled American Psychiatric Association Diagnostic and Statistical Manual (DSM IV) criteria for current nicotine dependence (305.1). Volunteers with any lifetime DSM-IV Axis I disorder other than nicotine dependence were excluded. Men who were seeking treatment for nicotine dependence, or who were wearing a nicotine patch were also excluded. All men selected for this study were in good physical health and had normal medical and laboratory screening examinations. Subjects in the high- and low-nicotine groups did not differ significantly with respect to age, years of smoking, number of cigarettes smoked per day, and body mass index (BMI) (Table 1). A total of 10 men were randomly assigned to smoking a high-nicotine

**Table 1** Subject Characteristics

	Age (years)	Years of smoking	Cigarettes smoked/day	BMI (KG/M2) ( $\bar{x} \pm SE$ )	Baseline CO levels	Baseline plasma nicotine levels
High-nicotine cigarette group (N = 10)	25.6 $\pm$ 1.1	6.1 $\pm$ 0.7	16.5 $\pm$ 1.0	24.1 $\pm$ 0.6	3.4 $\pm$ 0.8 ppm	1.61 $\pm$ 0.20 ng/ml
Low-nicotine cigarette group (N = 10)	25.2 $\pm$ 0.8	5.0 $\pm$ 0.3	15.0 $\pm$ 0.5	24.8 $\pm$ 0.4	3.7 $\pm$ 0.7 ppm	1.20 $\pm$ 0.18 ng/ml
ANOVA results	P = 0.77 (df = 1, F = 0.87)	P = 0.12 (df = 1, F = 2.73)	P = 0.16 (df = 1, F = 2.15)	P = 0.34 (df = 1, F = 0.96)	P = 0.71 (df = 1, F = 0.76)	P = 0.15 (df = 1, F = 2.1)

Demographic data, CO levels, and baseline plasma nicotine levels were analyzed using a one-factor ANOVA.

cigarette and 10 men were assigned to smoking a low-nicotine cigarette. A between-subjects design was used, because it was difficult to recruit nicotine-dependent men under 35 years of age. Moreover, an interval of at least 5 weeks between successive studies was required because of the volume of blood withdrawn for plasma nicotine and hormone analyses. It is usually difficult to maintain contact with subjects over this period.

The study procedures were explained during initial screening and again on the study day, and any questions or concerns were discussed. Subjects were admitted to the clinical research ward on the morning of the study day, and all studies were conducted at the same time of day. Subjects were asked to provide a urine specimen for drug testing, and levels of breath carbon monoxide (CO) and breath alcohol were measured. Subjects were told that they would be asked to smoke cigarettes with a low- or high-nicotine content under controlled conditions. After the study was completed, subjects remained on the clinical research ward for 2 or more hours. Lunch was provided and vital signs were measured at 30 min intervals. When subjects were medically stable and comfortable, they were discharged and transportation was provided. Subjects were paid for their participation in the study, consistent with NIH regulations.

### Smoking Abstinence Requirements

Cigarette smokers were asked to abstain from cigarettes and caffeinated beverages after midnight on the night before the study. CO levels were measured with a Vitalograph Breath CO Monitor (Vitalograph Inc., Lenexa, KS 66215) to assess compliance with smoking abstinence requirements. Cigarette smokers with a CO level above 10 ppm were not allowed to participate in the study and, as shown in Table 1, average CO levels were below 4 ppm. It is important for subject safety, as well as to avoid confounding of the dependent variables, to ensure that subjects have not used any drugs before cigarette smoking. On the morning of each study day, urines were collected and analyzed with a Triage<sup>®</sup> screen. The Triage<sup>®</sup> Panel for Drugs of Abuse (Biosite Diagnostics, San Diego, CA) is a rapid multiple immunoassay system for the qualitative detection of the major metabolites of these drugs of abuse in urine at the following cutoff concentrations as recommended by the Substance Abuse and Mental Health Services Administration: phencyclidine 25 ng/ml, benzodiazepines 300 ng/ml, benzoyllecgonine, a metabolite of cocaine, 300 ng/ml, amphetamines

1000 ng/ml, tetrahydrocannabinol 50 ng/ml, opiates 300 ng/ml, and barbiturates 300 ng/ml.

### Nicotine Dose Selection

A commercially available, high-nicotine cigarette and a low-nicotine cigarette were studied. The high-nicotine cigarette (Marlboro Red, Phillip Morris brand) contained 15.48 mg of nicotine and 16 mg of tar based on analysis by the Massachusetts Department of Public Health (MDPH, 1998). In our previous studies, smoking a Marlboro Red cigarette under the conditions described below produced peak plasma nicotine levels above 20 ng/ml (Mendelson *et al*, 2003). The low-nicotine cigarettes contained 1.1 mg of nicotine and 2.8 mg of tar based on analyses provided by the manufacturer. We refer to this cigarette as low nicotine rather than as denicotinized, because most 'denicotinized' cigarettes contain trace amounts of nicotine (<0.06–0.16 mg) (Pickworth *et al*, 1999; Shahan *et al*, 1999; Robinson *et al*, 2000). Low-nicotine cigarettes were acquired from Murty Pharmaceuticals Inc. (Lexington, KY 40509).

### Nicotine Administration Procedures

These studies were carried out on a clinical research ward. Subjects were studied in a semisupine position, and cigarettes were administered using a controlled smoking procedure designed to standardize puff volume and duration of inhalation (Griffiths *et al*, 1982). Every 30 s, subjects were asked to take one puff from a cigarette, hold the smoke for 5 s, and exhale at the end of 5 s. Subjects took 24 puffs over a 12 min smoking period with an interpuff interval of 25 s. This is equivalent to approximately two cigarettes. A new lighted cigarette was presented after every four puffs so that changes in cigarette length would not influence puff duration (Nemeth-Coslett and Griffiths, 1984a, b).

### Cardiovascular Measures and Safety Precautions

Heart rate, blood pressure, and electrocardiograms (EKG) were continuously monitored with a Hewlett-Packard EKG monitor (Model 78 352A) for 10 min prior to cigarette smoking and for 2 h following cigarette administration. A physician certified in cardiopulmonary resuscitation was present during each study, and a cardiac defibrillator and

appropriate emergency treatment medications were located in the study room.

### Sample Collection Procedures

Baseline samples for analysis of ACTH, cortisol, dehydroepiandrosterone (DHEA), epinephrine, and plasma nicotine levels were collected 10 min before the onset of cigarette smoking, and then at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 80, and 120 min following the onset of smoking a high- or low-nicotine cigarette. This rapid sampling frequency was based upon our previous observations that nicotine levels in plasma increase rapidly after smoking begins and reach peak levels within 14 min (Mendelson *et al*, 2003). The catheter site and collection tubes were covered during the smoking studies so that smoke in the air would not contaminate plasma samples. Blood samples for hormone analysis and for nicotine analysis were collected in vacutainer tubes without preservative. All samples were placed on ice immediately, centrifuged and plasma or serum was removed and frozen at  $-70^{\circ}\text{C}$  for nicotine and hormone analyses.

### Subjective Effects Measures

Subjects rated their reactions to smoking on a Visual Analogue Scale (VAS) that ranged from zero to 100. Subjects were asked to rate how 'high' they felt, if they felt a 'rush,' how much they 'liked' nicotine, and how much they 'craved' cigarettes. Subjects were also asked to rate feelings of 'sick,' 'jittery,' 'bad feeling,' 'dizzy,' and 'alert.' Subjects were asked to rate these subjective effects before smoking and every 2 min for the first 20 min after cigarette smoking began, and then at 25, 30, 40, 50, 60, 70, 80, and 120 min following smoking onset.

### Assay Procedures

**Adrenocorticotropin hormone.** Plasma ACTH concentrations were measured in duplicate using an immunoradiometric assay (IRMA) kit purchased from Nichols Institute (San Juan Capistrano, CA). The assay sensitivity was 0.37 pmol/l and the intra- and interassay CVs were 3.9 and 5.6%, respectively.

**Cortisol.** Plasma cortisol was determined in duplicate by the GammaCoat RIA method, using kits purchased from DIASORIN Corporation (Stillwater, MN). The assay sensitivity was 5.5 nmol/l and the intra- and interassay CVs were 5.9 and 9.6%, respectively.

**Dehydroepiandrosterone.** Plasma DHEA concentrations were determined in duplicate by Coated Tubes RIA method using kits purchased from Diagnostic Systems Laboratories Inc. (Webster, TX). The assay sensitivity was 0.4 ng/ml and intra- and interassay CVs were 5.8 and 10.2%, respectively.

**Epinephrine.** Plasma epinephrine concentrations were measured in duplicate by a double antibody RIA method, on previously extracted and acylated samples, using kits purchased from American Laboratory Products Company (Windham, NH). The assay sensitivity was 7.6 pg/ml

and the intra- and interassay CVs were 4.3 and 6.0%, respectively.

**Plasma nicotine analysis.** Plasma nicotine levels were measured in duplicate using a gas chromatography-mass spectrometry method described by Jacob *et al* (2000). The nicotine assay sensitivity was 1.0 ng/ml and the intra- and interassay CVs were 4.7 and 11.5%, respectively. Nicotine assays were conducted in the laboratory of Dr Peton Jacob III at the University of California at San Francisco.

### Data Analysis

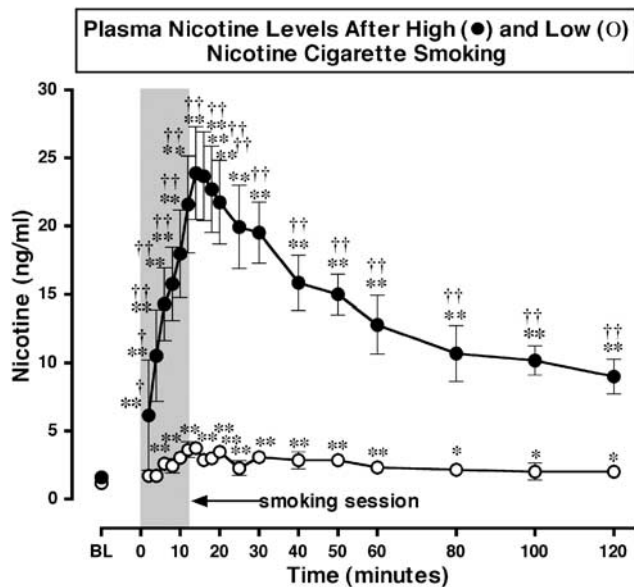
Comparisons between the subjective and physiological effects of high- and low-nicotine cigarettes were analyzed with ANOVA for repeated measures. Within each group, changes in each dependent variable from baseline were assessed with a one-factor repeated measures ANOVA. If significant main effects were detected, one-way ANOVAs were performed to determine which points differed significantly from baseline. Plasma nicotine levels, hormone levels, subjective measures, and cardiovascular parameters in the low- and high-nicotine groups were analyzed using a two-factor (dose group  $\times$  time) repeated measures ANOVA with time postsmoking as the repeated factor. If significant main effects were detected, one-way ANOVAs were performed to identify the time points that differed significantly between the two dose groups. The statistical significance of temporal covariance between ACTH, cortisol, DHEA and epinephrine and plasma nicotine levels were determined by Pearson's product moment correlation analyses. The statistical significance of the temporal covariance between subjective measures and heart rate was also evaluated with a Pearson's product moment correlation analyses.

Estimates of time to peak plasma concentrations ( $T_{\text{max}}$ ), and half-life ( $T_{1/2}$ ) of plasma nicotine and hormone data were calculated with GraphPad Prism software (version 4, San Diego, CA) by fitting the equation for one-phase exponential decay to the data. These pharmacokinetic parameters were analyzed with an ANOVA to determine whether there were any significant differences between plasma nicotine and HPA hormones after smoking a high-nicotine cigarette. Low-nicotine cigarette smoking produced minimal changes in HPA axis hormones, so pharmacokinetic analyses were not performed.

## RESULTS

### Plasma Nicotine Levels (Figure 1 and Table 1)

There were no significant differences between groups in baseline nicotine levels or baseline CO levels (Table 1). Plasma nicotine levels increased significantly within 2 min, or four puffs on a high-nicotine cigarette, and remained significantly above baseline throughout the 120 min sampling period ( $P = 0.009\text{--}0.0001$ ). Peak plasma nicotine levels of  $23.90 \pm 2.68$  ng/ml were detected within 14 min, and then plasma nicotine levels gradually decreased to  $9.01 \pm 1.25$  ng/ml at the end of the sampling period. Plasma nicotine levels also increased significantly above baseline levels after smoking a low-nicotine cigarette ( $P = 0.03\text{--}0.0003$ ). Peak



**Figure 1** Plasma nicotine levels after smoking a low- or high-nicotine cigarette. Plasma nicotine levels after smoking a high-nicotine cigarette (filled circles) and a low-nicotine cigarette (open circles) are shown on the left ordinates. Time (min) is shown on the abscissae. Points above BL were collected 10 min before cigarette smoking began at time 0. The 12 min cigarette smoking period is indicated by a gray rectangle. Each data point is the average ( $\pm$  SEM) of 10 subjects. Statistical analyses indicated significant changes in plasma nicotine levels from baseline in both low- ( $df=18$ ,  $F=6.96$ ,  $P=0.03$ ) and high- ( $df=18$ ,  $F=21.8$ ,  $P<0.0001$ ) nicotine cigarette groups. Significant changes from the presmoking baseline are indicated by asterisks (\* $P<0.05$ ; \*\* $P<0.01$ ). Daggers indicate points at which plasma nicotine levels after smoking a low-nicotine cigarette differed from plasma nicotine levels after smoking a high-nicotine cigarette ( $^{\dagger}P<0.05$ ;  $^{\ddagger}P<0.01$ ) ( $df=1$ ,  $F=23.9$ ,  $P=0.0004$ ).

plasma nicotine levels of  $3.63 \pm 0.59$  ng/ml were detected within 12 min after low-nicotine cigarette smoking began. At the end of the sampling period, plasma nicotine levels averaged  $2.02 \pm 0.31$  ng/ml. Plasma nicotine levels were significantly higher after high-nicotine cigarette smoking than after low-nicotine cigarette smoking throughout the 120 min sampling period ( $P=0.05$ – $0.0001$ ) (Figure 1).

Pharmacokinetic analysis of plasma nicotine levels confirmed that the  $C_{max}$  was significantly higher after smoking a high-nicotine cigarette than after smoking a low-nicotine cigarette ( $P<0.0001$ ). However, there were no significant differences in the time to reach maximum plasma levels of nicotine ( $T_{max}$ ) or in the nicotine half-life ( $T_{1/2}$ ) after low- and high-nicotine cigarette smoking. The  $T_{max}$  was  $15.2 \pm 1.5$  min and  $14.6 \pm 1.2$  min after smoking low- and high-nicotine cigarettes, respectively. The  $T_{1/2}$  was  $49.8 \pm 13.3$  and  $62.9 \pm 11.5$  min after smoking low- and high-nicotine cigarettes, respectively.

#### Hypothalamic–Pituitary–Adrenal Hormones after Low- and High-Nicotine Cigarette Smoking (Figure 2 and Table 2)

Baseline levels of ACTH, cortisol, epinephrine, and DHEA did not differ significantly before low- and high-nicotine cigarette smoking. However, high-nicotine cigarette smoking produced rapid and significant increases in all four

measures. Low-nicotine cigarette smoking did not alter ACTH or epinephrine levels, but both cortisol and DHEA levels decreased after smoking. The temporal profiles of these changes are described separately for each measure.

#### Adrenocorticotropin Hormone Levels after Low- and High-Nicotine Cigarette Smoking (Figure 2)

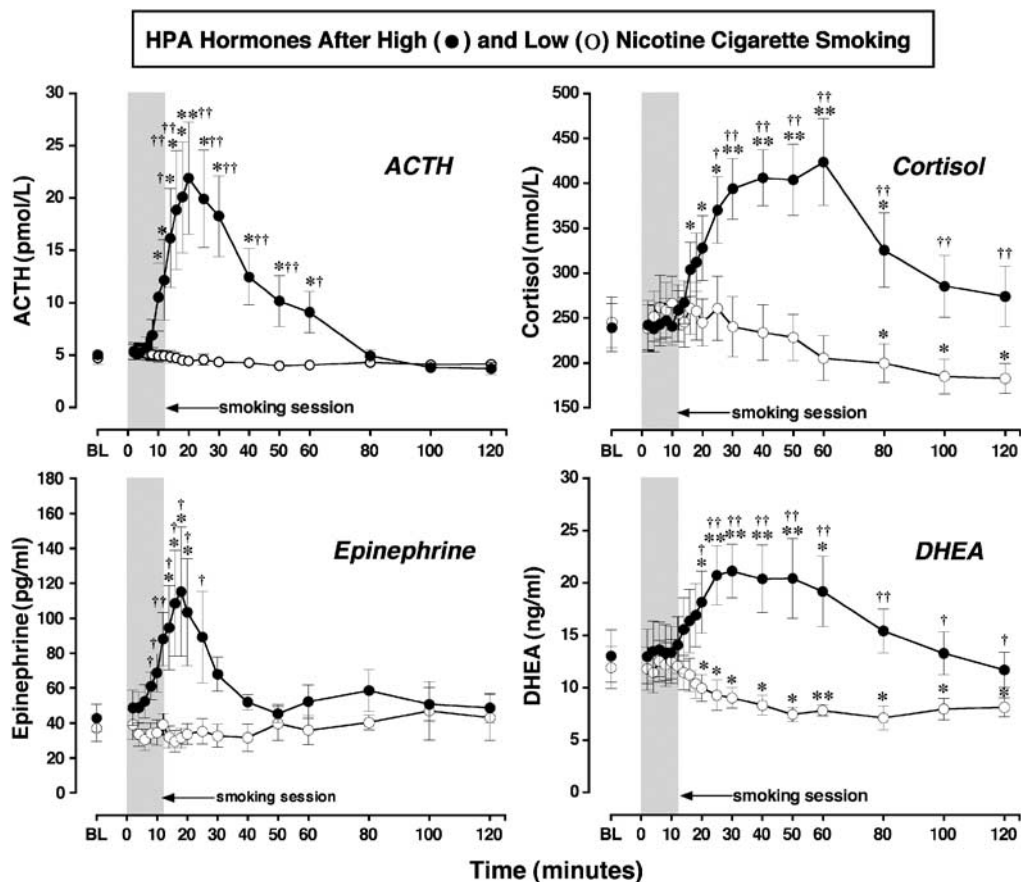
Before smoking began, average baseline ACTH levels were equivalent in the low- and high-nicotine cigarette groups. After low-nicotine cigarette smoking, ACTH levels did not change significantly from baseline, and were significantly lower than after high-nicotine cigarette smoking between 14 and 60 min ( $P=0.029$ – $0.002$ ). After smoking a high-nicotine cigarette, ACTH levels increased significantly above baseline within 12 min and peak ACTH levels of  $21.88 \pm 5.34$  pmol/l were measured at 20 min when plasma nicotine levels averaged  $21.76 \pm 2.10$  ng/ml. The increase in ACTH was significantly correlated with the increase in plasma nicotine levels shown earlier in Figure 1 ( $r=0.85$ ;  $P<0.0001$ ). ACTH remained significantly higher than baseline for 60 min ( $P=0.048$ – $0.008$ ). At 80 min, ACTH levels were equivalent in the low- and high-nicotine groups.

#### Cortisol Levels after Low- and High-Nicotine Cigarette Smoking (Figure 2)

Baseline cortisol levels were equivalent before low- and high-nicotine cigarette smoking. Cortisol levels decreased significantly from baseline at 80, 100, and 120 min after smoking a low-nicotine cigarette ( $P=0.03$ – $0.02$ ), and were significantly lower than after high-nicotine cigarette smoking between 25 and 120 min ( $P=0.016$ – $0.0003$ ). Cortisol increased significantly within 18 min and remained significantly above baseline for 80 min ( $P=0.05$ – $0.006$ ). Cortisol reached peak levels of  $424 \pm 48$  nmol/l within 60 min, that is, 40 min after peak ACTH levels were detected.

#### Dehydroepiandrosterone Levels after Low- and High-Nicotine Cigarette Smoking (Figure 2)

Baseline DHEA levels were equivalent before low- and high-nicotine cigarette smoking. After low-nicotine cigarette smoking, DHEA levels decreased significantly from baseline within 20 min and remained below baseline throughout the study ( $P=0.043$ – $0.007$ ). DHEA levels were significantly lower after low-nicotine cigarette smoking than after high-nicotine cigarette smoking. Significant differences in DHEA levels between the low- and high-nicotine cigarette groups were detected at 20 min and persisted throughout the sampling period ( $P=0.03$ – $0.0003$ ). After high-nicotine cigarette smoking, DHEA increased significantly above baseline within 20 min, and peak levels of  $21.13 \pm 2.55$  ng/ml were detected at 30 min. DHEA remained significantly above baseline until 60 min after high-nicotine cigarette smoking ( $P=0.044$ – $0.005$ ). Increases in DHEA and cortisol were significantly correlated ( $r=0.92$ ;  $P<0.0001$ ). DHEA was also significantly correlated with plasma nicotine levels ( $r=0.46$ ;  $P=0.049$ ).



**Figure 2** ACTH, cortisol, DHEA, and epinephrine levels after smoking a low- or high-nicotine cigarette. Hormone levels after smoking a high-nicotine cigarette (filled circles) and a low-nicotine cigarette (open circles) are shown on the left ordinates. Time (min) is shown on the abscissae. Points above BL were collected 10 min before cigarette smoking began at time 0. The 12 min cigarette smoking period is indicated by a gray rectangle. Each data point is the average ( $\pm$ SEM) of 10 subjects. Statistical analyses indicated significant changes from baseline in the levels of ACTH ( $df=18$ ,  $F=8.8$ ,  $P=0.005$ ), epinephrine ( $df=18$ ,  $F=3.9$ ,  $P=0.05$ ), DHEA ( $df=18$ ,  $F=8.7$ ,  $P=0.0006$ ), and cortisol ( $df=18$ ,  $F=9.0$ ,  $P=0.002$ ) after high-dose nicotine. ACTH (pmol/l) and epinephrine (pg/ml) are shown in the left column; cortisol (nmol/l) and DHEA (ng/ml) are shown in the right column. Asterisks indicate points that were significantly different from baseline (\* $P<0.05$ ; \*\* $P<0.01$ ). Daggers indicate points at which hormone levels were significantly different after high-nicotine cigarette smoking than after low-nicotine cigarette smoking († $P<0.05$ ; †† $P<0.01$ ); ACTH ( $df=1$ ,  $F=7.0$ ,  $P=0.016$ ), epinephrine ( $df=1$ ,  $F=5.8$ ,  $P=0.026$ ), DHEA ( $df=1$ ,  $F=4.8$ ,  $P=0.048$ ), and cortisol ( $df=1$ ,  $F=5.5$ ,  $P=0.03$ ).

**Table 2** Time Course of Nicotine, ACTH, Epinephrine, Cortisol, and DHEA after High-Nicotine Cigarette Smoking

	$T_{max}$ (min)	$T_{1/2}$ (min)
Nicotine (ng/ml)	$14.6 \pm 1.2$	$62.9 \pm 11.5$
ACTH (pmol/l)	$20.9 \pm 1.2^{***}$	$15.8 \pm 1.6^{**}$
Epinephrine (pg/ml)	$22.3 \pm 6.9$	$19.1 \pm 8.1^{**}$
Cortisol (nmol/l)	$47.0 \pm 4.2^{***}$	$29.7 \pm 10.5$
DHEA (ng/ml)	$37.0 \pm 3.9^{***}$	$24.0 \pm 9.3^*$

Significant differences from plasma nicotine are indicated by asterisks: \* $P=0.01$ ; \*\* $P=0.001$ ; \*\*\* $P<0.001$ .

### Epinephrine Levels after Low- and High-Nicotine Cigarette Smoking (Figure 2)

Baseline epinephrine levels were equivalent before low- and high-nicotine cigarette smoking. Epinephrine levels did not change significantly from baseline after low-nicotine cigarette smoking, and were significantly lower than after high-

nicotine cigarette smoking between 8 and 25 min after smoking began ( $P=0.047-0.008$ ). At the end of the sampling period, epinephrine averaged  $48.6 \pm 8.4$  and  $43.0 \pm 13.1$  pg/ml after high- and low-nicotine cigarette smoking, respectively. Epinephrine began to increase immediately during high-nicotine cigarette smoking and levels were significantly above baseline within 14 min ( $P=0.043$ ). Peak epinephrine levels of  $115.2 \pm 37.0$  pg/ml were detected at 18 min after smoking began ( $P=0.048$ ) and epinephrine remained significantly above baseline for 8 min, that is, until 20 min after smoking began. The increases in epinephrine were significantly correlated with the increases in ACTH ( $r=0.86$ ;  $P<0.0001$ ). Epinephrine was also significantly correlated with plasma nicotine levels ( $r=0.83$ ;  $P<0.0001$ ) after high-nicotine cigarette smoking.

### Comparison of the Time Course of Plasma Nicotine, ACTH, Epinephrine, and DHEA (Table 2)

Pharmacokinetic analyses indicated that after smoking a high-nicotine cigarette, nicotine reached maximum plasma

levels ( $T_{\max}$ ) significantly faster than ACTH, cortisol, and DHEA ( $P < 0.0001$ ). The half-life ( $T_{1/2}$ ) of plasma nicotine was significantly longer than the half-life of ACTH, epinephrine, or DHEA ( $P < 0.01-0.001$ ). Taken together with Figures 1 and 2, these data suggest that the rapid increase in plasma nicotine levels stimulates ACTH and epinephrine release. Cortisol and DHEA began to increase during the ascending limb of the increase in ACTH and reached peak levels within 16–26 min after peak levels of ACTH were detected.

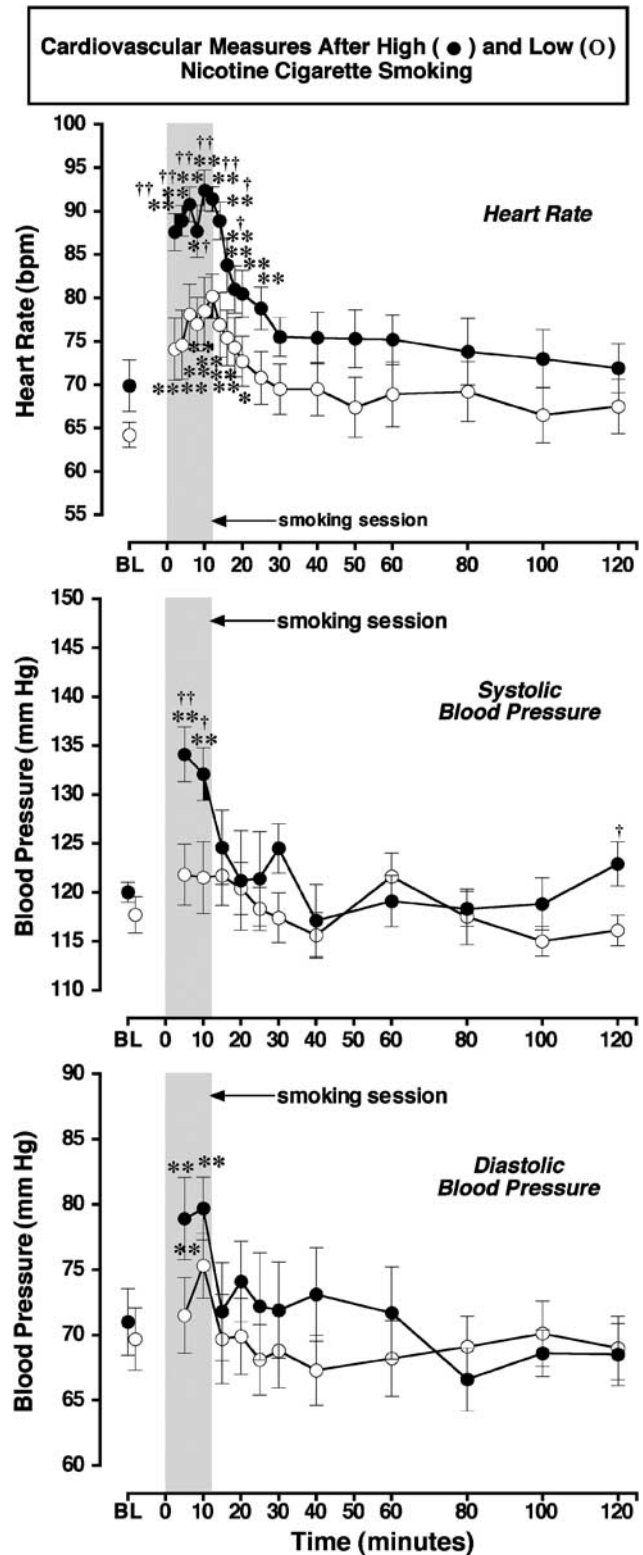
### Cardiovascular Effects of Low- and High-Nicotine Cigarette Smoking (Figure 3)

**Heart rate.** There were no significant differences in heart rate between groups before smoking began. Heart rate increased significantly within 2 min after cigarette smoking began in both nicotine dose groups ( $P < 0.0001-0.0005$ ). However, heart rate was significantly higher after the high-nicotine cigarette smoking than after the low-nicotine cigarette smoking between 2 and 16 min ( $P = 0.048-0.001$ ). After high-nicotine cigarette smoking, peak heart rates averaging  $92.4 \pm 2.3$  bpm were detected within 10 min ( $P < 0.0001$ ), and heart rate remained significantly above baseline for 30 min ( $P = 0.032-0.0001$ ). After low-nicotine cigarette smoking, peak heart rates of  $80.2 \pm 2.6$  bpm were detected within 12 min ( $P < 0.002$ ). Heart rate remained significantly above baseline levels for 20 min ( $P = 0.037-0.0003$ ).

**Systolic blood pressure.** Baseline systolic blood pressure did not differ before low- and high-nicotine cigarette smoking. Systolic blood pressure increased significantly from baseline at 5 and 10 min after smoking a high-nicotine cigarette ( $P = 0.002-0.0005$ ), but there were no significant changes after smoking a low-nicotine cigarette. Systolic blood pressure was significantly higher after smoking a high- than a low-nicotine cigarette at 5 and 10 min after smoking began ( $P = 0.03-0.009$ ).

**Diastolic blood pressure.** Baseline diastolic blood pressure did not differ before low- and high-nicotine cigarette smoking. Diastolic blood pressure increased significantly

from baseline at 5 and 10 min after high-nicotine cigarette smoking began ( $P = 0.001-0.0005$ ) and at 10 min after low-nicotine cigarette smoking began ( $P = 0.003$ ), but there were no significant differences in diastolic blood pressure between the two groups.

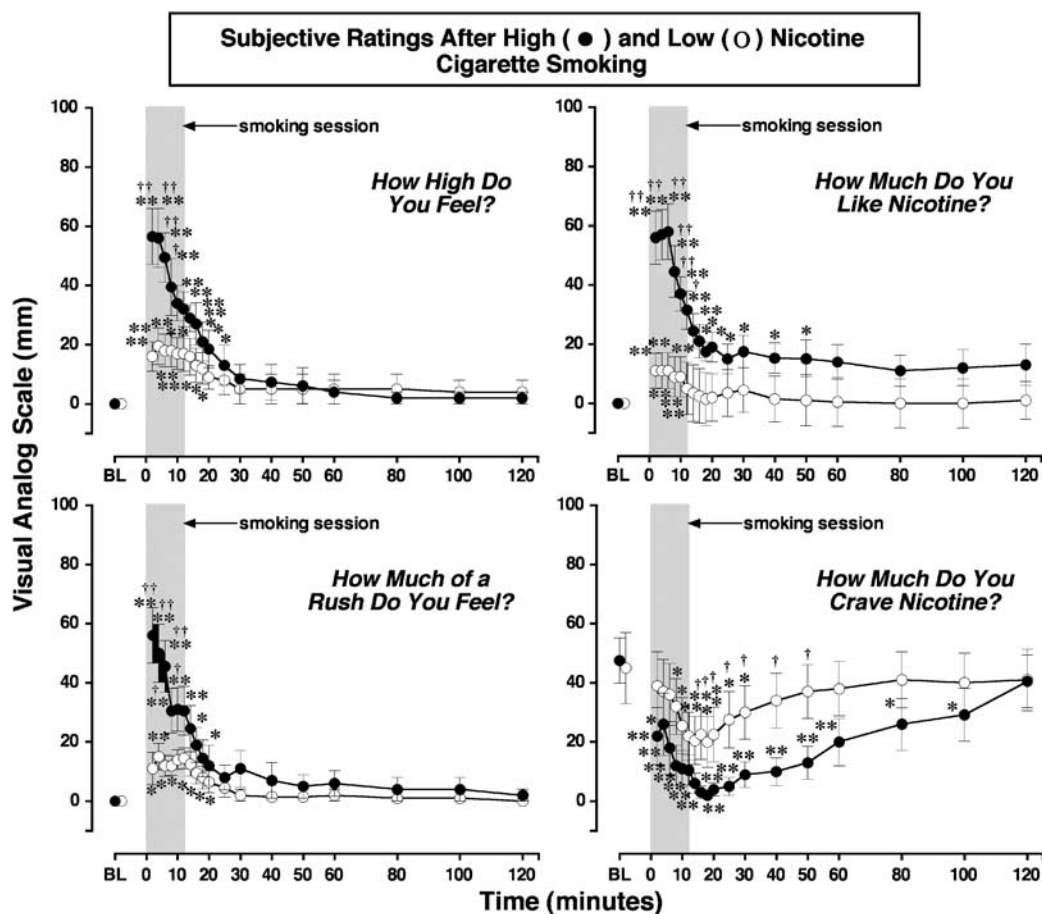


**Figure 3** Cardiovascular measures after smoking a low- or high-nicotine cigarette. Heart rate (bpm) and systolic and diastolic blood pressure (mm/Hg) after smoking a high-nicotine cigarette (filled circles) and a low-nicotine cigarette (open circles) are shown on the left ordinates in the first, second, and third rows, respectively. Time (min) is shown on the abscissae. Points above BL were collected 10 min before smoking began at time 0. The 12 min cigarette smoking period is indicated by a gray rectangle. Each data point is the average ( $\pm$  SEM) of 10 subjects. Statistical analyses indicated significant changes from baseline in heart rate ( $df = 18$ ,  $F = 18$ ,  $P < 0.0001$ ), systolic blood pressure ( $df = 11$ ,  $F = 6.1$ ,  $P = 0.001$ ), and diastolic blood pressure ( $df = 11$ ,  $F = 7.0$ ,  $P = 0.0006$ ) after high-nicotine cigarettes. Asterisks indicate points that were significantly different from baseline ( $*P < 0.05$ ;  $**P < 0.01$ ). Daggers indicate points that were significantly different after high-nicotine cigarette smoking than after low-nicotine cigarette smoking ( $^{\dagger}P < 0.05$ ;  $^{\dagger\dagger}P < 0.01$ ); heart rate ( $df = 1$ ,  $F = 6.3$ ,  $P = 0.021$ ), systolic blood pressure ( $df = 1$ ,  $F = 5.9$ ,  $P = 0.026$ ), and diastolic pressure ( $df = 1$ ,  $F = 5.8$ ,  $P = 0.03$ ).

### Subjective Effects of Low- and High-Nicotine Cigarette Smoking (Figure 4)

VAS ratings on four subjective effect measures during and after smoking a low- or a high-nicotine cigarette are shown in Figure 4. Baseline levels of these subjective measures did not differ significantly between the high- and low-nicotine cigarette groups. When high-nicotine cigarette smoking began, VAS ratings of 'high,' 'rush,' and 'nicotine liking' increased significantly within the first 2 min or four puffs, and reached peak levels within 4–6 min. These VAS ratings were significantly intercorrelated during the first 20 min after smoking began ( $r = 0.96$ – $0.98$ ;  $P < 0.0001$ ). Increases in ratings of 'high,' 'rush,' and 'nicotine liking' were also significantly correlated with increases in heart rate ( $r = 0.86$ – $0.88$ ;  $P < 0.0001$ ). After low-nicotine cigarette smoking, the VAS ratings of 'high,' 'rush,' and 'nicotine liking' also increased significantly ( $P = 0.002$ – $0.026$ ), but these increases were significantly less than after high-nicotine cigarette smoking ( $P < 0.05$ – $0.001$ ).

In contrast, VAS ratings of 'craving' for nicotine were high in both groups before cigarette smoking began, and decreased significantly during smoking. High-nicotine cigarette smoking decreased 'craving' scores significantly more than low-nicotine cigarette smoking between 16 and 50 min after smoking began ( $P = 0.05$ – $0.032$ ). 'Craving' ratings decreased to a nadir of  $2.0 \pm 1.3$  mm within 18 min after high-nicotine cigarette smoking began, and remained significantly below baseline for 100 min ( $P < 0.03$ – $0.0003$ ). 'Craving' ratings decreased to a nadir of  $20.0 \pm 8.6$  mm within 16 min after low-nicotine cigarette smoking began and remained significantly below baseline for 30 min ( $P < 0.02$ – $0.003$ ). There were no significant changes from baseline in VAS ratings of 'sick,' 'jittery,' 'bad feeling,' and 'alert,' in either the high- and low-nicotine cigarette groups (data not shown). The high-nicotine cigarette group reported significantly higher ratings of dizziness than the low-nicotine cigarette group during the first 8 min after smoking began ( $P < 0.01$ ) (data not shown).



**Figure 4** Reports of subjective effects after smoking a low- or high-nicotine cigarette. Subjective ratings on a VAS (0–100) are shown on the left ordinates and time (min) is shown on the abscissae. Points above BL were collected 10 min before smoking began at time 0. Each data point is the average ( $\pm$  SEM) of 10 subjects. The 12 min cigarette smoking period is indicated by a gray rectangle. Asterisks indicate points that were significantly different from baseline ( $*P < 0.05$ ;  $**P < 0.01$ ). Statistical analyses indicated significant changes from baseline in reports of 'high' after high-nicotine cigarettes ( $df = 18$ ,  $F = 16.9$ ,  $P < 0.0001$ ) and low-nicotine cigarettes ( $df = 18$ ,  $F = 5.2$ ,  $P = 0.01$ ), reports of 'liking' after high-nicotine cigarettes ( $df = 18$ ,  $F = 14.1$ ,  $P < 0.0001$ ) and low-nicotine cigarettes ( $df = 18$ ,  $F = 3.9$ ,  $P = 0.05$ ), reports of 'rush' after high-nicotine cigarettes ( $df = 18$ ,  $F = 13.2$ ,  $P < 0.0001$ ) and low-nicotine cigarettes ( $df = 18$ ,  $F = 5.1$ ,  $P = 0.009$ ), and reports of 'craving' after high-nicotine cigarettes ( $df = 18$ ,  $F = 8.1$ ,  $P = 0.0007$ ) and after low-nicotine cigarettes ( $df = 18$ ,  $F = 5.6$ ,  $P = 0.007$ ). Daggers indicate points that were significantly different after high-nicotine cigarette smoking than after low-nicotine cigarette smoking ( $^{\dagger}P < 0.05$ ;  $^{\ddagger}P < 0.01$ ); 'high' ( $df = 1$ ,  $F = 4.5$ ,  $P = 0.049$ ), 'like' ( $df = 1$ ,  $F = 6.2$ ,  $P = 0.023$ ), 'rush' ( $df = 1$ ,  $F = 6.3$ ,  $P = 0.02$ ), and 'craving' ( $df = 1$ ,  $F = 5.6$ ,  $P = 0.04$ ).



## DISCUSSION

We believe that this is the first study to use rapid sampling procedures to examine the covariance between endocrine, cardiovascular, and subjective measures both during and after cigarette smoking. Cigarette smoking produced nicotine dose-dependent increases in anterior pituitary and adrenal hormones as well as cardiovascular and subjective responses in nicotine-dependent men. The low-nicotine cigarette did not increase pituitary or adrenal hormones, and increases in heart rate and VAS ratings of positive subjective effects were significantly lower than after smoking a high-nicotine cigarette. Although smoking behavior, defined in terms of duration of cigarette exposure and number and duration of puffs, was equivalent for high- and low-nicotine cigarettes, nicotine plasma levels were significantly higher after smoking high-nicotine cigarettes. Thus, it is likely that the significant differences in hormonal and subjective effects were attributable to the relative dose of nicotine. The remainder of this discussion examines the temporal interactions between cigarette smoking and changes in HPA axis hormones, VAS ratings of subjective effects, and cardiovascular measures. Some possible implications of these findings for the neurobiology of nicotine's abuse-related effects are considered.

### Nicotine Plasma Levels after Smoking High- and Low-Nicotine Cigarettes

Nicotine plasma levels increased significantly above baseline within 2 min (four puffs) and reached peak levels within 12–14 min after cigarette smoking began. This time course was similar to that reported after 10 min of smoking at one puff per minute ( $T_{\max} = 11.9 \pm 1.9$  min) (Gourlay and Benowitz, 1997). Nicotine plasma levels and pharmacokinetic profiles in the present study were also similar to those previously reported under similar smoking conditions (Mendelson *et al*, 2003). Although the low-nicotine cigarette produced measurable levels of nicotine (less than 4 ng/ml), these levels were significantly lower than peak plasma levels produced by high-nicotine cigarettes (over 20 ng/ml). Moreover, the small but significant increase in plasma nicotine occurred later (after 6 min or 12 puffs) after smoking a low- than a high-nicotine cigarette and remained at relatively stable, low levels for 120 min. In a study designed to determine the threshold dose at which subjects could discriminate nicotine nasal spray from placebo, nicotine-dependent smokers were able to distinguish a dose of 3  $\mu$ g/kg from placebo (Perkins *et al*, 2001). This dose of nicotine nasal spray produced nicotine plasma levels of 2.6 ng/ml, or slightly lower than the low-nicotine cigarette in the present study. Despite differences in routes of nicotine administration (nasal spray and smoking), these data converge to suggest that very low doses of nicotine are discriminable.

### Subjective Responses to Smoking High- and Low-Nicotine Cigarettes

*High, rush, and liking.* Significant increases in ratings of positive subjective effects occurred within four puffs over 2 min after smoking both a low- and a high-nicotine cigarette. However, VAS ratings were significantly higher

during high- than low-nicotine cigarette smoking, and this suggests that subjects could detect the relative nicotine levels within four puffs. These data are consistent with the failure of low-yield cigarettes to reduce smoking behavior (Benowitz, 2001). It is well established that smokers adjust their smoking topography and smoke more cigarettes to try to compensate for a low nicotine yield (Benowitz, 2001).

VAS ratings were highest during the initial ascending limb of the plasma nicotine curve, and then gradually diminished during the smoking period as plasma nicotine approached peak levels. In contrast, after smoking a low-nicotine cigarette, positive VAS ratings remained relatively stable across the 12 min smoking period. Interestingly, the time course of increases in VAS ratings of positive subjective effects was similar during smoking a high-nicotine cigarette and after i.v. nicotine administration. When i.v. nicotine was infused over 10 s, peak VAS ratings of high, rush, and liking (Jones *et al*, 1999) and strength of drug effect (Soria *et al*, 1996) occurred within 2 min. In the present study, VAS ratings of high, rush, and liking began to decrease within 4 min after high-nicotine cigarette smoking began. The relatively rapid decrease in reports of positive subjective effects during smoking occurred while plasma nicotine levels were increasing. A similar rapid decrease in positive subjective reports was also observed after i.v. nicotine administration, although plasma nicotine levels were not measured (Soria *et al*, 1996; Jones *et al*, 1999). It is unlikely that the decrease in positive VAS ratings reflected an increase in the aversive properties of smoking, because ratings of sick, jittery, and bad feeling did not change significantly from baseline.

One limitation of the present study is that nicotine and hormone levels were measured in venous rather than arterial blood. Arterial nicotine levels are 6- to 10-fold higher than venous nicotine levels (Benowitz, 1996). It is estimated that nicotine from cigarette smoke reaches peak levels in the arterial circulation within 20 s (Rose *et al*, 1999). Although significant increases in subjective ratings and plasma nicotine were detected within four puffs in the present study, arterial nicotine, CRH, and ACTH levels may have increased significantly after a single puff.

*Craving.* Ratings of cigarette craving decreased rapidly during and after smoking in both the low- and high-nicotine cigarette groups. Smoking a high-nicotine cigarette reduced ratings of craving significantly more than smoking a low-nicotine cigarette, and craving scores remained significantly below baseline for much longer (100 vs 30 min). Decreases in cigarette craving often are reported after smoking low-nicotine cigarettes that contain <0.06–0.1 mg of nicotine (Gross *et al*, 1997; Pickworth *et al*, 1999; Robinson *et al*, 2000; Rose *et al*, 2000). The salient effects of low-nicotine cigarettes on reports of craving, liking, and reduction of acute withdrawal symptoms are usually interpreted as evidence that complex sensory cues are important in maintaining cigarette smoking behavior (Butschky *et al*, 1995; Gross *et al*, 1997; Pickworth *et al*, 1999; Shahan *et al*, 1999; Rose *et al*, 2000). In the present study, the greatest reductions in ratings of craving occurred within the first 20 min after smoking, and then craving ratings began to increase. The extent to which increases in craving may prompt the next smoking episode is poorly

understood. However, during several weeks residence on a clinical research ward, men usually initiated smoking within 20–30 min after the last cigarette (Mello *et al*, 1985; Mutschler *et al*, 2002).

### Cigarette Smoking, Heart Rate, and the HPA Axis

**Heart rate.** The rate and magnitude of heart rate increases after cigarette smoking were similar to those reported after i.v. nicotine administration (Soria *et al*, 1996; Jones *et al*, 1999). After high-nicotine cigarette smoking, the ascending limb of the plasma nicotine curve was highly correlated with significant increases in ACTH, epinephrine, and heart rate. However, significant increases in heart rate occurred within 2 min after smoking began, whereas significant increases in epinephrine were detected 12 min later. These data are consistent with previous reports, and are usually interpreted to suggest that the initial effects of nicotine on heart rate are mediated by release of norepinephrine in the cardiovascular system, rather than release of epinephrine from the adrenal medulla (Gourlay and Benowitz, 1997).

**ACTH and epinephrine.** Increases in ACTH after cigarette smoking have been reported under a number of conditions where samples for analysis were collected once a day or at relatively infrequent intervals, often without concurrent plasma nicotine measurement (Seyler *et al*, 1984, 1986; Baron *et al*, 1995; Pickworth and Fant, 1998; Coiro and Vescovi, 1999; del Arbol *et al*, 2000). The present study confirms the generality of earlier findings and examines ACTH and epinephrine levels at 2 min intervals during as well as after smoking. ACTH and epinephrine began to increase within 6 min after high-nicotine cigarette smoking began and reached peak levels within 4–6 min after peak nicotine plasma levels were detected. This time course was similar to our previous report of the temporal covariance between ACTH, epinephrine, and plasma cocaine levels after i.v. cocaine administration in men (Mendelson *et al*, 2002). In that study, peak levels of ACTH, epinephrine, euphoria, and plasma cocaine were measured within 8–10 min after i.v. cocaine injection. We interpreted those data to suggest that the HPA hormone increases may be significant concomitants of the abuse-related effects of cocaine in humans (Mendelson *et al*, 2002). As described earlier, CRH from the hypothalamus stimulates ACTH release from the anterior pituitary. Antagonism of CRH release by a CRH-1 antagonist selectively reduced i.v. cocaine self-administration by rats (Goeders and Guerin, 2000). The possible influence of HPA axis activation, as indicated by ACTH and cortisol increases, on the abuse-related effects of cocaine has been discussed in several reviews (Goeders, 1997, 2002a,b; Mello and Mendelson, 2002). The similar patterns of HPA axis activation and subjective responses after i.v. cocaine and smoked nicotine are provocative.

**Cortisol and DHEA.** It is also interesting to consider the possible contribution of cortisol and DHEA to the abuse-related effects of cigarette smoking. Cortisol began to increase significantly when ACTH levels were highest, and reached peak levels between 30 and 60 min after smoking when ACTH levels were decreasing. This time course is

consistent with established feedback relationships between ACTH and cortisol (Yen *et al*, 1999). The increases in cortisol and DHEA were significantly correlated, and each had a similar half-life after high-nicotine cigarette smoking. These data are consistent with previous reports that smokers have higher basal cortisol and DHEA levels than nonsmokers (Pomerleau *et al*, 1992; Field *et al*, 1994; del Arbol *et al*, 2000; al'Absi *et al*, 2003). Cigarette smoking usually induces an increase in plasma cortisol levels (Cryer *et al*, 1976; Winternitz and Quillen, 1977; Spohr *et al*, 1979; Wilkins *et al*, 1982; Seyler *et al*, 1984; Gossain *et al*, 1986; see for review Pickworth and Fant, 1998), and cortisol may remain elevated as a consequence of repeated cigarette smoking throughout the day. It has also been suggested that nicotine may inhibit the diurnal decrease in plasma cortisol levels (Pomerleau *et al*, 1992). The contribution of high cortisol levels to the maintenance of smoking behavior is unclear, but decreases in cortisol on the first day of smoking abstinence were associated with a higher rate of relapse during the first week (al'Absi *et al*, 2004). The importance of cortisol for the reinforcing effects of cocaine has been clearly demonstrated in rodents (Goeders, 2002a).

DHEA is an adrenal androgen precursor of testosterone (Yen *et al*, 1999). DHEA formulations are sold over the counter as dietary supplements, and DHEA is believed to improve a sense of well-being and sexuality in the elderly, and in persons with adrenal insufficiency. Although the evidence is conflicting (see for review Spark, 2002), two placebo-controlled clinical trials support the notion that DHEA treatment improves mood and alleviates depression (Morales *et al*, 1994; Schmidt *et al*, 2005). In a 6-month, placebo-controlled cross-over trial, DHEA administration at physiological levels (50 mg, p.o.) consistently increased energy and improved mood and feelings of well-being in both men and women (Morales *et al*, 1994). In a 6-week, placebo-controlled cross-over trial in men and women with major or minor depression, DHEA administration (90 mg/day for 3 weeks; 450 mg/day for 3 weeks) significantly improved mood and depression ratings in comparison to pretreatment baseline and placebo conditions (Schmidt *et al*, 2005). In the present study, peak levels of DHEA after smoking a high-nicotine cigarette ( $21.13 \pm 2.55$  ng/ml or  $73.32 \pm 8.84$  nmol/l or  $2113.0$  ng/dl) were over four times higher than average DHEA levels achieved after 3 months of DHEA replacement in men ( $14.72 \pm 1.4$  nmol/l) (Morales *et al*, 1994) and over twice as high as average DHEA levels after 6 weeks of high-dose DHEA treatment ( $1047.2 \pm 709.1$  ng/dl) (Schmidt *et al*, 2005). Taken together, these data suggest that increases in DHEA levels may contribute to the mood-elevating effects reported after cigarette smoking.

The effects of cigarette smoking and i.v. cocaine on DHEA were also similar. The significant increases in DHEA and cortisol after smoking a high-nicotine cigarette observed in the present study were similar in time course and magnitude to the increases in DHEA and cortisol measured after i.v. cocaine in men (Mendelson *et al*, 2002). It is interesting to note the temporal covariance between changes in DHEA and cortisol levels and craving for nicotine. Nicotine craving increased sharply between 60 and 80 min after cigarette smoking began, when both cortisol and DHEA were decreasing. Although relatively little is

known about the interactions between DHEA and cocaine or nicotine, DHEA administration produced a dose-dependent enhancement of cocaine conditioned place preference in mice (Romieu *et al*, 2003).

**Cigarette smoking and the HPA axis.** The data reported in the present study are consistent with the interpretation that the hormonal milieu may modulate the abuse-related effects of cigarette smoking. The rapid increases in positive subjective ratings and the rapid release of HPA axis hormones were nicotine dose-related. The temporal covariance between plasma nicotine levels and HPA axis activation parallels patterns reported after i.v. cocaine administration under similar experimental conditions (Mendelson *et al*, 2002). HPA axis activation is also a response to 'stress' variously defined, and there has been considerable interest in the relationship between 'stress' and vulnerability to drug addiction (Koob and Le Moal, 2001; Sinha, 2001; Grammatopoulos and Chrousos, 2002; Marinelli and Piazza, 2002; Heinrichs and Koob, 2004), as well as other psychiatric disorders (see for review Tsigos and Chrousos, 1994, 2002). HPA axis hormones, as well as gonadal steroid hormones, appear to influence some behavioral responses to cocaine (Goeders, 1997, 2002a,b; Mello and Mendelson, 2002). Studies of the generality of these findings to women at the follicular and luteal phases of the menstrual cycle are in progress (Mendelson *et al*, 2004). Although much remains to be learned about the interactions between cigarette smoking and the HPA axis, medications that mimic or attenuate the hormonal effects of cigarette smoking may be useful for treatment of this addictive disorder.

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