Relationship of Smoking Cessation and Nicotine Gum Use to Salivary Androstenedione and Testosterone in Middle-Aged Men

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Cross-sectional studies have associated cigarette smoking in men with elevated androstenedione and little net effect on other sex steroids. However, it is not clear if such findings reflect the impact of nicotine exposure or if sex hormone levels change following smoking cessation. The relationship of the reported number of cigarettes smoked per day and salivary cotinine to salivary testosterone and androstenedione was examined in 221 men aged 35 to 59 years at baseline and 1 year following randomization into a clinical trial including a smoking-cessation intervention. At baseline, salivary cotinine was related to increased salivary androstenedione and testosterone following control for age, body mass, alcohol intake, and time of day of specimen collection (partial $r = +.14$ and $+.30$, $P < .05$ and $.01$, respectively). The reported number of cigarettes smoked per day was unrelated to either hormone. At the first annual visit, there was a significant decrease in the salivary androstenedione of men who had quite smoking and were currently using nicotine gum (94 *v* 60 pg/mL, $P < .05$, n = 34) and of men who had quit smoking and were not exposed to nicotine (86 *v* 56 pg/mL, $P < .05$, n = 48), whereas the salivary androstenedione of men who remained smokers at the first annual visit was unchanged (83 *v* 85 pg/mL, n = 139). Salivary testosterone levels were not significantly affected by a change in smoking status. These findings suggest that cigarette smoking has a primary effect that serves to increase salivary androstenedione, whereas neither cigarette smoking nor nicotine exposure per se has a clear effect on salivary free testosterone levels in men.

ALTHOUGH CIGARETTE smoking is associated with premature mortality from numerous diseases, the greatest number of excess deaths associated with smoking are due to coronary heart disease.[1] Based largely on the observation that the prevalence of coronary heart disease in premenopausal women is lower than in men, it has been hypothesized that a likely factor contributing to the increased coronary heart disease risk in cigarette smokers is increased androgenicity or decreased estrogenicity.[2]

Indeed, the majority of studies examining hormone levels in smokers and nonsmokers appear to indicate that cigarette smoking has an androgenic and/or antiestrogenic effect in women.[3-5] Comparatively less is known about the influence of cigarette smoking on sex steroids in men. Although an in vitro study found that both nicotine and cotinine inhibit steroidogenesis in mouse Leydig cells,[6] epidemiologic investigations of smoking-related differences in sex steroids among men have provided mixed results. Cross-sectional studies of men have generally shown that cigarette smokers have increased estradiol and/or estrone levels as compared with nonsmokers,[7-11] although not all evidence is unequivocal.[12,13] Levels of androstenedione appear to be greater in smokers than in nonsmokers in both men and postmenopausal women.[3,4,7,12,14] Some cross-sectional studies have also found that levels of total or free testosterone are greater in male smokers than in nonsmokers.[12,15-20] However, other studies have found the reverse[21] or no significant differences.[7,9,11,22,23] A change in smoking status has also been associated with an increase in plasma testosterone[24] or no net change.[16]

To some degree, these inconsistent findings may have resulted from variability in exposure to the constituents of cigarette smoke. Due to differences in such factors as type of cigarette smoked, puffing intensity, puff volume, and cigarette rod length, the number of cigarettes smoked may be only an approximate index of exposure to nicotine or other smoke constituents.[25,26] Indeed, since it has been hypothesized that constituents of cigarette smoke such as polycyclic aromatic hydrocarbons[27] or carbon monoxide[24] may affect the production or metabolism of sex hormones, it is unclear if smoking-associated effects are mediated by nicotine exposure. In a clinical setting, some indication of the importance of nicotine-related effects of cigarette smoking may be at least partially provided by comparing hormone levels in individuals while they are either smoking cigarettes or receiving nicotine substitution therapy (ie, nicotine gum or a transdermal nicotine patch) following cessation of cigarette smoking, and assessing salivary cotinine levels as an indicator of nicotine exposure.

An opportunity to examine the relation of cigarette smoking and nicotine exposure to salivary androgen levels was offered by the Lung Health Study, a 5-year study of the effectiveness of early intervention for chronic obstructive pulmonary disease. The goal of the present investigation was to explore the relationship of participation in a smoking-cessation intervention program to salivary levels of testosterone and androstenedione in a subsample of middle-aged men participating in this trial. In addition, we examined the association of salivary cotinine to hormone levels in continuing smokers and ex-smokers either currently using nicotine gum or not exposed to nicotine.

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SUBJECTS AND METHODS

Subjects and Study Design

The sample for these analyses was drawn from the Lung Health Study, a 5-year, 10-center, National Institutes of Health–supported clinical trial investigating the efficacy of smoking cessation and bronchodilator therapy in early intervention for chronic obstructive pulmonary disease. Recruitment for this trial was initiated in October 1986 and concluded in January 1989. The data for these analyses were obtained at the second and third screening visits, before randomization of participants to a usual care (UC) or special intervention (SI) condition, and at the first annual follow-up visit.

Individuals eligible for participation in this trial were current cigarette smokers between the ages of 35 and 59 years at the initial screening. Screenees who currently smoked 10 or more cigarettes per day and had a forced expiratory volume in 1 second between 50% and 90% of the predicted value based on age, gender, height, and race and a maximum forced expiratory volume in 1 second to maximum forced vital capacity ratio not exceeding 75% were invited to a second visit at a clinic site. At the second visit, weight, height, and medical and smoking history were assessed, and all screenees provided a 1-mL saliva sample. All saliva samples were frozen at −20°C following collection, and cotinine concentrations were determined at the American Health Foundation in Valhalla, NY, using radioimmunoassay procedures described by Langone et al.[28]

Weight in excess of 150% of normal for gender and height based on data from the National Health and Nutrition Examination I[29] was a criterion for exclusion. Individuals who reported a weekly alcohol consumption of 25 drinks or more or an intake of eight or more drinks on one occasion at least once a month during the past year were also eliminated. Thus, this trial included nondrinkers, and light and moderate drinkers and moderate to heavy smokers, and excluded the most severely overweight. A more complete description of recruitment and screening procedures for the Lung Health Study is available elsewhere.[30]

The final randomized sample for the Lung Health Study consisted of 3,702 men and 2,185 women. Because of the lack of information regarding the menstrual phase or menopausal status of women in this trial, these analyses were restricted to male participants.

Participants were randomized on a 2:1 basis to SI or UC conditions at the conclusion of the third screening visit. Half of all SI participants received a metered-dose inhaler containing placebo, and half received an anticholinergic bronchodilator (Atrovent; Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT). Use of the bronchodilator continued throughout the duration of the trial. UC participants returned for yearly examinations only. All SI participants were offered a smoking-cessation treatment that included a behaviorally based stop-smoking program and the use of 2 mg Nicorette chewing gum (Marion Merrell Dow, Kansas City, MO). Most participants who quit smoking during the 3-month smoking-cessation program stopped using the gum during or shortly after the conclusion of this program. Overall, 38% of SI men and 10% of UC men were not smoking at the first annual visit, and 36% of men who quit smoking were using nicotine gum. Placebo or active-drug bronchodilator status was otherwise ignored in the analyses described here.

Three groups of participants were defined: (1) *ex-smokers not using gum* reported no use of nicotine gum, cigarettes, or other tobacco substances within 30 days of the first annual visit, and had a salivary cotinine level of less than 20 ng/mL and an expired carbon monoxide level of less than 10 ppm; (2) *ex-smokers using nicotine gum* reported current nicotine gum use at the first annual visit and no exposure to cigarettes or other tobacco substances within the past 30 days. Smoking status for this group was verified by an expired carbon monoxide level of 10 ppm or lower; and (3) *current smokers* reported smoking one or more cigarettes within the past 24 hours, had not stopped smoking for 24 hours or more within the past month, and had no current use of nicotine gum, snuff, chewing tobacco, or pipes or cigars.

The total number of men in the Lung Health Study that could be categorized in groups 1, 2, or 3 at the first annual visit was 404, 203, and 1,270, respectively. Because of budgetary constraints, all saliva specimens for this trial collected before March 1988 were destroyed. Also, subsequent to November 1988, collection of saliva specimens at annual visits was limited to all subjects denying current smoking plus a 10% random sample of all remaining subjects. The total number of men remaining in groups 1, 2, and 3 with saliva specimens from both baseline and the first annual visit was 85, 57, and 199, respectively.

Visits to the Lung Health Study clinics were scheduled at times convenient for the participants and could potentially occur anytime within approximately a 12-hour period (7 AM to 7 PM). Salivary androstenedione and testosterone levels decrease rapidly during the morning hours and continue to decline at a diminished rate throughout the remainder of waking hours.[31,32] Because of this diurnal variation, intrasubject variability (ie, comparisons between baseline and the first annual visit) was reduced by restricting the sample to men who attended both second-screening and first annual visits within 1 clock hour of each other if either visit preceded 12 noon and within 90 minutes of each other if both visits followed 12 noon. Two hundred twenty-eight of 341 participants fulfilled this criterion. Missing data for one or more variables reduced this sample size to 221. Following sample selection, an error in data coding was discovered that resulted in the visits of a number of subsample cases (55) being slightly outside this time window. However, because the time of the baseline and annual visits remained highly correlated in the full subsample including these participants (Pearson $r = +.95$) and because the mean absolute value of the time difference between the baseline and first annual visits remained less than 1 hour (39.5 minutes), all participants in this subsample were retained.

Steroid Analysis

Steroids were extracted with diethyl ether from samples ($\approx$450 μL) of saliva provided as coded samples from the American Health Foundation. The ether extracts were chromatographed on 2.5-g Sephadex LH-20 microcolumns[33] using a hexane:benzene:methanol (85:15:5 vol/vol/vol) elution mixture to isolate androstenedione and testosterone from other steroids that might cross-react with the antisera used in the radioimmunoassay (androstenedione lot no. RI-P9 from ICN Biomedicals, Costa Mesa, CA; testosterone lot no. A-1 from Holly Hills Biologicals, Hillsboro, OR). In the radioimmunoassay, bound and free steroids were separated by addition of dextran-coated charcoal followed by centrifugation after overnight incubation of appropriate standards and unknown samples with the specific antisera and appropriate tritiated steroids. The isotope remaining in the supernatant was determined by scintillation counting, the plots of picogram standard versus counts per minute isotope were linearized with a logit-log transformation, and the unknown samples were quantified by extrapolation from the appropriate standard curve. Tritiated steroids were added to independent saliva samples processed in parallel with the unknown samples to monitor and correct for purification losses. Solvent blanks and quality-control samples were processed with each assay,

Table 1. Relation of Salivary Cotinine and Reported Number of Cigarettes Smoked Per Day to Salivary Testosterone and Androstenedione in a Subsample of Men From the Lung Health Study, 1988-1989

	Pearson r		Partial r*	
	Testosterone	Androstenedione	Testosterone	Androstenedione
Salivary cotinine	+.11	+.26‡	+.14†	+.30‡
No. of cigarettes per day	+.03	+.06	+.02	+.06

*Adjusted for age, weekly alcohol intake, time of day, and body mass.
†$P < .05$.
‡$P < .01$.

and reported values were corrected for solvent blanks, recoveries, and aliquot factors. Average blank, recovery, and intraassay/interassay coefficients of variation for androstenedione and testosterone assays (n = 3) were 6.2 pg, 80.2%, 7.0%/8.5%, and 8.4 pg, 83.5%, and 6.2%/6.0%, respectively.

Statistical Analysis

The cross-sectional relationship of salivary testosterone and salivary androstenedione at baseline to the reported number of cigarettes smoked per day and salivary cotinine was explored with Pearson bivariate *r*s and multiple linear regression techniques. The change in salivary androstenedione and testosterone between baseline and the first annual visit was determined with a repeated-measures analysis of covariance, with Bonferroni procedures used in post hoc comparisons. Age, body mass, reported weekly alcohol intake, and time of day of specimen collection were used as covariates in cross-sectional analyses, and age, body mass, and reported weekly alcohol intake were used as covariates in analyses of change between baseline and the first annual visit. Kolmogorov-Smirnov tests of normality showed that salivary cotinine and both salivary androstenedione and testosterone had non-normal positively skewed distributions. Therefore, analyses for these variables were conducted with natural log transformations. However, all means reported here are untransformed values.

RESULTS

Cross-Sectional Association of Baseline Characteristics to Salivary Androstenedione and Testosterone

The relationship of salivary testosterone and androstenedione to the number of cigarettes smoked per day and salivary cotinine with and without adjustment for age, body mass, number of cigarettes per day, alcoholic drinks per week, and time of day of the second screening visit is shown in Table 1. Salivary testosterone was unrelated and salivary androstenedione was positively related to salivary cotinine in univariate analyses. Following adjustment for all covariates, androstenedione remained significantly related to cotinine and salivary testosterone had a significant but weak positive relation to salivary cotinine. Although not shown here, salivary testosterone and androstenedione were also both significantly related to the time of day following adjustment for all covariates ($r_p = -.14$ and $-.30, P < .05$, respectively). The only other significant association in these analyses was a negative relationship observed between salivary androstenedione and number of drinks per week ($r_p = -.15$, $P < .05$), although there were marginal but nonsignificant tendencies for salivary testosterone to decline with age ($r_p = -.11, P = .10$) and increase with body mass ($r_p = +.11, P = .10$).

The number of cigarettes smoked per day was not independently associated with either hormone in these analyses. If it can be assumed that the relationship of testosterone and androstenedione levels to cotinine reflects an effect of nicotine exposure, the lack of an association between the reported number of cigarettes smoked per day and either testosterone or androstenedione may be a function of the modest relation between salivary cotinine and the number of cigarettes smoked per day at baseline (Pearson $r = +.24, P < .01$).

Change in Salivary Androstenedione and Testosterone in Relation to First Annual Visit Smoking Status

Table 2 shows the characteristics of each group at both the second screening visit and the first annual visit. In

Table 2. Selected Characteristics of Subjects Defined by First Annual Visit Smoking Status

	Quitters		Continued Smokers (n = 139)
	No Gum Use (n = 48)	Nicotine Gum (n = 34)	
Body mass (kg/m²)			
Baseline	27.47 ± .47	25.82 ± .55	26.11 ± .30
Annual visit	29.04 ± .51	27.30 ± .56	26.33 ± .31
No. of cigarettes per day			
Baseline	29.38 ± 1.70	33.97 ± 1.86	33.77 ± 1.30
Annual visit	0	0	25.63 ± 1.10
Salivary cotinine (ng/mL)			
Baseline	293 ± 23	351 ± 23	322 ± 10
Annual visit	1 ± .01	233 ± 21	333 ± 12
Alcohol intake (drinks/wk)			
Baseline	5.00 ± .89	5.47 ± 1.16	4.98 ± .50
Annual visit	4.16 ± .83	3.91 ± 1.07	4.38 ± .50

NOTE. Results are the mean ± SEM.

ANOVAs of all baseline characteristics, men who remained nonsmokers without using nicotine gum weighed more than quitters using nicotine gum ($P < .05$ in Bonferroni comparison), and the baseline salivary cotinine level of ex-smokers not using nicotine gum at the first annual visit was lower than that of ex-smokers using gum at the first annual visit ($P < .05$). There was a significant interaction effect between group status and visit for body mass index ($F = 28.80$, $P < .001$). Both groups of men who quit smoking gained weight, whereas continued smokers did not. Finally, there was also a significant decrease in alcohol intake between baseline and the first annual visit ($F = 9.52$, $P < .01$), but no interaction between group status and time of assessment ($P > .20$).

Overall Pearson correlations between hormone levels assessed at baseline and at the first annual visit were +.36 for testosterone and +.34 for androstenedione (both $P < .01$). Separate 2 (baseline/annual visit) ×3 (smoking status) repeated-measures analyses of covariance of salivary testosterone and androstenedione using age, body mass, and number of drinks per week as covariates produced both a significant main effect for time ($F = 17.18$, $P < .001$) and a significant time-by-group interaction ($F = 11.43$, $P < .001$) for androstenedione, and no significant effects for salivary testosterone (all $P > .10$). Figure 1 displays the mean androstenedione and testosterone levels in each group at both assessment points. As shown in Fig 1, there were substantial decreases in salivary androstenedione in both groups of men who stopped smoking, but no change in salivary testosterone. Post hoc comparisons showed that this decrease was significant for both quitters using gum and quitters not using gum (both $P < .05$), whereas salivary androstenedione levels of continued smokers were unchanged.

Finally, because the analysis of change in sex hormones related to smoking cessation might have been confounded by weight gain in the two groups who stopping smoking, these analyses were also performed without including body mass as a covariate. However, removing body mass as a control factor had a negligible effect on all analyses.

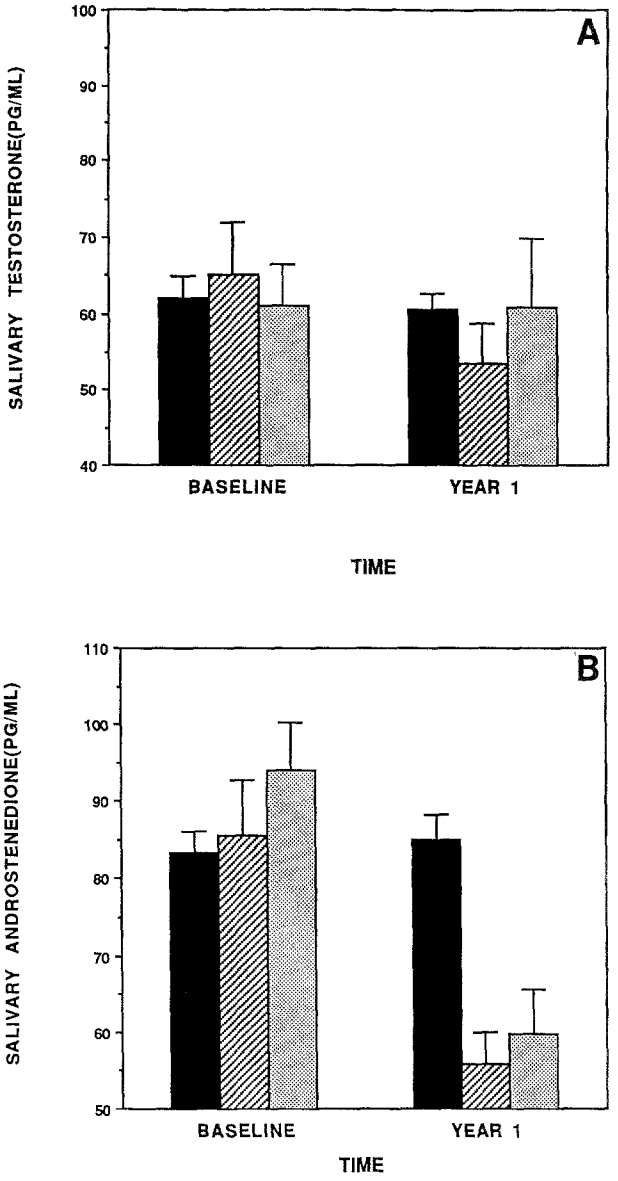

Fig 1. Mean ± SEM (A) salivary testosterone and (B) salivary androstenedione in (■) continued smokers, (▨) quitters using nicotine gum, and (▩) quitters not using nicotine gum at baseline and 1 year following randomization in the Lung Health Study.

DISCUSSION

In this group of middle-aged men, salivary androstenedione was cross-sectionally related to increases in cotinine and smoking cessation was accompanied by a decrease in androstenedione. Salivary testosterone, in contrast, was associated with individual differences in salivary cotinine, but was not significantly affected by a change in smoking status. Considered together, these findings suggest that a stimulus provided by cigarette smoke exposure is responsible for increases in androstenedione secretion or decreases in metabolism, and that withdrawal of this stimulus (ie, smoking cessation) results in decreased androstenedione levels, whereas cigarette smoking has little net effect on gonadal androgens. Inasmuch as nicotine has been found to stimulate the secretion of other adrenal hormones,[34] it might be reasonable to postulate that exposure to the nicotine in cigarette smoke provides this stimulus. However, arguing against this notion is the similar decrease in salivary androstenedione in men who had quit smoking and were not nicotine-exposed and men who had quit smoking but maintained substantially elevated cotinine levels (nicotine gum users).

In contrast to findings for salivary androstenedione, no change in salivary testosterone was observed in either group of men who quit smoking, although there was a weak cross-sectional association between salivary testosterone and salivary cotinine at baseline. This finding is consistent with several other studies that have found a weak positive association between cigarette smoking and either free or total testosterone levels in men,[12,15-17,19,20] and with a previous study of Multiple Risk Factor Intervention Trial participants that found a weak but significant cross-sectional

association between serum thiocyanate and plasma total and free testosterone.[16] In a follow-up investigation to this latter study,[12] a change in the number of cigarettes smoked (presumably among individuals who were current smokers at baseline) was also positively related to a change in plasma testosterone over a 3-year period. Although inconsistent with our overall findings, an analysis of continued smokers only in the current study revealed that a change in salivary cotinine but not in the number of cigarettes smoked was positively related to a change in salivary testosterone ($r = +.21$, $P < .05$). There is no obvious explanation for this association in both the study by Dai et al[12] and our study.

The use of saliva specimens in clinical studies of sex steroids is still relatively uncommon. There typically is a strong correlation (.81 to .94) between plasma free testosterone and salivary testosterone in men.[35-37] Although there are somewhat less data regarding the relationship of salivary androstenedione to plasma androstenedione, the observed relations[38-41] have varied in the same general range (.70 to .97). Because blood was not drawn at either baseline or the first annual visit in the Lung Health Study, plasma levels of testosterone or androstenedione are unknown, and there is some possibility that our outcomes could have been affected by alterations in protein binding associated with cigarette smoke exposure. However, to date, there appears to be no evidence that smokers have lower levels of sex hormone–binding globulin than nonsmokers.[11,26,27]

The finding that salivary androstenedione is cross-sectionally related to cotinine and longitudinally related to smoking status provides further support for the central role of nicotine contained in cigarette smoke in smoking-associated elevations of adrenal androgens. Conversely, the absence of any clear association of salivary testosterone with either cigarette smoking or cotinine casts further doubt on the role of cigarette smoking as a determinant of gonadal androgen levels in men.

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