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Differentiation in the short- and long-term effects of smoking on plasma total ghrelin concentrations between male nonsmokers and habitual smokers

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Abstract

To explore the association between the anorexigenic effects of nicotine and the orexigenic properties of ghrelin, plasma total ghrelin levels were measured in nonsmokers and habitual smokers before and after short-term exposure to cigarette smoke. Thirty-one male smokers and 23 nonsmoking volunteers were matched for age and body mass index. After an overnight fast and abstinence from smoking, they all smoked 2 cigarettes consecutively (same brand, rate of inhalation, and duration of smoking). Total ghrelin concentrations were measured by radioimmunoassay before smoking (baseline), immediately afterward, and 30, 60, and 90 minutes after the second cigarette. Baseline ghrelin levels were not different between smokers and nonsmokers. Smoking did not have an immediate influence on ghrelin concentrations in smokers (analysis of variance for repeated measurements, $P = 0.74$), whereas there was a progressive decline in nonsmokers, reaching statistical significance at 30 minutes ($P = .04$) and a nadir at 60 minutes ($P = .04$) after smoking. Moreover, the area under the curve for the changes of ghrelin over time after smoking was lower in nonsmokers than in smokers (-287.2 ± 167.1 vs 29.2 ± 125.3 ng·min/L, $P = .03$). In conclusion, fasting plasma total ghrelin concentrations are not different between male smokers and nonsmokers. Smoking does not provoke any short-term change in ghrelin levels in smokers, but induces a decline in nonsmokers. If the anorectic effect of smoking is ghrelin induced, this effect may be present only in people not habituated to smoke exposure. In habitual smokers, ghrelin suppression by short-term smoking could be blunted as a result of desensitization due to prolonged nicotine exposure.

1. Introduction

The detrimental effects of smoking on health have been unequivocally proven in the last decades. There seems to be, however, one interesting "beneficial" facet of tobacco smoking, namely, its effect on body weight. An overwhelming wealth of evidence shows that smokers weigh less than nonsmokers and smoking cessation leads to weight gain.

An analysis of the Second National Health and Nutrition Examination Survey, examining an impressive sample of 12,103 men and women aged 19 to 74 years, demonstrated that smokers were leaner than nonsmokers of the same sex and age [1]. Moreover, leanness seemed to increase with duration but not with intensity of smoking. Nonsmokers and ex-smokers experienced a much larger weight gain after the age of 25 than current smokers [1]. Furthermore, a study on 1911 pairs of male monozygotic twins showed that nonsmoking subjects weighed substantially more than did their smoking siblings and had an increased risk of clinically significant obesity [2].

What is really alarming is the perception that the public has of the contribution tobacco use could have on weight maintenance. Adolescents who desire to lose weight may initiate smoking as a method of appetite control, whereas among overweight females, smoking initiation is more likely [3]. Even more ominously, it seems that expectancies for the effect of tobacco use on appetite and weight control can predict smoking status and smoking rate [4]. Adults younger than 30 years are more likely to smoke if they are trying to lose weight [5].

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Given not only the epidemiologically proven effect of cigarette smoking on weight, but also its catastrophic effects on other health aspects, it is of particular interest to explore the mechanisms underlying these actions and especially the influence of smoking on the concentrations of appetite-modulating mediators.

The purpose of this study was to investigate the effect of smoking on ghrelin, a recently discovered potent orexigenic hormone, which is mainly produced in the gastric fundus and stimulates food intake through the release of neuropeptide Y and agouti-related protein from the arcuate nucleus of the hypothalamus [6]. We examined the long-term effect of cigarette smoking on ghrelin levels through the comparison of fasting plasma total ghrelin levels in smokers and nonsmokers, as well as the short-term effect of tobacco smoke through the comparison of ghrelin levels before and after consumption of 2 cigarettes by both smokers and nonsmokers.

2. Subjects and methods

Thirty-one male habitual smokers and 23 male nonsmokers, all volunteers, were consecutively recruited. The nature and details of the study were fully explained to them orally and in writing and all subjects gave their informed consent before being included in the study. The experimental protocol was approved by the ethics committee of our hospital. All subjects were aged 21 to 45 years, healthy, and not taking any medication.

On the morning of the study, the subjects arrived between 8:00 and 8:30 AM at the metabolic unit of our department after an overnight fast and abstinence from smoking. The subjects were weighed with an electronic scale, and their height and waist and hip circumferences were measured. Body mass index (BMI) and waist-hip ratio were calculated accordingly. A venous cannula for blood sampling was then placed in a superficial forearm vein and kept patent by 0.9% saline water infusion.

For smokers, smoking habits were recorded and expressed as smoking years (years of continuous smoking until the present), cigarettes per day, and pack years, which is an index of overall tobacco exposure (pack years = packs per day × smoking years or cigarettes per day × smoking years/20).

After a 20-minute resting interval for acclimatization, a sample of blood was drawn for the measurement of baseline total ghrelin concentrations and serum glucose. Each subject was then given 2 cigarettes of the same brand (Winston Classic Red, 10 mg "tar," 0.9 mg nicotine, 10 mg carbon monoxide per cigarette, JT International, Geneva, Switzerland) and was instructed to smoke these consecutively, with a 5-minute interval between them. Subjects were observed and timed while smoking and maintained a constant rate of inhalation; each cigarette was smoked in 6 to 7 minutes and after 12 to 14 inhalations (one inhalation every 30 seconds). Additional blood samples for the measurement of total ghrelin were drawn immediately after the second cigarette was smoked (0 minutes), and 30, 60, and 90 minutes later while the subjects were resting.

Samples for the measurement of ghrelin were cryocentrifuged immediately after collection; plasma was separated and stored promptly at −80°C until assayed.

2.1. Analytical methods

Fasting serum glucose was measured by using the oxidase-peroxidase method (Zafiropoulos, Athens, Greece). Total plasma ghrelin concentrations were measured with a commercially available radioimmunoassay kit (Linco Research, St Louis, MO; intra-assay coefficient of variation, 6.4 ± 3.0, at a sensitivity of 21.6 ± 10.2 ng/L).

2.2. Statistical methods

Statistical analyses were performed with software provided in the SPSS 12.0 statistical package (SPSS, Chicago, IL). Analysis of variance (ANOVA) for repeated measurements was performed to test the effect of smoking on ghrelin concentrations over time. The Greenhouse-Geisser adjustment was used when the sphericity assumptions were not fulfilled. A paired Student *t* test was performed to compare ghrelin values at baseline and after smoking with those obtained after 30, 60, and 90 minutes. Unpaired *t* tests were performed to compare the values of the study variables between smoking and nonsmoking subjects. Spearman correlation coefficient was used to look for associations between the duration and pack years of smoking with baseline plasma ghrelin concentrations. The overall response in plasma ghrelin levels after smoking over the study period was calculated as incremental or nonincremental area under the curve (ΔAUC) by use of the trapezoid rule. The ΔAUC adjusted for baseline values was calculated by subtracting the baseline value from each value after smoking before the area calculation. Statistical significance was assumed at $P < .05$.

3. Results

Baseline characteristics of the study subjects are presented in Table 1. There were no differences between the 2

Table 1
Baseline characteristics of the study subjects

	Smokers	Nonsmokers	*P*
n	31	23	–
Age (y)	32.0 (5.6)	33.5 (4.0)	.28
Weight (kg)	83.1 (10.0)	80.2 (12.1)	.35
Height (m)	1.77 (0.05)	1.77 (0.05)	.88
BMI (kg/m^2)	26.6 (2.9)	25.5 (3.1)	.22
Waist circumference (cm)	95.1 (9.2)	93.3 (8.9)	.48
Hip circumference (cm)	105.3 (5.2)	103.2 (6.2)	.19
Waist-hip ratio	0.90 (0.06)	0.91 (0.05)	.87
Smoking years	13.6 (6.1)	–	–
Pack years (packs × years)	15.3 (8.3)	–	–
Fasting total plasma ghrelin (ng/L)	1214.2 (406.5)	1239.8 (453.9)	.83
Serum glucose (mg/dL)	92.6 (9.9)	95.4 (12.9)	.36

Values are presented as mean (SD).