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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).

groups in any anthropometric variable. Moreover, baseline total plasma ghrelin and fasting glucose levels did not differ between smokers and nonsmokers.

In smokers, there was a trend for a positive association between the years of smoking and fasting plasma ghrelin levels (r = 0.31, P = .09). Pack years of smoking were not associated with fasting plasma ghrelin levels (r = 0.06, P = .71).

After the end of the second cigarette, smokers did not exhibit significant changes in ghrelin levels at any time point of measurement, as compared with baseline values (1221.7 ± 388.8, 1255.6 ± 473.5, 1226.5 ± 470.7, and 1207.6 ± 508.4 ng/L [mean ± SD] at 0, 30, 60, and 90 minutes, respectively; ANOVA for repeated measurements, P = .74). In nonsmokers, however, a progressive although nonsignificant (ANOVA for repeated measurements, P = .08) decline in plasma ghrelin throughout the study was observed. This reached statistical significance at 30 minutes and a nadir at 60 minutes after smoking (1249.7 ± 439.2, 1174.7 ± 443.2, 1155.8 ± 419.4, and 1149.5 ± 462.4 ng/L [mean ± SD] at 0, 30, 60, and 90 minutes, respectively; P value for the comparison between time 0 and 30 minutes and the comparison between time 0 and 60 minutes = 0.04) (Fig. 1).

Moreover, the overall change of plasma ghrelin concentrations over time, expressed as ΔAUC, was significantly lower in nonsmokers than in smokers (−287.2 ± 167.1 vs 29.2 ± 125.3 ng·min/L; P = .03).

When subjects in both groups were stratified into 4 groups according to their BMI (lean, BMI ≤25 kg/m^2; overweight, BMI >25 kg/m^2) and smoking status, no differences in baseline ghrelin levels between the 4 resulting groups were observed (data not shown). In smokers, stratification according to BMI did not reveal any significant differences in ghrelin values over time. In the 2 resulting groups of nonsmokers, although the decrease in ghrelin levels that was observed in the whole group still remained, this became of borderline significance ($P \leq .08$) because the statistical samples were quite diminished.

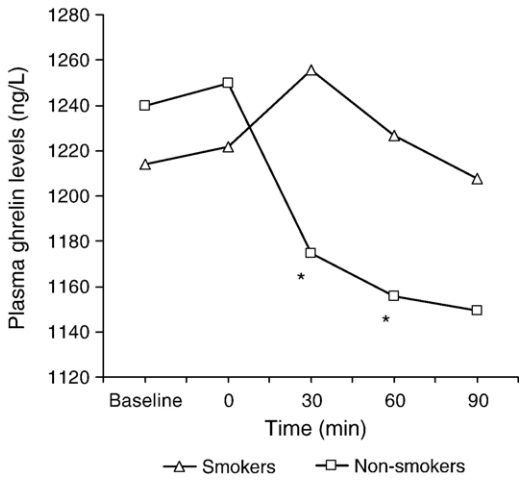

Fig. 1. Mean plasma total ghrelin concentrations over time in smokers and nonsmokers. ANOVA for repeated measurements: P = .74 for the effect of time in smokers; P = .08 in nonsmokers. *P = 0.04 for the comparison of total plasma ghrelin levels between time 0 and 30 minutes and the comparison between time 0 and 60 minutes.

4. Discussion

The mechanism by which smoking leads to lower body weight and its cessation to weight gain has been extensively examined. Some studies denote a reduction in appetite as the main driving force behind the effects of nicotine [7-10], an influence that could be mediated through the modulation of dopaminergic and serotoninergic activity in the hypothalamus [8,10]. Another large proportion of studies identify an increase in metabolic rate as the cause of weight reduction [11-13], pointing out the sympathetic nervous system as the obvious mediator [11,13]. There are also reports favoring a lowering of the body weight set point by nicotine [14-16], an increase in cytokine activity [17,18], or even the modulation of lipoprotein lipase activity [19,20].

The most probable etiology is that smoking produces both a reduction in food intake and an increase in metabolic rate that lead to the lowering of body weight [21]. The importance, therefore, of the identification of the pathways that mediate the anorexigenic effects of smoking is evident.

In the present study, we have shown that fasting plasma total ghrelin levels are not influenced by long-term smoking status by demonstrating that baseline plasma ghrelin levels were not different between 2 groups of male nonsmokers and habitual smokers matched for age and BMI. However, a short-term effect of exposure to tobacco smoke was evident when subjects smoked 2 cigarettes. Interestingly, this effect was present only in nonsmokers; namely, smoking decreased ghrelin levels, whereas in smokers no difference was observed over time.

One can only speculate as to the cause of the short-term differences we observed between smokers and nonsmokers. If the anorectic effect of smoking could be attributed to a modulation of ghrelin concentrations, then, according to our findings, this would hold true only for nonsmokers and in the setting of short-term exposure to tobacco smoke. Along these lines, a recent study reported a greater hunger-suppressing effect of nicotine on nonsmokers than on smokers [7]. One possible explanation for our findings could be desensitization to the short-term ghrelin-suppressing effects of nicotine in habitual smokers. Indeed, Pidoplichko et al [22] demonstrated that although initial nicotine administration can increase the activity of dopaminergic neurons, prolonged exposure can cause desensitization of nicotinic receptors and tolerance to its effects.

The short-term effect we observed in nonsmokers could also be possibly explained by the adverse physiologic effects of tobacco smoke, especially in subjects not accustomed to its influence. Ghrelin has been implicated in the regulation of gastrointestinal motility [23], so an obvious “culprit” could be cigarette-induced nausea. How-

ever, of our 23 nonsmoking subjects, only 4 experienced mild nausea after smoking, and 8 experienced dizziness as a result of exposure to carbon monoxide. Furthermore, all of these symptoms had resolved within 20 minutes, a time point quite apart from the observed decrease in ghrelin concentrations. There is virtually no literature on the effects of dizziness or gastrointestinal symptoms, such as nausea and vomiting, on ghrelin levels, but given their frequency, intensity, and time of manifestation in our study, we consider their involvement in the suppression of ghrelin, although not impossible, quite improbable. Moreover, statistical comparison of the 4 subjects who exhibited nausea with the rest of the nonsmokers who did not showed no difference in baseline ghrelin levels or ghrelin concentrations in response to smoking.

There is a scarcity of studies dealing with the relationship between smoking and ghrelin. In fact, only one other study, by Bouros et al [24], has investigated the short-term effect of smoking on ghrelin concentrations. Using an experimental model similar to ours, the authors found no differences in baseline fasting ghrelin levels between smokers and nonsmokers, but demonstrated a short-term increase in ghrelin levels after one cigarette in both groups that occurred quite early, a fact that might suggest a neural mechanism behind this effect. The contrasting results with our study could be attributed to differences in methodology in the study by Bouros et al: only one cigarette with a lower nicotine content, double the frequency of inhalations and hence a stronger possibility for adverse effects in nonsmokers, different sampling intervals (0, 2, 5, 15, and 60 minutes after initiation of smoking), and a smaller sample of subjects that also included women. Despite these differences, the fact that both studies used a similar experimental model with markedly contrasting results is quite interesting and warrants further elucidation in future studies.

An earlier study reported that ghrelin was positively associated with current smoking in a population of 58-year-old healthy men [25]. However, after adjustment for body fat (instead of waist circumference), this relationship became nonsignificant. Furthermore, the inference of an effect of short-term smoking on ghrelin levels in that study was made without the use of strictly controlled smoking conditions. Thus, the results are not comparable with the present study.

Another study reported that educational level, alcohol intake, and smoking history were positively associated with ghrelin levels, independently of age, BMI, and sex [26], while also observing that frequent exercisers had lower ghrelin levels, which, according to the authors, could be explained by their smoking status. This relationship could actually be inverse, though; specifically, if exercise were the cause of low ghrelin levels, and nonsmokers were naturally more likely to exercise frequently, then their low ghrelin levels could be explained by their increased physical activity. Therefore, the long-term effect of smoking on ghrelin cannot be unequivocally determined by the results of this study.

Finally, another recent study corroborates the findings of our study regarding the long-term effect of smoking on ghrelin levels by demonstrating no correlation between smoking status and ghrelin concentrations [27].

In the present study, we decided to measure total ghrelin (the sum of both the acylated [or active] and the desacylated [or supposedly inactive] form of the molecule) not only because controversy still exists regarding the physiologic role of desacylated ghrelin [28], but also because total ghrelin concentrations have been found to correlate well with those of the acylated (active) form [29]. It is evident that the investigation of the short- and long-term effects of smoking on the concentrations of other gut peptides involved in the regulation of appetite and satiety (eg, peptide YY, oxyntomodulin, and glucogon-like peptide 1) is warranted and would be especially interesting.

A possible limitation of the present study should be pointed out: nonsmokers may not have smoked as effectively as smokers because they may not be acquainted with the process. The measurement of plasma cotinine (the major metabolite of nicotine) might have helped in the determination of the degree of nicotine exposure. However, our smoking protocol was quite strict in terms of observation and inhalation frequency; moreover, all nonsmoking subjects were instructed on how to inhale and were monitored quite closely while doing so. These facts, along with the self-evident effect on ghrelin concentrations and the adverse effects experienced by the nonsmokers, make it clear that the nonsmoking group was substantially exposed to nicotine, although maybe not to the same extent as experienced smokers.

Because in the present study a short-term decrease in ghrelin after smoking was observed only in nonsmokers and, moreover, because baseline ghrelin levels did not differ between the 2 groups of subjects, one would be tempted to dismiss the modulation of ghrelin concentrations as the mechanism for nicotine-induced anorexia. Still, the blunting of ghrelin suppression by short-term smoking in habitual smokers as a result of desensitization after prolonged nicotine exposure cannot be excluded as an explanation of our findings. Finally, nicotine could possibly exert its anorectic effect through modulation of ghrelin signaling or regulation of ghrelin receptors. Given the enormity of the health hazard that smoking to control body weight poses, further exploration of these pathways is evidently important.

References

[1] Albanes D, Jones DY, Micozzi MS, et al. Associations between smoking and body weight in the US population: analysis of NHANES II. Am J Public Health 1987;77:439-44.

[2] Eisen SA, Lyons MJ, Goldberg J, et al. The impact of cigarette and alcohol consumption on weight and obesity. An analysis of 1911 monozygotic male twin pairs. Arch Intern Med 1993;153:2457-63.

[3] Cawley J, Markowitz S, Tauras J. Lighting up and slimming down: the effects of body weight and cigarette prices on adolescent smoking initiation. J Health Econ 2004;23:293-311.

[4] Copeland AL, Carney CE. Smoking expectancies as mediators between dietary restraint and disinhibition and smoking in college women. Exp Clin Psychopharmacol 2003;11:247-51.
[5] Wee CC, Rigotti NA, Davis RB, et al. Relationship between smoking and weight control efforts among adults in the United States. Arch Intern Med 2001;161:546-50.
[6] Kamegai J, Tamura H, Shimizu T, et al. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. Diabetes 2001;50: 2438-43.
[7] Jessen A, Buemann B, Toubro S, et al. The appetite-suppressant effect of nicotine is enhanced by caffeine. Diabetes Obes Metab 2005;7:327-33.
[8] Miyata G, Meguid MM, Fetissov SO, et al. Nicotine's effect on hypothalamic neurotransmitters and appetite regulation. Surgery 1999; 126:255-63.
[9] Jo YH, Talmage DA, Role LW. Nicotinic receptor-mediated effects on appetite and food intake. J Neurobiol 2002;53:618-32.
[10] Yang ZJ, Blaha V, Meguid MM, et al. Infusion of nicotine into the LHA enhances dopamine and 5-HT release and suppresses food intake. Pharmacol Biochem Behav 1999;64:155-9.
[11] Hofstetter A, Schutz Y, Jequier E, et al. Increased 24-hour energy expenditure in cigarette smokers. N Engl J Med 1986;314: 79-82.
[12] Perkins KA, Epstein LH, Marks BL, et al. The effect of nicotine on energy expenditure during light physical activity. N Engl J Med 1989; 320:898-903.
[13] Perkins KA. Metabolic effects of cigarette smoking. J Appl Physiol 1992;72:401-9.
[14] Frankham P, Cabanac M. Nicotine lowers the body-weight set-point in male rats. Appetite 2003;41:1-5.
[15] Cabanac M, Frankham P. Evidence that transient nicotine lowers the body weight set point. Physiol Behav 2002;76:539-42.
[16] Perkins KA. Effects of tobacco smoking on caloric intake. Br J Addict 1992;87:193-205.
[17] Fernandez-Real JM, Broch M, Vendrell J, et al. Smoking, fat mass and activation of the tumor necrosis factor-alpha pathway. Int J Obes Relat Metab Disord 2003;27:1552-6.
[18] Langhans W, Hrupka B. Interleukins and tumor necrosis factor as inhibitors of food intake. Neuropeptides 1993;33:415-24.
[19] Carney RM, Goldberg AP. Weight gain after cessation of cigarette smoking. A possible role for adipose-tissue lipoprotein lipase. N Engl J Med 1984;310:614-6.
[20] Sztalryd C, Hamilton J, Horwitz BA, et al. Alterations of lipolysis and lipoprotein lipase in chronically nicotine-treated rats. Am J Physiol 1996;270:E215-23.
[21] Moffatt RJ, Owens SG. Cessation from cigarette smoking: changes in body weight, body composition, resting metabolism, and energy consumption. Metabolism 1991;40:465-70.
[22] Pidoplichko VI, DeBiasi M, Williams JT, et al. Nicotine activates and desensitizes midbrain dopamine neurons. Nature 1997;390:401-4.
[23] Peeters TL. Ghrelin: a new player in the control of gastrointestinal functions. Gut 2005;54:1638-49.
[24] Bouros D, Tzouvelekis A, Anevlavis S, et al. Smoking acutely increases plasma ghrelin concentrations. Clin Chem 2006;52:777-8.
[25] Fagerberg B, Hulten LM, Hulthe J. Plasma ghrelin, body fat, insulin resistance, and smoking in clinically healthy men: the atherosclerosis and insulin resistance study. Metabolism 2003;52:1460-3.
[26] Langenberg C, Bergstrom J, Laughlin GA, et al. Ghrelin and the metabolic syndrome in older adults. J Clin Endocrinol Metab 2005;90:6448-53.
[27] Poykko SM, Kellokoski E, Ukkola O, et al. Plasma ghrelin concentrations are positively associated with carotid artery atherosclerosis in males. J Intern Med 2006;260:43-52.
[28] Cummings DE, Foster-Schubert KE, Overduin J. Ghrelin and energy balance: focus on current controversies. Curr Drug Targets 2005;6: 153-69.
[29] Marzullo P, Verti B, Savia G, et al. The relationship between active ghrelin levels and human obesity involves alterations in resting energy expenditure. J Clin Endocrinol Metab 2004;89:936-9.