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ORIGINAL ARTICLE

Evaluation of oral mechanical and gustatory sensitivities and salivary cotinine levels in adult smokers

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ABSTRACT

Objective: The aim was to examine oral mechanical and gustatory sensitivities in adult smokers and to estimate salivary levels of cotinine by tobacco consumption. A total of 54 adults (20–45 years old; 28 males/26 females) were divided into two sex-paired groups: smoker group ($n = 27$), tobacco consumers with no other chronic disease/use of chronic medication, and a control non-smoker non-exposed group with similar age ($n = 27$).

Materials and Methods: 24 h-Recall was used to gather information about tobacco consumption, date of onset and duration of the habit. Oral mechanical evaluation comprised touch detection threshold (MDT) of upper and lower lips and tongue tip and two-point discrimination (TPD) assessments. Taste sensitivities for sweet, salty, sour and bitter were evaluated in four concentrations. Salivary cotinine was determined by high performance liquid chromatography. Statistical analysis comprised Mann-Whitney, Two-way ANOVA test and regression analysis.

Results: The mean smoking time was 13.6 years (mean 8.4 mg/day; 13 cigarettes/day). A sex-effect was observed on MDT of tongue tip (higher sensitivity in females), while group-effect was observed on TPD of lower lip, showing a smaller sensitivity among smokers ($p < .05$; moderate effect: Eta partial^2 = 0.076). Although the total score of gustatory sensitivity did not differ between groups, smokers exhibited an irregular pattern of correctly identified tastants among the different concentrations of salty, sour and bitter. The predictive model showed that salivary cotinine was dependent on "nicotine consumption on the day before" ($R^2 = 49\%$).

Conclusion: A difference in tactile sensitivity of the lower lip and qualitative changes in taste sensitivity were observed in smokers.

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KEYWORDS
Tobacco; cotinine; touch perception; taste

Introduction

Smoking is a major risk factor for the development of chronic diseases such as cancer, lung and cardiovascular diseases, thus being a major cause of preventable deaths [1]. It is also considered a public health problem, and disability caused by tobacco-related diseases is related to loss of productivity, and important health and economic consequences for individuals and society [2].

Nicotine is the largest constituent of tobacco of the genus *Nicotiana tabacum*; chemically speaking, nicotine is a tertiary amine composed of pyridine and pyrrolidine rings. Only 10–20% of all inhaled nicotine is excreted unchanged and among the main nicotine biotransformation products is cotinine [3], which can be measured non-invasively in saliva for purposes of tobacco use and tobacco cessation evaluations [4,5]. There is evidence that nicotine may influence the perception of various sensory stimuli through neural mechanisms [6,7] and studies suggest that the longer the time and cigarette consumption, the worse olfactory and taste performances [8], and the effect of alteration on taste perception ability (gustation) would be related to the amount and duration of smoking, and not just after the consumption of a single cigarette [9].

The sensory system is composed of sensory receptors, that is, structures responsible for the perception of stimuli from the environment and from the interior of the body. Taste is a chemical sense that allows the individual to select specific substances according to their wishes and according to the metabolic needs of the body tissues [10], which prepares the gastrointestinal system to receive food and influencing salivation and swallowing (or the pharyngeal reflex – choking – if the substance is unpleasant). According to Strapasson et al. [11], smell and taste disorders considerably reduce the quality of life and may even become severe, as people with reduced taste and odour sensitivity may try to compensate for these losses by increasing the intake of substances that may be harmful if ingested in excess.

Information on food temperature and texture is translated and sent from the mouth through somatic sensory receptors

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of the trigeminal cranial nerves and other sensorial nerves to the thalamus and somatic sensorial cortex. Mechanosensory processing of external stimuli is initiated by the activation of a diverse population of cutaneous and subcutaneous mechanoreceptors on the body surface that relays information to the central nervous system for interpretation and ultimately action. Cumulative exposure to tobacco is hypothesised to cause a reduced cutaneous blood flow that would lead to higher degeneration rates of peripheral nerve termination, lower mechanoreceptor density and lower tactile acuity [12].

Considering that sensory functions are also protective functions of the body, it is important to recognise the deviations and changes present in individuals exposed to tobacco, comparing sex-matched groups. Also, the use of saliva to understand the metabolism and excretion of the main nicotine biotransformation products is of interest, since when excreted in the oral cavity these products will be again swallowed and absorbed. Thus, the objective was to evaluate the tactile and gustatory sensitivities of adult smokers and to compare them with a sex-matched control group of non-smokers non-exposed, and to estimate the salivary cotinine levels through the tobacco consumption profile, thus expanding the knowledge of early tobacco consequences on oral tissues in young individuals.

Materials and methods

Study design and sample

This is a cross-sectional study with sex-matched groups approved by the Research Ethics Committee of the Federal University of São Paulo – UNIFESP (Protocol No. 1389/2016); the subjects were asked to provide verbal and written consent for their participation in the study, and they were informed in detail about all procedures and possible discomforts or risks, following the principles of the Declaration of Helsinki.

Adults aged between 20 and 45 years were invited, among students and employees of the University. After applying the inclusion and exclusion criteria, two sex-paired groups with similar age were comprised: smoker group with 27 adults, tobacco consumers with no other chronic disease or use of chronic medication; and non-smoker non-exposed group, with 27 healthy adults which have never consumed tobacco and were not passive smokers (who did not live with or have relationship with smokers).

By examining the results found by Santos et al. (2013), who evaluated the gustatory and olfactory perceptions smokers, and considering a difference of 2.59 points in the gustatory test between groups, standard deviation equal to 1.5, power of 80% and alpha level 5%, only 10 individuals would be needed in each group (smokers × non-smokers). As in the statistical practice we usually consider that 20 observations are necessary to observe a normal distribution, we opted to include a higher number of individuals.

The following was considered as inclusion criteria: adults (20–45 years) of both sexes. The study excluded: individuals with chronic diseases or who were taking any drug which could alter the variables under study, such as diabetes, hypertension, Parkinson disease, trauma, psychiatric disorders, alcohol, sleep disorders. Volunteers with dental caries, periodontal diseases, dental abscess, orthodontic appliances users, prosthetic users and/or those who reported xerostomia were also excluded.

Interview

By means of an interview, information concerning personnel, medical and dental experiences or treatments were recorded. A 24 h-Recall was used to gather information about tobacco consumption, such as the daily profile of cigarettes use, the tobacco consumption on the day and the day before of salivary collection, date of onset and duration of tobacco habit (years), and preferred cigarette trademark to estimate the consumption of nicotine/day (mg).

The physical examination comprised the evaluation of weight and height to calculate the body mass index (BMI) (Kg/m^2).

Saliva collection

Verbal and written instructions on the saliva collection procedure was offered to the volunteers; one *salivette* tube (Salivette, Sarstedt, Germany) was delivered to each volunteer containing a polyester roll inside for saliva collection at home. The collection was performed on waking up, with the subject still fasting, after a night of tobacco abstinence (~8 h) as a standard procedure, considering that the half-life of cotinine is approximately 20 h [13]. The polyester roll was placed on the tongue, keeping it inside the oral cavity until it is soaked in saliva. The subjects also informed verbally and in writing to avoid any physical activity, ingestion of alcohol derivatives, caffeine, soda, tea and corticosteroid and use of chewing gum in the 24 h before the day of salivary collection. Females were evaluated in the first days of the menstrual cycle (most stable phase).

After the collection, the volunteer delivered the *salivette* tube to the researcher on the same day, according to previous schedule. In the laboratory, samples were centrifuged at 5000 rpm for 10 min (4 °C), separated into eppendorfs and frozen (−80 °C) for further analysis.

Gustatory sensitivity

For the evaluation of the tastants detection threshold (sweet, salty, sour and bitter), a validated methodology called *three-drop-method* was used, a forced-choice method which is described in details in Mueller et al. [14]. Four liquid solutions were used in four different concentrations of each primary tastant: salty – sodium chloride (0.25, 0.1, 0.04, 0.016 g/mL), sweet – sucrose (0.4, 0.2, 0.1, 0.05 g/mL), acid - citric acid (0.075, 0.041, 0.0225, 0.0125 g/mL), bitter - quinine hydrochloride (0.0015, 0.0006; 0.0002; 0.0001 g/mL), which were delivered in drops (3 drops) on the back of the tongue, being 1 drop of the tastant and 2 drops of distilled water. According to the authors, the lowest concentration can be identified by only half of the healthy subjects (without taste

disorders), while the highest concentration can be detected by ~100% of the individuals.

The subjects were asked to refrain from eating, drinking, brushing teeth, smoking or chewing gum consumption 1 h prior to testing [15]. The order of presentation of the tests was drawn for each individual, with four different possibilities. The participant chose one of the four response options for each of the tests: sweet, salty, bitter or acid (sour), with no time limit for the test. All tastants were tested so that the administration of the tests respected the increasing order of concentrations. Between each test participants were instructed to rinse their mouths with mineral water to avoid residual taste that could confuse them. For each test correctly identified, the volunteer received 1 point, and the incorrect answers, either because they could not identify the tastant or because they confused it with another one, did not score points.

Mechanical somatosensory function

The mechanical somatosensory function evaluation comprised two aspects: the evaluation of mechanical detection threshold (MDT), tested on the upper and lower lips and tongue tip and the two-point discrimination (TPD) examined in the lower and upper lips. The examiner (MBR) was instructed and trained for at least one day with regard to MDT and TPD evaluations by an experienced professional and practiced in healthy subjects.

The MDT was measured by using the Semmes-Weinstein Monofilaments (Aesthesio® kit, California, EUA, DanMic Global); the kit contains 20 monofilaments, which exert different forces upon bending, with 20 target forces: 0.008 g; 0.02 g, 0.04 g, 0.07 g, 0.16 g, 0.40 g, 0.60 g, 1.0 g, 1.4 g, 2.0 g, 4.0 g, 6.0 g, 8.0 g, 10 g, 15 g, 26 g, 60 g, 100 g, 180 g, and 300 g.

After being properly guided on how the test would be performed and with the participant sitting in a quiet environment so that he/she could concentrate for the execution of the test and blindfolded, the test was performed in the vermilion areas of the lower lip (mental nerve) and upper lip (infraorbital nerve), on the dominant side of the volunteer (Figure 1); the tip of the tongue was also tested in its central region (lingual nerve).

The filaments were compressed at a 90° angle against the skin until curved, and thus held for 1.5 s (Figure 1). The tests started with the thinnest filament (0.008 g) and other filaments were applied sequentially (thicker filaments) until the volunteer verbally reported a light touch as instructed at the start of the test. This was considered as a positive (+) stimulus. After this positive report, the order was inverted and followed to the next thinner filament, until the volunteer no longer felt the application of the tactile stimulus (slight touch). This was considered as a negative stimulus (−). This measure was performed until five negative stimuli (descending) and five positive (ascending) stimuli were obtained and the geometric mean of these repetitions was calculated [16].

TPD is a technique that measures the minimum distance required to perceive two simultaneously applied stimuli as distinct [17]. The evaluation was carried out using a rigid instrument of tips arranged in pairs at different distances that were lightly pressed on the region to be evaluated (a rotating disk with variable 2-point discrimination). For this evaluation, a two-point discriminator (Touch-TestTM, model NC12776, North Coast Medical, Inc., Ireland) was used with measurements of 1–15 mm (Figure 2). The instrument was held in the same regions mentioned above, with the exception of the tongue. Participants were asked if they felt one or two tips touching the assessed region. Each distance was tested three times and in random order (by lot). The least perceived distance between the two points was considered as an answer, and it should present at least two coincident answers among the three tests.

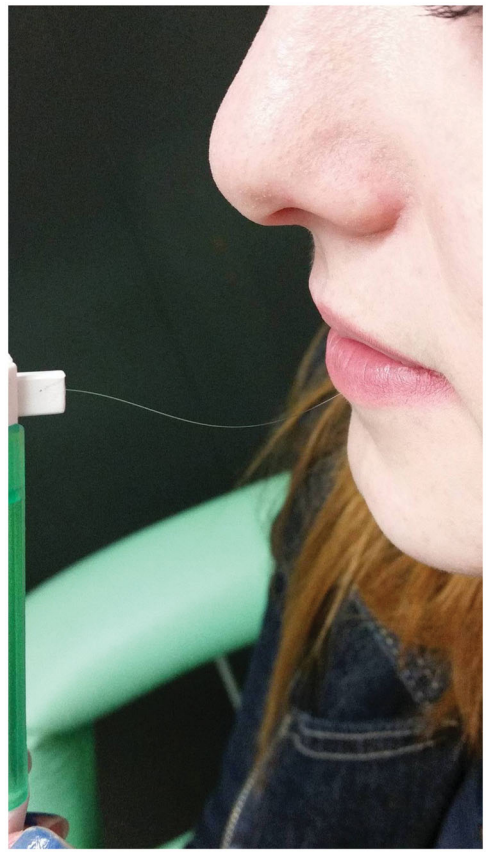

Figure 1. Evaluation of the mechanical detection threshold of the lower lip.

Salivary cotinine determination

Saliva samples were centrifuged in an Eppendorf Refrigerated Centrifuge (Eppendorf, Germany) for 10 min, at 5,000 rpm at 4 °C. Then, samples were filtered (Merck, Germany, 0.22 μm porous size), and transferred to HPLC vials for analysis.

Cotinine determination was performed using the reverse phase system by High Performance Liquid Chromatography (HPLC – LC-10A model, Shimadzu Corporation) coupled to a UV detector, at 260 nm, and a C18 column (150 mm length, 4.6 mm inner diameter, and 5 μm particle, Shim-pack CLC-ODS (M), Shimadzu Corporation. The phosphate buffer, pH 6.8, was prepared by weighing 1.940 g of anhydrous

monobasic potassium phosphate and 2.481 g of anhydrous dibasic potassium phosphate, and dissolved in 700 mL water HPLC grade. The pH was adjusted to 6.8 by adding formic acid solution and then, completed with water HPLC grade to 1,000 mL. The mobile phase consisted of phosphate buffer, pH 6.8, and acetonitrile (93:7, *v/v*). The mobile phase was previously filtered (Merck, Germany, 0.45 μm porous size), and the flow rate was 0.8 mL/min, with an injection volume of 50 μL.

The standard curve was built by using 24 known cotinine concentrations between 95 ng/mL and 475 ng/mL (Cotinine, Sigma-Aldrich, product 74003–25 mg, lot n. BCBT2945) added to centrifuged and filtered pure saliva from one of the researchers (a healthy adult who has never been a smoker or passive smoker). Under the selected chromatographic conditions, it was noted the method is suitable for cotinine determination in saliva. It was not observed any interference neither from endogenous compounds nor from caffeine and nicotine (Figure 3). The method presented good linearity, and a satisfactory correlation coefficient (r) of 0.9902 was obtained. For reliability purposes, cotinine levels were tested on a subgroup of non-smokers and they all showed concentrations below 9.42 ng/mL (similarly to the study of Etter et al. [18]).

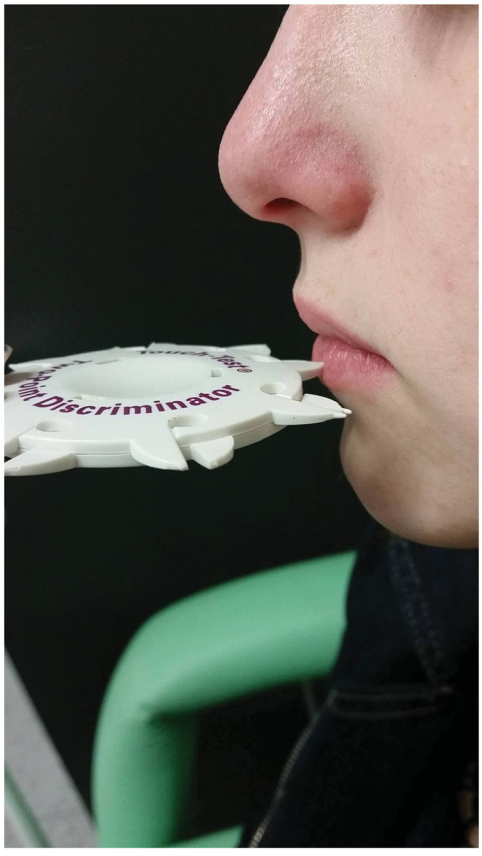

Figure 2. Evaluation of two-point discrimination of the lower lip.

Statistical analysis

Data were statistically analysed using SPSS 24.0 software (IBM Corp., NY, USA), considering an alpha level of 5%, by one of the authors (PMC, Applied Statistics Specialist). The exploratory statistics consisted of percentages, means, standard deviation, and medians. Normality was tested using Shapiro Wilk test and Quantile-quantile-plot (QQ-plot) analysis. The similarity of the groups according to age and BMI was tested using Mann-Whitney test.

A general linear model – Two-way ANOVA – was used to test the effects of *group* and *sex* and the effect of interaction *group***sex* in the observed variance of the mechanical somatosensory function. The effect size (partial *Eta* squared) and power of the test were also obtained and the results of Levene equality of variances test were evaluated as premises of ANOVA.

Additionally, a linear regression model was adjusted by using the stepwise method to obtain a predictive model for salivary cotinine levels (as the dependent variable), considering as explanatory variables: age, sex (male = 0 and female = 1), BMI, years of smoking and calculated nicotine consumption on the day before. The changes in the adjusted R^2 and F-values were considered for the adjustment of the final model, as well as the assumptions of the test: normality, collinearity (VIF and tolerance), independence of errors (Durbin-Watson) and homoscedasticity (residual analysis).

Results

The description of the two sex-paired groups is shown in Table 1. Age and BMI did not differ between groups ($p = .528$ and $p = .829$, respectively), which demonstrates the homogeneity of the sample.

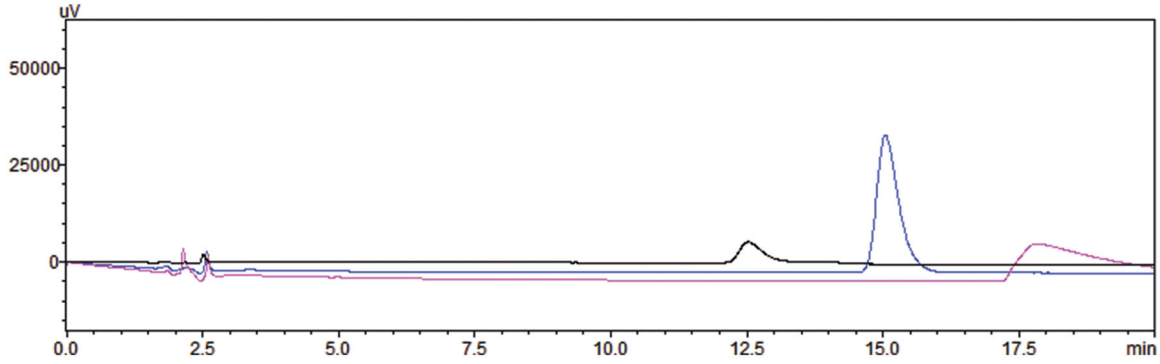

Figure 3. Chromatogram of cotinine injection with concentration of 9.5 ng/mL (black/left peak), caffeine 12.4 ng/mL (blue/middle) and nicotine 100 ng/mL (pink/right peak). (Color figure online.)

Table 1. Demographic characteristics of the sample and smokers' tobacco consumption.

	Fem/ Males		Age (years)	BMI (Kg/m2)	Frequency of smoking (cigarettes/day)	Estimated nicotine consumption (per day)	Estimated nicotine consumption (day before)	Years of smoking	Salivary cotinine levels (ng/mL)
Smokers	13/14	*Mean (SD)*	29.4 (1.7)	23.8 (0.8)	12.9 (8.1)	8.4 (5.2)	6.3 (4.7)	13.6 (8.6)	247.0 (113.3)
n = 27		*Median (25–75%)*	28.0 (22.0-37.0)	23.4 (21.0-26.0)	10.0 (6.0-20.0)	8.0 (4.8-12.0)	6.0 (2.0-9.0)	12.0 (6.0-21.0)	235.9 (177.5-341.2)
Non-smokers	13/14	*Mean (SD)*	27.6 (1.5)	24.3 (0.9)	–	–	–	–	–
n = 27		*Median (25–75%)*	24.0 (22.0-32.0)	23.5 (21.5-25.7)	–	–	–	–	–

Table 2. Smoking and sex effects on mechanical sensory: a Two-way ANOVA analysis.

		Mean (SD)				
	n	MDT upper lip	MDT lower lip	MDT tongue tip	TPD upper lip	TPD lower lip
Smokers	27	0.011 (0.016)	0.101 (0.005)	0.009 (0.004)	4.519 (0.975)	4.704 (1.235)
Non-smokers	27	0.009 (0.003)	0.010 (0.003)	0.008 (0.0030)	4.074 (1.269)	4.074 (1.035)
Group effect	*p-value*	0.531	0.828	0.459	0.153	**0.048**
Sex effect	*p-value*	0.276	0.217	**0.001**	0.759	0.361
Group*sex effect	*p-value*	0.221	0.598	0.459	0.448	0.669
F†	–	1.068	0.635	4.192	0.903	1.693

MDT: mechanical detection threshold; TPD: two-point discrimination.

Additionally, the tobacco consumption in terms of frequency (cigarettes per day) and years of smoking and estimated salivary cotinine levels are described. The average of smoking time was equal to 13.6 years, with a mean consumption of 13 cigarettes daily. This estimate was made by means of the information gathered on the commercial packages of the 9 (nine) different trademarks cited by the participants, which indicated the average amount of nicotine in a cigarette. Smokers showed a mean salivary cotinine levels of 247.0 ng/mL (and median equal to 236 ng/mL).

Table 2 shows the results of mechanical sensory sensitivity evaluation of tongue tip and lips for both groups. According to the Two-way ANOVA analysis, a significant *sex* effect was observed on MDT of the tongue tip (with higher sensitivity observed in females; power = 91%) (Table 2). Additionally, a significant group effect on TPD of the lower lip was observed, showing a smaller lower lip sensitivity among smokers with a moderate effect size (*Eta* partial squared= 0.076) [19]. No significant *sex*group* interaction effect was observed.

The percentages of correctly identified tastants (sweet, sour, bitter and salty) in both groups are shown in Table 3. By reviewing the records, it was observed that the most common taste quality confusion was sour-bitter confusion in both groups (calling citric acid as 'bitter').

The median total score of gustatory sensitivity did not differ between groups. However, smokers exhibited an irregular pattern of correctly identified tastants among the different concentrations of salty, sour and bitter: while an almost linear decrease in the percentage of correct answers could be seen in the non-smokers group for all tastants, in the smoker group conflicting results were observed for salty and sour tastants, with the level 3 achieving lower percentage of correct answers (85 points) than the level 4 (the lowest concentration; 93 and 96 points, respectively). Additionally, in the smoker group the percentage of correct answers for bitter 1 (the highest concentration) was lower than bitter 2.

In order to understand the predictors of cotinine concentrations measured in saliva, a linear regression model was adjusted using the stepwise method to obtain a linear model of salivary cotinine. The explanatory variable which remained in the final model using the backward procedure was the reported nicotine consumption on the day before (Table 4): *(Ln) salivary cotinine = 4.795 + 0.091*nicotine consumption day before*. The final model presented a good fit, and an adjusted R^2 of 0.492 was found, indicating that this model explained 49% of the variation in salivary cotinine level. In addition, the residuals showed a normal distribution, fulfilling one of the premises of the test to obtain a good model.

Table 3. Descriptive analysis and percentages of correctly identified tastes according to groups.

	Range	Correct (%) Non-smokers *n* = 27	Correct (%) Smokers *n* = 27
Sweet 1 (highest)	–	100	100
Sweet 2	–	100	100
Sweet 3	–	96	96
Sweet 4 (lowest)	–	74	82
Sweet total	0–4	–	–
Salty 1 (highest)	–	96	89
Salty 2	–	96	93
Salty 3	–	96	85
Salty 4 (lowest)	–	85	93
Salty total	0–4	–	–
Sour 1 (highest)	–	96	93
Sour 2	–	96	93
Sour 3	–	96	85
Sour 4 (lowest)	–	82	96
Sour total	0-4	–	–
Bitter 1 (highest)	–	100	93
Bitter 2	–	100	100
Bitter 3	–	96	93
Bitter 4 (lowest)	–	74	70
Bitter total	0–4	–	–
Total score (median)	0–16	15	15

Discussion

The present study aimed to compare the mechanical and taste sensitivities between young smokers and non-smokers, using a study design with sex-matched groups. The main

Table 4. Predictive model used to estimate salivary cotinine level.

					Model		
	B	Standard error	*t*	*p*-value	F (*p*-value)	Adj R^2	Durbin- Watson
constant	4.795	0.142	33.775	–	25.176 (<.001)	0.492	2.226
Nicotine consump_day before	0.091	0.018	5.018	<.001			

findings were: (1) the tactile acuity of the lower lip, observed through TPD test, was significantly lower in the group of smokers, regardless of sex; (2) the tactile detection threshold on the tongue was significantly higher in women, regardless of cigarette use; (3) qualitative differences in taste sensitivity between groups were observed and (4) the reported nicotine consumption in the day before explained almost 50% of the cotinine level variation measured in saliva.

The average number of cigarettes consumed per day in the sample was 13 cigarettes, and the estimated amount of nicotine consumed per day was 8.6 mg on average. Brazilian official data showed that in the 27 cities evaluated, the frequency of adults who reported smoking 20 or more cigarettes a day was 2.6%, being higher in males (3.8%) than in females (1.6%); in the total population, the frequency of smokers tended to be lower among young adults (up to the age of 34) and among those aged 65 years and over, the prevalence decreased with increasing schooling [20].

Tactile acuity and smoke

Smoking has been associated with a variety of diseases in the oral cavity; however, few studies evaluated the influence of cigarette smoking on somatosensory sensitivity in orofacial structures. In addition, tobacco users are often unaware of tobacco effects on oral health and evidence of a potential negative effect on sensory perception may reveal a real threat they would like to avoid [21], as sensory functions are also discriminative and protective functions of the body that must be preserved. A previous study showed a reduction in thermal sensitivity, but not mechanical sensitivity, in the region innervated by the lingual nerve in smokers, possibly caused by degeneration of thermosensitive receptors as a consequence of smoking [6], a decrease that seems to persist in former smokers [7]. The present results corroborate those previous observations, since the tactile detection threshold did not differ between cigarette and non-cigarette consumers, regardless of whether the evaluation was performed in the lip or tongue region. However, women presented, regardless of cigarette use, a higher tactile detection threshold in the tongue, but not in the lip. In a previous study, women were more sensitive than men for warm stimuli in the lingual region; the observed lower threshold for those stimuli thus suggests evidence for a neuronal-based higher sensitivity in female's tongue [6]. In that same study, it was found an impaired somatosensation only in the tongue mucosa of adult smokers, while extraoral trigeminal branches of the chin did not seem to be affected.

In contrast, the TPD test differed between groups, as lower tactile acuity was observed in the lower lip of young adult smokers compared to non-smokers, pointing out that early changes may be present among smokers. The use of the discriminator does not cause pain or discomfort and is considered as a valid measure of functional sensitivity [22], in which it is possible to perceive two points instead of one because two distinct populations of neurons are activated; in this sense, this test complements the MDT to determine possible changes in tactile thresholds. Probably, the lower lip was the region that showed this change because it suffers more injuries when compared to the upper lip [16,23].

In addition, it has been shown that chronic smoking induces significant changes in tongue mucosa morphology, calibre and number of capillaries [24], which could also influence the sensitivity of oral cavity structures. Nicotine present in tobacco smoke is a powerful inhibitor of peripheral vasodilation [12], that is, it has a specific effect on microcirculation leading to reduced sensory neuron function and degeneration [6]. According to Stevens et al. [12], individuals who have good overall health and healthy habits seem to have a lower loss of tactile sensitivity due to a greater resistance to environmental and endogenous stressors, although more studies are needed to understand these changes.

Gustatory sensitivity

Smell and taste are sensory functions that can be affected by external agents such as smoking, which through their local irritants seems to increase the taste detection threshold [9,21]. Although of great clinical importance, very few studies attempted to evaluate the possible effects of smoking on taste sensitivity and its impact on nutrition [25]. Smoking can not only reduce the taste of foods, but also make tasty foods perceived as unpleasant due to changes in perception threshold for bitter, salty and sweet substances, decreased overall hedonic impression (pleasure) and possibly aversion to sweet substances [11].

The taste system encodes information about the quantity as well as the quality of the stimuli and, in healthy individuals, the higher the stimulus concentration, the greater the perception of flavour intensity [26]. Although the total score of gustatory sensitivity did not differ between groups, qualitative differences were observed with smokers exhibiting an irregular pattern of correctly identified tastants among the different concentrations of salty, sour and bitter. It was noted an almost linear decrease in the percentage of correct answers among non-smokers from the highest to the lowest concentration of all tastants, while in the smoker group the percentages of corrected answers varied between the different concentrations of salty, sour and bitter, with some lower concentrations being correctly identified more frequently than higher concentrations. In healthy female university students, a previous study [15] showed that there were monotonic increases in the perceived suprathreshold intensity as the concentration of stimuli was increased, similar to that

observed among non-smokers of the present study. These findings corroborate some previous results which mentioned a reduced overall hedonic impression (pleasure) among smokers [11], which ultimately may lead to food aversions and/or taste preferences changes [27].

Thus, one may hypothesise that greater taste confusion in suprathreshold tastant intensities is frequent among smokers due to changes in the number of fungiform papillae [28,29] and/or changes in the function and morphology of the tongue mucosa (neuronal and vascular tissues), as mentioned before [6,7]. Furthermore, the literature suggests that nicotine inhibits neural activity in the tongue mucosa and nasal pathways [9,21]; also, tobacco products of combustion form a layer inside the mouth and airways, preventing olfactory and taste stimuli from coming into contact with receptors [30]. Inhibition of neural activity may occur after prolonged use of tobacco, while formation of a film or layer in the oral and nasal cavity may result from single exposure to combustion products [21,31]. Thus, the recognition of taste changes can positively impact the smoker in an attempt to encourage her/him to stop the habit.

On the other hand, the sweet tastant showed a regular pattern of correctly responses in both groups, probably because this is the most acceptable flavour since birth [32] and subjects in general have more experience with salty and sweet foods than other flavours [33]. Taste quality confusion was frequent in both groups, especially sour-bitter confusion. According to Doty et al. [34], taste confusions can be influenced by procedural factors, such as the way stimuli are presented and the number of response alternatives, as well as by subject factors, such as age and sex; in that study, the most common was also sour-bitter confusion (∼19%) and which causes are poorly understood.

Changes in the perception of tastes that may be a consequence of tobacco use make us reflect on possible changes in eating habits, such as seeking foods with higher intensity of taste, sweeter, salty or fatty, which may endanger those individuals who are prone to diabetes, hypertension and dyslipidemia, respectively [10,11,35]. In addition, such sensory changes seem to influence the swallowing pattern which, consequently, may also impact eating behaviour and should be considered [9].

Salivary cotinine determination

In humans, about 70–80% of nicotine is converted to cotinine, which accumulates in the body as a result of tobacco exposure, crosses the blood-brain barrier and has different pharmacological properties compared with nicotine [36,37]. Although without evidence of addictive or cardiovascular effects in humans [36], the understanding of the metabolism and excretion of cotinine in saliva is of interest, since when excreted in the oral cavity, nicotine metabolism products will again be swallowed and absorbed. In addition, salivary, urine and serum cotinine measurements have been used to validate self-reported smoking status [38,39], with saliva representing an easily obtained source, highly correlated with blood, which can be assayed using gas or liquid chromatography, radioimmunoassay and enzyme immunoassays [13,18,40].

In order to understand the predictors of cotinine concentrations measured in saliva, a linear regression model was obtained and after adjustment only the variable "nicotine consumption on the day before" was included; cotinine salivary levels were not dependent on age, sex, BMI and years of smoking. Similarly, in the study of Ozdener et al. [40] a significant correlation between the reported frequency of cigarette use and cotinine levels measured in nasal lavage fluid was observed, although it is important to note that the amount of nicotine varies between different trademarks, which was considered and quantified to be tested in the present study. The result shows that 24 h-Recal data provided very consistent information about exposure to tobacco smoke, as the predicted model explained almost 50% of the variation in salivary cotinine level. Although useful as a biomarker of nicotine intake, cotinine dosage is not perfect because of the individual variation in nicotine metabolism, which can be affected by race, sex, age, genetic variation in the liver enzyme CYP2A6, and/or by the presence of pregnancy, liver or kidney disease [37].

The inclusion criteria and the needed compliance in providing saliva and information about the smoking habits restricted the number of participants, which may have limited the observation of other clinical changes. However, it is important to emphasise the study strengths, such as the paired sample design controlling for sex effect, a homogeneous age-range, and a "health" sample of smokers, free of chronic diseases or chronic use of medications, which increase the power of the analysis. It is also important to consider that this study included only young adults, as older individuals may present confounding factors that are difficult to control or to exclude from the study, such as cognitive losses, polypharmacy and chronic diseases, which may impact significantly the taste and oral mechanical sensitivities thus making difficult to examine the effect of tobacco specifically. In addition, an in-depth and detailed assessment of the mechanical and gustatory sensitivities, tobacco consumption in terms of years of smoking, number of cigarettes and trademarks consumed was performed to ensure that the results were as reliable as possible.

Although in the present study few mechanical sensory changes were observed, it would be important to investigate in future studies the full battery of quantitative sensory testing [41], including pain and thermal threshold assessments. It should be considered that nicotine as well as other ingredients of tobacco smoke may profoundly affect the function and morphology of the different orofacial structures [6,7]; these sensory changes may result from axonal degeneration or even neuronal demyelination and may not only be restricted to the oral cavity, but also having a systemic effect, which would need to be investigated in a future study.

Conclusion

It can be concluded that the mechanical detection threshold of the tongue and upper lip were not affected by cigarette

consumption; however, a lower tactile acuity in the lower lip was observed in a sample of young smokers. Although the total score of gustatory sensitivity did not differ between groups, smokers exhibited an irregular pattern of correctly identified tastants among the different concentrations of salty, sour and bitter. Finally, 50% of the variation in salivary cotinine was explained by tobacco consumption on the day before.

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