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Salivary evaluation of young habitual caffeine consumers: biochemical and taste characteristics

Avaliação salivar de jovens consumidores habituais de cafeína: características bioquímicas e gustativas

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ABSTRACT

Objective: Caffeine may be related to systemic effects, but information on sex differences and its influence on salivary characteristics is scarce. This study assessed the relationship between caffeine consumption, saliva and gustatory characteristics and heart rate in healthy adults. **Material and Methods:** The sample was composed of 60 individuals (30 men/30 women; 18-27y). Caffeine-based foods, beverages and medicines intake were assessed in three pre-set days. Gustatory sensitivity, resting heart rate, and salivary flow, pH, amylase (AMY), total protein (TP), and caffeine levels were evaluated in stimulated (SS) and unstimulated saliva (US). The data were analyzed using paired t-tests/Wilcoxon tests, one-way and two-way ANOVA, Spearman correlation, and hierarchical regression ($\alpha = 0.05$). **Results:** The reported caffeine intake was higher in males and caffeine concentration was higher in SS compared to US ($p=0.029$). Salivary caffeine correlated with pH ($rho=0.29$; $p=0.025$), while SS AMY and TP concentrations were lower in caffeine consumers. No difference was observed in gustatory sensitivity and resting heart rate considering caffeine intake. According to the hierarchical regression model, caffeine salivary concentration was predicted by age, caffeinated food/beverages intake on the day of collection, and female sex ($p=0.001$; $R^2=0.25$). **Conclusion:** Caffeine intake showed subtle effects on salivary pH and TP. However, a relationship between salivary caffeine concentrations and sex was evidenced, with women consuming less but showing higher salivary caffeine concentration, information useful when considering saliva as a source of caffeine biomarkers.

KEYWORDS

Caffeine; Heart rate; Saliva; Sex differences; Taste perception.

RESUMO

Objetivo: A cafeína pode estar relacionada a efeitos sistêmicos, mas informações sobre diferenças sexuais e sua influência nas características salivares são escassas. Esse estudo avaliou a relação entre consumo de cafeína, características salivares e gustativas e frequência cardíaca em adultos saudáveis. **Material e Métodos:** A amostra foi composta por 60 indivíduos (30 homens/30 mulheres; 18-27 anos). A ingestão de alimentos, bebidas e medicamentos à base de cafeína foi avaliada em três dias pré-definidos. Sensibilidade gustativa, frequência cardíaca em repouso e fluxo salivar, pH, amilase (AMY), proteína total (PT) e níveis de cafeína foram avaliados em saliva estimulada (SE) e não estimulada (SNE). Os dados foram analisados por meio dos testes t pareado/Wilcoxon, ANOVA one-way e two-way, correlação de Spearman e regressão hierárquica ($\alpha = 0,05$). **Resultados:** A ingestão de cafeína relatada foi maior em homens e a concentração de cafeína foi maior na SE em comparação com SNE ($p = 0,029$). A cafeína salivar correlacionou-se com o pH ($rho = 0,29$; $p = 0,025$), enquanto as concentrações de AMY e TP em SE foram menores em consumidores de cafeína. Nenhuma diferença foi observada na sensibilidade gustativa e frequência cardíaca em repouso considerando a ingestão de cafeína. De acordo com o modelo de regressão hierárquica, a concentração salivar de cafeína foi prevista pela idade, ingestão de alimentos/bebidas com cafeína no dia da coleta e sexo feminino ($p=0,001$; $R2=0,25$). **Conclusão:** A ingestão de cafeína mostrou efeitos sutis no pH salivar e PT. No entanto, uma relação entre as concentrações de cafeína salivar e sexo foi evidenciada, com mulheres consumindo menos, mas apresentando maior concentração de cafeína salivar, informação útil ao considerar a saliva como uma fonte de biomarcadores de cafeína.

PALAVRAS-CHAVE

Cafeína; Frequência cardíaca; Saliva; Diferenças sexuais; Percepção gustativa.

INTRODUCTION

Caffeine, an alkaloid belonging to the group of trimethylxanthines, is the most widely used psychoactive substance in the world [1]. Its main source is coffee, however, it may also be present in other commonly consumed foods and beverages, including tea, soft drinks and chocolate [2]. Caffeine is also incorporated into prescription medications, particularly those used to treat headaches and colds, where it acts as an adjuvant to enhance the analgesic efficacy of other compounds [3]. Among young adults and physically active individuals, the use of various caffeine-containing products has increased, including energy drinks, pre-workout supplements, caffeinated chewing gum, energy gels and chews, aerosols, and many other novel caffeinated food products [4]

Caffeine influences various physiological functions, and its effects can vary depending on the dose, the type of product consumed, and individual characteristics such as sex, age, and dietary habits [1,5]. In the nervous system, low (~40 mg or ~0.5 mg kg^{-1}) to moderate (~300 mg or 4 mg kg^{-1}) caffeine doses improves alertness, vigilance, attention, reaction time and attention [6]. However, high doses may lead to anxiety, restlessness, and sleep disturbances [7,8]. In the cardiovascular system, caffeine can temporarily raise heart rate and blood pressure, particularly in people who are not regular consumers [9]. Caffeine also acts as a short-term respiratory stimulant and this effect is especially beneficial in certain medical conditions, such as apnea in premature infants [10]. In terms of metabolism, caffeine may improve energy balance by reducing appetite and increasing the basal metabolic rate and food-induced thermogenesis, and it may have a mild diuretic effect [2]. In addition, caffeine has consistently been shown to improve exercise performance when consumed in doses of 3-6 mg/kg body mass [4].

Over the past decades, saliva has emerged as a valuable diagnostic fluid, capable of reflecting both health conditions and ongoing physiological processes [11,12]. Despite the well-documented systemic effects of caffeine and its important action as a central stimulant [13], little is known about its effect at the salivary glands level and how it would qualitatively and quantitatively modulate salivary secretion and gustatory sensitivity. There is also little information on the excretion of caffeine in saliva. Measuring salivary caffeine concentrations allows researchers and clinicians to assess caffeine intake, clearance rates, and individual sensitivity. Clinically, this approach has potential utility in pharmacokinetic studies, behavioral or sleep-related assessments, and personalized recommendations regarding caffeine consumption, particularly in individuals with sleep disorders or cardiovascular risks. A key advantage of using saliva as a diagnostic matrix lies in its non-invasive nature, making it especially suitable for populations with restrictions to venipuncture, such as children, aged, and hemophiliac patients [14]. Moreover, previous studies have demonstrated a strong correlation between serum and salivary caffeine levels ($r > 0.8$), supporting the reliability of saliva as a biological fluid for monitoring caffeine exposure [10,15,16].

Although no sex differences have been found in caffeine salivary levels following acute administration [17,18], no information was found related to habitual intake of caffeine-based foods/drinks. In this sense, we hypothesized that caffeine may have an influence on salivary characteristics, which may differ between habitual and non-habitual consumers and between sexes. Thus, the objective was to assess the salivary and gustatory characteristics of young adults according to the intake of caffeinated-based foods and beverages.

MATERIAL AND METHODS

Study design and participants

Ethical approval was obtained from Ethics Committee of the Federal University of São Paulo – UNIFESP (CAAE 18250313.8.0000.5505 and 319.793/13), and all participants provided written informed consent in accordance with the Declaration of Helsinki (1964) and its later revisions.

A convenience sample of participants was selected and comprised 60 young healthy adults (30 males and 30 females), aged between 18 and 27 years-old (undergraduate students). This age group was selected intentionally to represent young healthy adults with high and consistent consumption patterns, especially from coffee and energy drinks, which are common in university-aged individuals. Participants were interviewed concerning their dental and medical history. The exclusion criteria were:

presence of systemic, neurological or chronic disease; individuals presenting tooth decay or gingivitis; dental prosthesis users; current orthodontic treatment; smokers; use of chronic medications that could influence the salivary analysis; hormonal contraceptive use. Females were examined during the most stable menstrual cycle phase (early follicular: 3 - 4 first days of the menstrual cycle).

Clinical examination

Participants were clinically examined on a dental chair with artificial light, explorer and a dental mouth mirror by a dentist (PMC). Clinical examination was performed in individuals in order to verify the oral tissues and teeth conditions. Only individuals with complete permanent dentition and absence of caries lesions, dental pain or any kind of oral diseases were included.

Weight and height were measured using a digital electronic scale and a portable stadiometer, following standardized procedures for adults as outlined in the NHANES Anthropometry Procedures Manual (CDC, 2020) [19]. Participants were positioned upright, with their body aligned to the vertical axis, for the calculation of body mass index (BMI = kg/m^2). The maximum and average resting heart rate were recorded using a portable heart rate monitor (CS100, Polar, Finlândia) during saliva collection always in the afternoon (between 3-5 pm) to avoid the influence of the circadian rhythm [20].

Evaluation of caffeine consumption

Caffeine consumption was recorded in detail using a standardized form with the same structure of the 24-hour recall, regarding the day of saliva collection and the day before collection (full days); a second assessment was performed on an additional day, two to seven days prior to the saliva collection, which would reflect the habitual consumption of foods and/or beverages containing caffeine. Participants provided information about all foods, candies, drinks and medicines consumed; the forms were provided in advance to be filled out during the scheduled days, thus preventing recall bias. The three 24-hour recalls were analyzed by a nutritionist using the DietWin software (Dietwin® professional) to estimate caffeine consumption in mg. For statistical analysis of the data, the sample was divided in two subgroups according to caffeine consumption: *habitual* and *non-habitual consumers* (the latter who achieved a consumption of zero mg of caffeine in the three pre-set days according to the 24-Recall).

Saliva collection

Stimulated and unstimulated saliva samples were collected at the laboratory, always in the afternoon, at least 2h after the last meal and 1h after toothbrush. Participants were instructed not to use alcohol and to not perform physical exercises in the day before sampling.

First, they were seated comfortably in an upright position and rinsed their mouth with distilled water, being asked to lean forward and spit all the unstimulated saliva produced for five minutes into a cooled tube, through a glass funnel. The stimulated saliva was collected with the participant chewing on 0.3 g of an inert and tasteless material (Parafilm, Merifeld, USA) for 5 minutes. The average flow rate was calculated from the total volume by time of secretion (g/min and mL/min) [21].

Immediately after saliva collection the salivary pH was measured using a portable pHmeter for 30 seconds (Tecnal, TEC5, Piracicaba, SP, Brasil) (Eckley et al.) Further, the saliva samples were centrifuged at 14,000 rpm for 10 minutes at 4 °C and stored at -80 °C until analysis.

Gustatory sensitivity

After saliva collection, taste sensitivity was assessed using the 'there-drop-method', as previously described [22]. Four concentrations of the four basic flavors were used: 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), sour – citric acid (0.3, 0.165, 0.09, 0.05 g/mL), bitter – quinine hydrochloride (0.006, 0.0024, 0.0009, 0.0004 g/mL), totaling 16 tastant solutions diluted on distilled water which were administered using a pipette at the middle of the tongue (in a blinded procedure), being one drop of the taste solution and the two others of distilled water. All solutions were stored in amber glass bottles with no visible identification of the taste.

Immediately after administration of the three drops, the participant selected the flavor among four options: sweet, salty, bitter or acid (sour). The administration sequence was randomized through simple randomization trials and the flavors were tested at increasing concentrations.

Between tests, the participant drank a sip of water to avoid interactions between the gustatory stimuli. For each test correctly identified, the participant received 1 (one) point and incorrect answers, either or not identifying the flavor or confusing with another flavor, he/she did not receive a point (maximum of 16 points).

Salivary analysis

Alpha-amylase concentration on stimulated and unstimulated saliva was determined by measuring the enzymatic activity in diluted saliva (1:25), while total protein concentration was determined by colorimetric method (ELI Tech, Seppim SA, Sees, France) in pure saliva and using automated technique (Vital Scientific, Dieren, Switzerland), as described earlier [22]

Salivary caffeine concentration was measured by high-performance liquid chromatography (HPLC) based on the method described in Scott et al. (1984) [23]; a Shimadzu analytical system was used which consisted of a controller (model CBM-20a), a pump (model LC-10Ai), an automatic injector (model SIL-10Ai), detector (model DAD SPD-M20A), and ODS C18 (159 mm x 4,6 mm) column. The mobile phase was composed of acetonitrile:tetrahydrofuran:acetic acid:water (20:20:5:955). The run was carried out under a flow rate of 1 ml/min with the column at 35°C and the detector set to read at 273 nm.

For sample preparation, 400 μL of saliva was homogenized with 800 μL of mobile phase in na eppendorf under vortexing for 1 minute. The eppendorfs were then kept on ice for 15 minutes and then vortexed again for 1 minute and centrifuged at 9,000 rpm for 10 minutes at 4 °C. The supernatant was removed and transferred to the vial for analysis [21].

The calibration curve was obtained with known concentrations: Y = -142.092 (98515.6) x; R=0.9999746. Figure 1 shows the chromatogram of the caffeine standard at a concentration of 1μg/ml, showing the retention time around 14 minutes (left). On the right is the chromatogram of a saliva sample from a healthy individual who habitually consumes caffeine-containing beverages (retention time of around 14 minutes).

Statistical analysis

Data were statistically analyzed using SPSS 27.0 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 means, standard deviation, medians and quartiles. Normality was tested by the Shapiro-Wilk test. Tests were chosen based on the type of data (categorical/continuous) and on the distribution of the data (if symmetrical, parametric tests were chosen).

Differences in salivary aspects between stimulated and unstimulated saliva samples were evaluated by means of paired *t*-test/Wilcoxon (with or without normal distribution, respectively) and differences between sexes were tested using one-way ANOVA. Spearman's correlation test was applied to verify the correlation between variables.

The two-way ANOVA was used to test the effect of caffeine consumption (*habitual versus non-habitual consumers)*, sex (males x females), and the interaction between these two factors on salivary characteristics, gustatory sensitivity and heart rate. The equality of variances was tested using the Levene's test. In this analysis, one participant was excluded due to missing value.

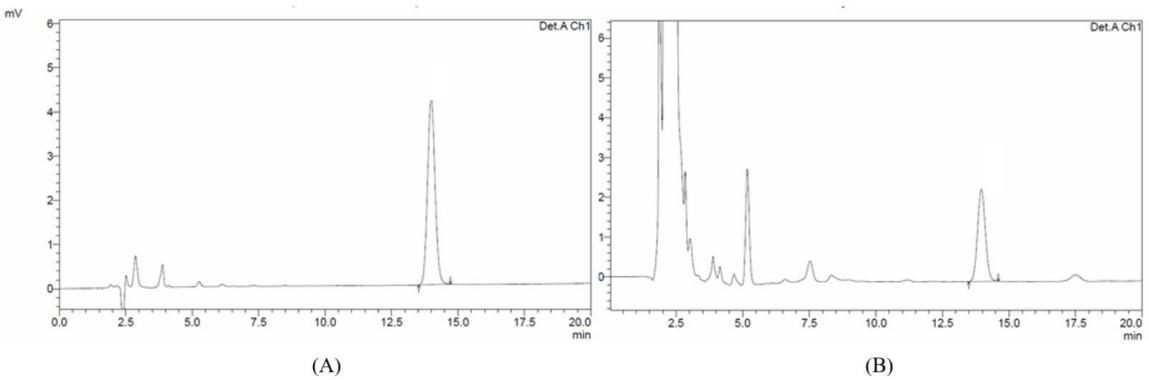

Figure 1 - Profile of the high-performance liquid chromatogram of the caffeine standard solution 1 µg/ml (A) and the profile chromatogram of a healthy volunteer habitual caffeine consumer (B). Retention time of approximately 14 minutes and wavelength of 273 nm.

A linear regression model was adjusted by the hierarchical method to obtain a predictive model of salivary caffeine concentration in unstimulated saliva (as the dependent variable), considering as independent variables the consumption of food/beverages containing caffeine on the day of collection and the day before, age and sex. For the adjustment of the final model, the changes in the adjusted R^2 and F-values were considered for each new independent variable added, as well as the assumptions of the test: normality, collinearity (VIF and tolerance), independence of errors (Durbin-Watson) and homoscedasticity (residual analysis).

RESULTS

This study included healthy young adults, undergraduate students free of chronic diseases or other medical condition that could have an influence in the analyses. Divided by sex, the subgroups were similar according to age, BMI, resting heart rate, and gustatory sensitivity (Table I).

The consumption of caffeine was extracted from the 24h-Recall applied to participants, mainly foods and drinks (coffee, tea, soft drinks). Only one participant reported having ingested an analgesic that contained caffeine. The reported intake of caffeine was higher in males in the three preset days, and this difference was significant in the day before saliva collection (p=0.033), what was not reflected in the salivary caffeine concentration. The caffeine consumption of the day of saliva collection should be considered as half a day, as the saliva sample was collected between 3-5 pm. Most of the participants showed low to moderate caffeine intake.

Considering the total sample of participants, caffeine concentrations differed between unstimulated (median and 25-75%: 0.20 and 0.1-0.6) and stimulated saliva (0.17 and 0.1-0.5; p=0.029). Besides, by examining the correlation matrix between caffeine concentration and salivary characteristics, it was observed that unstimulated salivary caffeine concentration correlated with salivary pH (r=0.29; p=0.025), but not with flow rate, amylase and total protein concentrations (Table II).

Table I - Description of the sample divided by sex

	Age (years)	BMI (Kg/m²)	Average heart rate (bpm)	Maximal heart rate (bpm)	Gustatory sensitivity score	[Caffeine] US (µg/mL)	[Caffeine] SS (µg/mL)	Caffeine consumption 1 week before (mg/day)	Caffeine consumption day before (mg/day)	Caffeine consumption day of collection (mg/half a day)
	Mean (SD)	*Median (25-75%)*	*Mean (SD)*	*Mean (SD)*	*Median (25-75%)*	*Median (25-75%)*	*Median (25-75%)*	*Mean (SD)*	*Mean (SD)*	*Mean (SD)*
Females (n=30)	21.4 (2.0)	21.2 (20.2-23.3)	83.3 (8.6)	97.8 (11.0)	16 (16-16)	0.29 (0.1-0.6)	0.24 (0.1-0.5)	41.2 (109.0)	27.4* (59.8)	18.0 (57.4)
Males (n=30)	21.93 (2.4)	24.3 (21.2-29.4)	82.2 (11.7)	105.9 (28.2)	16 (14-16)	0.12 (0.1-0.5)	0.12 (0.1-0.5)	106.5 (189.2)	102.6* (178.8)	52.7 (99.6)
Total (n=60)	21.7 (2.2)	22.4 (20.6-25.5)	82.8 (10.2)	101.2 (21.6)	16 (15-16)	0.20 (0.1-0.5)	0.17 (0.1-0.5)	73.3 (155.9)	64.4 (136.6)	35.0 (82.1)

BMI = body mass index; US = unstimulated saliva; SS = stimulated saliva; SD = standard deviation.
*p=0.033 (ANOVA, F=4.761; partial η^2 = 0.08 [medium]; power=60%).

Table II - Matrix of correlation between caffeine concentration and salivary characteristics

[Caffeine] Unstimulated saliva			[Caffeine] Stimulated saliva		
Flow rate US	rho	0.24	Flow rate SS	rho	0.15
	p-value	0.066		p-value	0.247
pH US	rho	**0.29**	pH SS	rho	0.03
	p-value	**0.025**		p-value	0.835
AMY US	rho	0.09	AMY SS	rho	-0.12
	p-value	0.547		p-value	0.402
Total protein US	rho	-0.05	Total protein SS	rho	-0.12
	p-value	0.705		p-value	0.402

US = unstimulated saliva; SS = stimulated saliva; AMY = amylase; rho = Spearman correlation coefficient.

Table III shows the results of the sample divided according to caffeine intake: habitual (n=38) and non-habitual consumers (n=21) (the latter who achieved a consumption of zero mg of caffeine in the three pre-set days according to the 24-Recall). There was a significant interaction effect group*sex for the concentration of amylase in stimulated saliva: the concentration was higher in men non-habitual caffeine consumers (p=0.004). The concentration of total protein in stimulated saliva was also higher in non-habitual consumers (p=0.048; *eta* partial^2=0.07), with no significant effect of sex (Table III).

The scores of gustatory sensitivity for the four basic tastes were not different between habitual consumers (mean total score 15.3±1.3) and non-habitual caffeine consumers (mean total score=15.5±1.1) (p=0.499), nor it was the mean (81.7±10.6 bpm and 85.0±9.7 bpm; p=0.293) and maximal (100.5±21.0 bpm and 103.3±23.1; p=0.365) resting heart rate, respectively; in both cases, no significant effect of sex was observed.

The linear regression model used to predict the salivary caffeine concentration (Appendix 1) showed that caffeine concentration was dependent on the amount of caffeine consumed in the day of salivary collection, but not the day before collection. The addition of caffeine consumption on the day before collection did not show a significant change (R^2 change=.001; F change=.071). Besides, the independent variable 'sex' was significant, and it is expected an increase of 0.323 μg/mL in the caffeine concentration in females (compared to males), keeping the other independent variables constant.

The final model showed a good fit, as observed by the parameters of tolerance, VIF, residual analysis, and independence of errors (Durbin-Watson); an R^2 of 0.25 was found, meaning that 25% of the variation in the concentration of caffeine was explained. The regression coefficients are also depicted in Figure 2.

Table III - Description of the sample divided according to caffeine consumption

	n male/ female	[Caffeine] US (µg/mL)	[Caffeine] SS (µg/mL)	US flow rate (mL/min)	SS flow rate (mL/min)	US pH	SS pH	US AMY (U/L)	SS AMY (U/L)	US total protein (mg/dL)	SS total protein (mg/dL)
Habitual consumers	23/15	0.63 (0.78)	0.59 (0.72)	0.6 (0.3)	1.5 (0.7)	6.8 (0.3)	7.2 (0.2)	2068.5 (114.0)	1989.6 (997.2)	84.9 (59.3)	62.4* (18.8)
Non-habitual consumers	6/15	0.15 (0.16)	0.11 (0.13)	0.6 (0.4)	1.2 (0.7)	6.8 (0.3)	7.2 (0.3)	2138.8 (1369.1)	2040.2 (980.4)	76.8 (34.6)	74.5* (27.6)

US = unstimulated saliva; SS = stimulated saliva; AMY = amylase. *Group effect (2-way ANOVA; p = 0.048; partial η^2 = 0.07.

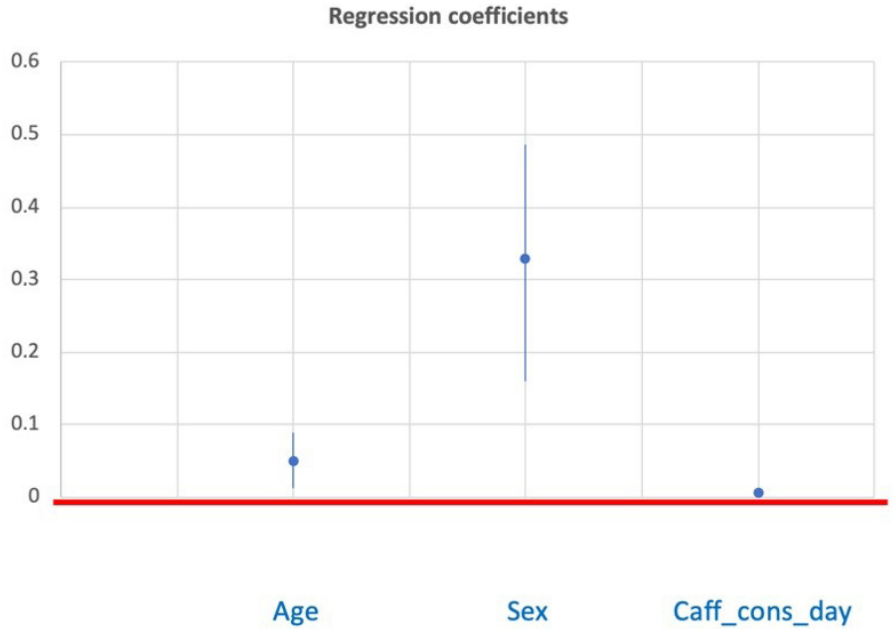

Figure 2 - Regression coefficients (SE) of independent variables age, sex, and caffeine consumption the day of saliva collection that predicts caffeine concentration in non-stimulated saliva (constant B = -0.834; p = 0.001; R^2 = 25%).

DISCUSSION

By including a healthy sample of young students, this study showed that caffeine consumption may have subtle effects on salivary characteristics, but evidenced important differences in caffeine salivary levels between sexes, with females consuming less caffeinated-based foods and beverages but showing higher salivary caffeine concentration. To our knowledge, this is the first study that described the sex differences in caffeine salivary levels related to habitual ingestion.

The study did not find differences in the sensitivity of the four basic tastes according to habitual caffeine consumption, while the study by Tanimura and Mattes [24] observed that caffeine non-consumers showed greater sensitivity to bitter taste than regular consumers (moderate and high), probably due to a tolerance effect. Conversely, Ong et al. [25] stated that the consumption of coffee, tea and alcohol is shaped by genetic variants associated with the perception of bitter substances. According to Masi et al. [26], the density of fungiform papillae also plays a determining role in taste sensitivity to bitter compounds, such as coffee, what emphasizes the complex interactions of many biological and environmental aspects that shape taste sensitivity and food choices.

Similarly, the mean and maximum resting heart rates did not differ between habitual consumers and non-consumers of caffeine. According to the literature, caffeine has a central stimulant action by secreting catecholamines, which would raise blood pressure [27]; however, this effect appears to be more evident in susceptible individuals, such as those who have hypertension [28-30]. There is also the possibility that the individual develops tolerance to caffeine through regular consumption [31-33].

Salivary amylase and total protein did not correlate with the salivary levels of caffeine. While stress can stimulate alpha-amylase secretion by activating the sympathetic nervous system, previous studies have shown that caffeine alone does not change salivary alpha-amylase activity [29,30,34]. The study by Klein et al. [30] showed that caffeine administered alone did not change salivary alpha-amylase activity even when exposing participants to a cognitive (but not stressful) task. Also, caffeine administration did not affect the levels of salivary immunoglobulin A during training sessions performed by athletes in a placebo double-blinded trial [35]. Conversely, the concentration of total protein in stimulated saliva was higher in non-habitual consumers, as well the amylase levels, although the latter was observed only in men. However, it is important to consider that alpha-amylase is involved in oral sensory perception, namely in sweet taste sensitivity [36] and the perception of starchy foods [37], and its secretion is also influenced by dietary patterns, taste perception, and nutritional status [36].

No correlation was found between caffeine levels and saliva flow, which is in line with a previous study [38] that investigated the effect of the intake of caffeine-based soft drinks on the salivary flow in healthy adults; although the literature suggests a possible vascular and diuretic effect of caffeine [27,39], the authors did not observe significant changes in salivary flow one hour after ingestion. The present results showed a correlation between caffeine concentration and pH in unstimulated saliva, probably due to the action of caffeine as an alkaloid; however, this correlation was not observed in the stimulated one, probably because of pH variation due to increased salivary flow; this finding is relevant when it is hypothesized that higher consumption of foods/beverages containing caffeine can increase resting salivary pH.

There are few studies that have described the secretion of caffeine in saliva. The present study found a subtle difference in caffeine concentration between unstimulated and stimulated saliva, the latter with lower concentration, probably because of a dilution effect; however, caffeine concentration did not correlate with salivary flow. In the study of Biederbick et al. (1997), no difference was found between sublingual and parotid salivary glands. Thus, caffeine levels do not seem to be dependent on salivary flow and gland [40]. In preterm infants who are treated with caffeine for apnea of prematurity, in whom blood sampling must be severely restricted, salivary sampling demonstrated to be a valid non-invasive alternative that could be used to individualize and optimize caffeine dose [10]. Decreased salivary caffeine concentrations was found in patients with Parkinson disease, and the reason remains unclear; one of the hypotheses would be the reduced albumin levels in old age, that might lead to a higher proportion of free caffeine subject to faster metabolism [41].

According to Suzuki et al. [42], salivary caffeine concentration is lower than plasma and probably reflects the unbound plasma fraction that diffuses passively and rapidly [43] The binding of caffeine to plasmatic albumin is around 27-30% [44], and albumin is the most abundant plasma protein that acts as a transporter for many substances, such as calcium and some drugs. In the present study, females had an increase of 0.323 μg/mL in unstimulated salivary caffeine concentration compared to males. On the contrary, the study of Leodori et al. [41] found no gender effect on the salivary caffeine concentration, caffeine absorption, or caffeine metabolism of Parkinson's disease patients (mean age 66y). However, both results can be explained by the sex variation in serum albumin concentration observed by Weaving et al. [45], who found a consistently lower serum albumin concentration in women from the age 16, which becomes close to male values at 60 years. Taken together, this information justifies the higher concentration of caffeine found in the saliva of female participants considering that they reported lower intake of caffeine-based products.

Conversely, a previous study did not find sex differences in salivary caffeine clearance after 2h and 14h post-caffeine acute administration [18]. Considering that the caffeine excreted in the saliva is swallowed again, there is a possibility that the caffeine circulates for a longer time in the female organism, justifying the higher concentration of caffeine in the saliva related habitual ingestion found in the present study and raising the possibility of long-lasting effects of caffeine in women.

Urinary elimination of caffeine is low [43], and a previous study found higher urine caffeine concentrations and excretion rates in men and individuals aged 40-59y [46]. Besides, considering that males and females differ in their subjective and physiological responses to caffeine administration [47], it is important to highlight the differences and specificities between biological samples and sexes to ensure the collection of the most reliable caffeine biomarkers in sport studies and when testing exercise conditions.

The strength of the study was the inclusion of sex-paired groups of healthy, non-medicated participants; in addition, the main confounding factors known to affect caffeine levels were controlled: age, BMI, caffeine-based food, beverages and medicine consumption, tobacco smoking, and oral health. However, the use of a convenience sample composed exclusively of young university students may limit the generalizability of the findings to broader and more diverse populations. Therefore, the results should be interpreted and generalized considering this limitation.

CONCLUSION

This study highlights significant relationships between caffeine consumption, salivary characteristics, and sex differences. While caffeine intake was higher in males, females exhibited higher salivary caffeine concentrations, suggesting that sex plays an essential role in caffeine metabolism or clearance. The findings also indicate that caffeine consumption has a modest influence on salivary pH and total protein levels, but no significant impact on gustatory sensitivity or resting heart rate was observed. These results contribute to the understanding of caffeine's systemic effects and offer valuable insights when using saliva as a biomarker source for caffeine assessment.

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Author's Contributions

KGOS: Conceptualization, Writing – Original Draft Preparation. LMC, TLI, CR: Methodology, Data Curation. FLAF: Project Administration, Funding Acquisition. ECP: Conceptualization, Methodology. PMC: Conceptualization, Formal Analysis, Data Curation, Writing – Review & Editing, Supervision.

Conflict of Interest

The authors report there are no competing interests to declare.

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Regulatory Statement

The present study was conducted in accordance with all the provisions of the local human subjects' oversight committee guidelines and policies of the Ethics Committee of the Federal University of São Paulo – UNIFESP (CAAE 18250313.8.0000.5505 and 319.793/13).

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Appendix 1. Predictive model obtained by hierarchical method to estimate the concentration of caffeine in unstimulated saliva (US)

Table A1 - Predictive model obtained by hierarchical method to estimate the concentration of caffeine in unstimulated saliva (US)

[caffeine] unstimulated saliva	B	t	Sig	F (p-value)	R^2
constant	-0.834	-1.024	0.310	6.052 (0.001)	0.25
Age	0.046	1.253	0.216		
Sex (female)	0.323	2.009	0.050		
Caffeine consumption day of collection	0.004	3.724	0.000		

Tolerance = 0.936; VIF = 1.068.