

ORIGINAL ARTICLE

Descriptive analysis of the masticatory and salivary functions and gustatory sensitivity in healthy children

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ABSTRACT

Objective: The objective of this study is to better understand salivary and masticatory characteristics, this study evaluated the relationship among salivary parameters, bite force (BF), masticatory performance (MP) and gustatory sensitivity in healthy children. The secondary outcome was to evaluate possible gender differences.

Materials and methods: One hundred and sixteen eutrophic subjects aged 7–11 years old were evaluated, caries-free and with no definite need of orthodontic treatment. Salivary flow rate and pH, total protein (TP), alpha-amylase (AMY), calcium (CA) and phosphate (PHO) concentrations were determined in stimulated (SS) and unstimulated saliva (US). BF and MP were evaluated using digital gnathodynamometer and fractional sieving method, respectively. Gustatory sensitivity was determined by detecting the four primary tastes (sweet, salty, sour and bitter) in three different concentrations. Data were evaluated using descriptive statistics, Mann–Whitney/*t*-test, Spearman correlation and multiple regression analysis, considering $\alpha = 0.05$.

Results: Significant positive correlation between taste and age was observed. CA and PHO concentrations correlated negatively with salivary flow and pH; sweet taste scores correlated with AMY concentrations and bitter taste sensitivity correlated with US flow rate ($p < 0.05$). No significant difference between genders in salivary, masticatory characteristics and gustatory sensitivity was observed. The regression analysis showed a weak relationship between the distribution of chewed particles among the different sieves and BF.

Conclusions: The concentration of some analytes was influenced by salivary flow and pH. Age, saliva flow and AMY concentrations influenced gustatory sensitivity. In addition, salivary, masticatory and taste characteristics did not differ between genders, and only a weak relation between MP and BF was observed.

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Introduction

Saliva is an exocrine secretion of the salivary glands, containing water, electrolytes, proteins and enzymes. It provides sensory perception of food, and aids chewing, swallowing and digestion of food.[1] The salivary flow rate is critical for the maintenance of whole body health; it helps bolus formation by moistening food, protects the oral mucosa against mechanical damage, plays a role in preliminary digestion and has defence functions against pathogen micro-organism.[2] In addition, increased remineralization and decreased demineralization of enamel depend on salivary fluoride, PHO and CA contents.[3]

The reduction of food particles is determined by a complex multifactorial process, which depends on factors such as bite force (BF), the coordination of chewing muscles, craniofacial morphology and the number of occluding pairs of teeth.[4] BF is one of the components of the stomatognathic system and has been used as a reliable parameter to evaluate

the functional characteristics of the masticatory muscles. According to the literature, it is assumed that BF is related to the integrity of the mastication muscles; therefore, a greater BF would result in a more efficient mastication.[5]

The fragmentation and humidification of food is the primary function of mastication, when taste and food texture are perceived, which influence chewing process.[6] Taste is a primary aspect by which children determine food acceptance and plays an essential role in eating behaviour.[7] Taste substances should be dissolved in the salivary fluid layer to reach and stimulate taste receptors during the initial process of perception; in addition, saliva plays a role in the maintenance of taste buds and taste-sensing cells.[8] As there is a mutual relationship between saliva flow and diet, food does not only influence salivary flow, but saliva can also affect sensory perception.

Proper salivary, chewing and swallowing functions are of great importance, since it affects food's digestion and has

potential influence on subject's nutritional status. The oral phase is the shortest stage of the digestion, although little attention has been given to the understanding of salivary and masticatory characteristics in healthy children. Thus, the aim of this study was to describe the characteristics and evaluate the relationship between salivary function and composition, BF, masticatory performance (MP) and gustatory sensitivity in caries-free children; the secondary outcome was to evaluate possible gender differences in the studied parameters.

Material and methods

Sample

At the beginning of the study, students from three public schools of Piracicaba (SP, Brazil) were invited to participate; from those who agreed formally to participate, 116 prepubertal eutrophic subjects aged 7–11 years were included (64 girls/52 boys) considering the inclusion/exclusion criteria.

The procedures, possible discomforts or risks, as well as the possible benefits, were fully explained to the children and their parents/guardians, and the Ethics Committee of Piracicaba Dental School (University of Campinas) approved the study (Protocol no. 017/2012). Informed consent was obtained from all individual participants included in the study.

The inclusion criterion was prepubertal children with mixed dentition. The exclusion criteria were pain of dental origin, premature tooth loss, anomalies of shape, number, structure, tooth decay, trauma and soft tissue abnormalities; symptoms of temporomandibular and/or masticatory muscles pain; presence of systemic disturbance, cerebral palsy, among others; current use of medications; use of dental prostheses; previous orthodontic treatment and girls who had been through menarche.

The sample size calculation was based on the results from a pilot study, which included 19 healthy children aged 7–9 years, caries-free and with no definite orthodontic treatment need, which were not included in the final sample. Considering a correlation coefficient between BF and MP equal to -0.30 , power $=0.80$ and alpha level 0.05 , it was found that 86 subjects would satisfy the adopted criteria. A second calculation considered a correlation coefficient between gustatory total score and salivary flow rate equal to 0.37 , power $=0.80$ and alpha level 0.05 ; it was found that 56 subjects would be sufficient. The largest sample size was chosen, and the final sample included was 20% higher to prevent losses.

Anthropometric evaluation involved the measurements of stature and weight by means of a digital stadiometer. The body mass index ($BMI = kg/m^2$) was calculated in order to include only eutrophic subjects, according to the reference data BMI-for-age and sex (5–19 years).[9]

During sample selection, caries experience was evaluated by determining the number of decayed, missing and filled teeth for permanent and primary dentitions (DMFT and dmft-t, respectively).[10] Children with decayed and/or missing teeth were not included.

The Index of Orthodontic Treatment Need – Dental Health Component (IOTN-DHC) was used for occlusion evaluation. The DHC registers, using a millimeter probe, the occlusal characteristics of a malocclusion that harm the teeth and adjacent structures. There are five grades, from score one (there is no need for treatment) up to score five (there is a great need for treatment). This index serves as basic guide for an impartial judgment of the malocclusion.[11] Children with definite treatment need (scores 4 and 5) were not included.

Saliva collection and biochemical analysis

Stimulated (SS) and unstimulated (US) saliva were collected in the morning, with all subjects having refrained from eating, drinking or brushing their teeth for a minimum of 2 h before collection. They were trained to avoid swallowing saliva and asked to lean forward and spit all the US produced for 5 min into a cooled tube, through a glass funnel. After, SS was collected by subjects chewing on 0.3 g of an inert and tasteless material (Parafilm, Merifeld, VI), for approximately 70 cycles/min. SS and US flow rates were defined as the weight of saliva secreted per min (g/min).[12]

Saliva pH was determined immediately after collection, using a portable pH-meter (Orion 3 Star Benchtop, Thermo Electron Corporation, Waltham, MA). Next, the samples were centrifuged, fractionated and frozen for further analyses ($-80^{\circ}C$), according to the previous methodology.[12] Alpha-amylase (AMY) concentration was determined by measuring the enzymatic activity in diluted saliva (1:25). Total protein (TP), calcium (CA) and phosphate (PHO) concentrations were determined by colorimetric methods (ELI Tech, Seppim SA, Sees, France) in pure saliva and automated technique (Vital Scientific, Dieren, Switzerland) at the Clinical Analyses Laboratory of ABC Medical School (Santo André, Brazil).

Assessment of gustatory sensitivity

The perception of four different tastes was obtained at three different concentrations: salty – sodium chloride (0.1; 0.04; 0.016 g/mL), sweet – saccharose (0.2; 0.1; 0.05 g/mL), sour – citric acid (0.165; 0.09; 0.05 g/mL), bitter – quinine hydrochloride (0.0024; 0.0009; 0.0004 g/mL), totalling 12 taste solutions which were administered with a pipette (three drops) at the middle of the tongue. This methodology ("Three-drop-method") was adapted from Mueller et al.,[13] who described a validated method with four different concentrations for each taste. A reduction in the number of tests (16–12 tastants) was employed to adapt the method for children, and the solution of higher concentration of each flavour was excluded from the original method, since the use of 16 tastants showed to be very tiring in the pilot study.

The sequence of administration was randomized across trials by simple randomization; the examiner assigned subjects to each test (four options) randomly, using opaque envelopes, for every assignment (for more details, see [13]). The child was presented with each tastant and then selected the flavour among four options: sweet, salty, bitter or acid (sour). All flavours have been tested in increasing concentrations.

Between tests, the participant was instructed to drink a sip of mineral water in order to avoid interactions between the taste stimuli; the interval between the exposures of each taste was 1 min. For each test correctly identified, the child received 1 (one) point, and incorrect responses, either by failing to identify the flavour or have it confused with another flavour, not scored points (a maximum of 12 points). All tastants were stored in amber glass bottles without visible identification of the flavours.

Maximal bite force

Maximum unilateral BF was assessed with a digital gnathodynamometer (Digital Dynamometer DDK, Kratos Equip. Ind. Ltda., Cotia, Brazil), using a fork strength of 10 mm connected to a digital device. The children were instructed to bite the fork as forcefully as possible in each side of the dental arches (left/right), at the level of the first permanent molars. The recordings of each side were performed two times, with an interval of 2 min, and the maximum value was chosen, with an accuracy of 0.01 N.

Masticatory performance determination

MP was assessed by the determination of the individual capacity of fragmentation of a test material (Optocal). The components were blended and placed in metal moulds with cubic compartments measuring 5.6 mm, under mechanical pressure. The subjects received 10 cubes, which were chewed for 20 mastication cycles. After drying, the fragmented particles were removed from the paper filter, weighed and passed through a series of 10 granulometric sieves with meshes ranging from 5.60 mm to 0.71 mm, connected in decreasing order and closed with a metal base. The particles were placed in the first sieve of the series and the set was maintained under vibration for 20 min. The particles retained on each sieve were removed and weighed on an analytical scale with a precision of 0.001 g. The distribution of the particles by weight was described by a cumulative function (Rosim–Ramler equation). The degree of fragmentation of the material is given by the median particle size (X_{50}), which is the aperture of a theoretical sieve, through which 50% of the weight of the fragmented material could pass. The component “b” describes the distribution range of the chewed particles; higher “b” indicates that the particles were trapped in larger opening sieves.[11]

Reliability of the studied variables

For assessment of method error of the studied variables, the intraclass correlation coefficient (ICC) was calculated from data obtained from 25 children (7–10 years old) not included in the studied sample, on two separate occasions at an interval of 15 d (BioEstat 5.0; Mamirauá, Belém, PA, Brazil). ICCs for gustatory sensitivity and salivary parameters are shown in Table 1. The following criteria were adopted: ICC <0.2, poor; 0.21–0.40, weak; 0.41–0.60, moderate; 0.61–0.80, substantial, and 0.81–1.0, excellent to perfect.[14] For the evaluation of

Table 1. Reliability of the studied variables assessed by means of intraclass correlation coefficients (ICC).

Clinical variables	Intraexaminer ICC	Interexaminer Kappa
DMFT/dmf-t	–	0.97
Gustatory sensitivity	0.92	–
Saliva flow	0.81	–
Saliva pH	0.70	–

DMFT/dmf-t: number of decayed, missing and filled permanent/deciduous teeth.

dental status (dmf-t/DMFT index), agreement was assessed using the Kappa test.

Statistical analysis

Statistical analysis was performed using BioEstat 5.0 (Mamirauá, Belém, PA, Brazil) and SigmaPlot 11 (Systat Software Inc., Chicago, EUA, IL) with a 5% significance level. Descriptive statistics analysis (mean, standard deviation, median and interquartile range) and normality test (Shapiro–Wilk) were applied.

The differences in salivary, masticatory and gustatory variables between genders were analyzed using Mann–Whitney Rank Sum test or *t*-test, where appropriate. A Spearman correlation matrix was obtained between salivary flow rate, pH, salivary biochemical composition and gustatory sensitivity scores.

A regression analysis with stepwise backward elimination was used to test the relationship between the component “b” (distribution of chewed particles in the different sieves as the dependent variable) and the following independent variables: age, stage of dentition, gender, salivary flow rate and BF.

Results

The description of clinical and masticatory characteristics of the sample is shown in Table 2, with no significant difference between boys and girls. Table 3 shows the descriptive data of gustatory sensitivity for both genders. A significant positive correlation between total gustatory score and age ($r = 0.3026$; p value = 0.0014) was observed. That is, higher the age, greater the gustatory sensitivity.

Table 4 shows the descriptive data of salivary biochemical analysis; they also did not show significant difference between genders. According to the Spearman correlation matrix obtained (Table 5), it was observed that CA and PHO concentrations correlated negatively with salivary flow rate and pH.

The correlation between the four taste scores and the biochemical composition of saliva was also tested. It was observed that sweet scores correlated positively with AMY concentrations in US saliva ($r = 0.2119$; $p = 0.0487$). In addition, bitter taste sensitivity correlated positively with US flow rate ($r = 0.2250$; $p = 0.0310$).

The final multiple regression model obtained with the relationship between MP and the studied variables is shown in Table 6. A weak relationship between “b” (distribution of particles in the different screens) and BF was observed.

Table 2. Clinical and masticatory characteristics according to gender.

	Girls (n = 64)		Boys (n = 52)		p Value
	Mean (SD)	Median (25–75%)	Mean (SD)	Median (25–75%)	
Age (months)	111.97 (11.60)	–	112.35 (13.48)	–	0.88 ^a
BMI (kg/m ²)	16.40 (1.30)	–	16.12 (1.30)	–	0.26 ^a
X ₅₀	–	3.07 (2.56–3.69)	–	3.24 (2.90–4.20)	0.07 ^b
b	–	2.15 (1.89–2.46)	–	2.18 (1.86–2.47)	0.86 ^b
Bite force (N) (mean of left/right side)	–	259.76 (202.41–367.28)	–	268.51 (227.15–363.20)	0.41 ^b

BMI: body mass index, X₅₀: median size of test food particle; b: broadness/distribution of particles in the different sieves.

^at-test.

^bMann–Whitney Rank sum test.

Table 3. Descriptive data of gustatory sensitivity and gender comparison.

	25%	Median	75%	p Value ^a
Sweet taste scores (range 0–3)				0.24
Girls	2.0	3.0	3.0	
Boys	2.0	3.0	3.0	
Bitter taste scores (range 0–3)				0.64
Girls	2.0	3.0	3.0	
Boys	2.5	3.0	3.0	
Salt taste scores (range 0–3)				0.37
Girls	2.0	3.0	3.0	
Boys	1.0	3.0	3.0	
Sour taste scores (range 0–3)				0.44
Girls	3.0	3.0	3.0	
Boys	2.0	3.0	3.0	
Total score (range 0–12)				0.64
Girls	9.0	11.0	12.0	
Boys	8.0	10.0	12.0	

^aMann–Whitney rank sum test.

Age, gender, stage of dentition and salivary flow rate were not significantly associated with MP. The model accounted for only 7% of the variation of “b”, with a power of the test equal to 52.6%.

Discussion

It was observed a positive correlation between gustatory sensitivity and age, that is, higher the age, greater the gustatory sensitivity, corroborating the results found by Overberg et al.[15] Reasons for differences in taste sensitivity are poorly understood, and some findings about genetic, hormonal, learning and sociocultural factors suggest a multifactorial cause.[15,16] With regard to learning effect, the exposure to a variety of distinct taste stimuli in early childhood appears to be essential, and a better taste differentiation with increasing age is thought to be part of the normal development.[15] In adults, a clear distinction between men and women in their gustative perception was observed,[16] with women presenting a more accurate sensory perception, which was not observed in the pre-pubertal children enrolled in the present study. The sense of taste plays a critical role in an individual's food preferences, which ultimately guide the dietary behaviour and, thus, his nutritional status.[17]

CA and PHO are important electrolytes which provide protection against the development of dental caries. It was observed that CA and PHO concentrations decreased while saliva flow increased under masticatory stimulation, when the parotid glands participate to a greater extent, agreeing with the results found by de Campos et al.[12] Under stimulation and with the increase in saliva flow, the decrease in PHO concentration is compensated by the rise in pH, which occurs

due to the increased secretion of bicarbonate.[18] AMY concentrations did not significantly correlate with US and SS saliva flows, as it was shown previously that AMY concentration is independent on flow rate.[19]

A previous study [20] showed that high-protein concentration in saliva contributes to a greater adherence of *S. mutans*, one of the first inhabitants of dental plaque,[21] and, according to previous findings, subjects with reduction of the salivary flow rate and low salivary pH may show a high tendency towards dental caries.[21] In caries-free subjects, an increase in salivary pH [22] and a decrease in total protein levels [22,23] were observed when comparing with caries-active subjects. However, no significant correlation between total protein and salivary pH, and total protein and saliva flow was observed in caries-free children included in the present study, probably because total protein, salivary pH and flow may differ between clinical conditions (caries-free/caries-active), but probably they do not vary together.

Salivary flow rate can modify the concentration of tastants and various soluble taste perception-related mediators because of a dilution effect and by the fact that hydrophobic molecules may diffuse more easily into the epithelium, increasing or decreasing the perception.[24,25] The present study observed a positive correlation between bitter taste sensitivity and US flow rate; a previous study observed that subjects with higher salivary flow rates exhibited higher perceived intensity for acid solutions than subjects with low flow rates.[24] Also, elevation of taste thresholds and a reduction of the taste nerve responses were found after surgical removal of salivary glands in an animal model.[26]

A positive correlation between sweet taste and AMY concentrations in US saliva was also observed. AMY is responsible for starch hydrolysis in the oral cavity and accounts for up to 50% of salivary protein in human saliva. Although the majority of ingested starch is digested in the small intestine by pancreatic amylase, a previous study-related AMY levels with the oral perception of starchy foods attributes,[27] which may ultimately benefit nutrition.[28] Future studies are needed to clarify if starch-based food intake stimulates the output of AMY. Moura et al. [29] emphasized the need for more information on this subject by a multidisciplinary team, enabling early diagnoses and interventions in cases of taste changes.

The relationship between MP and gender was evaluated in previous studies, obtaining contradictory results. Some authors found no significant gender differences in masticatory efficiency and MP in subjects aged 6–15 years.[30,31] Maki et al. [32] also did not find differences between girls and boys aged 7–8 years old, but among 9 years old subjects

Table 4. Descriptive data of salivary biochemical analysis and gender comparison.

	Girls (n = 64) Median (25–75%)	Boys (n = 52) Median (25–75%)	p Value ^a
US flow rate (g/min)	0.51 (0.34–0.76)	0.48 (0.34–0.72)	0.67
SS flow rate (g/min)	0.80 (0.60–1.11)	0.79 (0.61–1.14)	0.77
US pH	7.02 (6.86–7.18)	7.12 (6.88–7.21)	0.38
SS pH	7.31 (7.17–7.44)	7.37 (7.25–7.47)	0.53
US amylase (U/L)	1977.50 (1114.75–2845.50)	1724.00 (978.50–2863.50)	0.83
SS amylase (U/L)	2122.00 (1366.00–3070.00)	1761.50 (1153.25–2638.75)	0.44
US calcium (mg/dL)	2.05 (1.43–3.13)	1.75 (1.00–3.15)	0.29
SS calcium (mg/dL)	1.30 (0.90–2.00)	1.45 (0.85–2.22)	0.79
US phosphate (mg/dL)	12.55 (9.25–16.23)	11.10 (9.83–13.78)	0.39
SS phosphate (mg/dL)	9.80 (7.70–12.20)	9.50 (8.20–10.88)	0.54
US total protein (mg/dL)	42.70 (32.45–57.85)	32.45 (26.60–55.10)	0.12
SS total protein (mg/dL)	39.90 (23.80–51.90)	31.65 (24.60–51.93)	0.55

US: unstimulated saliva; SS: stimulated saliva.

^aMann–Whitney rank sum test.

Table 5. Correlation matrix showing the coefficients between salivary flow rate and pH and biochemical parameters.

r (p value)	US calcium	SS calcium	US phosphate	SS phosphate	US amylase	SS amylase	US total protein	SS total protein
US flow rate	–0.12 (0.24)	0.06 (0.57)	–0.53 (0.00)*	–0.39 (0.00)*	0.04 (0.72)	–0.07 (0.52)	–0.12 (0.25)	0.03 (0.81)
SS flow rate	–0.26 (0.01)*	–0.17 (0.11)	–0.32 (0.00)*	–0.36 (0.00)*	0.11 (0.30)	0.13 (0.23)	–0.12 (0.25)	–0.09 (0.39)
US pH	–0.39 (0.00)*	–0.27 (0.03)*	–0.26 (0.02)*	–0.31 (0.00)*	0.01 (0.91)	0.05 (0.61)	–0.15 (0.17)	–0.15 (0.16)
SS pH	–0.25 (0.02)*	–0.29 (0.01)*	–0.16 (0.13)	–0.23 (0.03)*	0.17 (0.10)	0.11 (0.31)	–0.01 (0.96)	–0.09 (0.43)

* $p < 0.05$ (Spearman correlation test). r: Spearman correlation coefficient; US: unstimulated saliva; SS: stimulated saliva.

Table 6. Multiple regression analysis with stepwise backward elimination of the dependent variable *b* ($R^2 = 0.07$; power = 0.526).

Dependent variable	Independents variables	Coef.	p Value
Ln (<i>b</i>)	Constant	0.992	–
	Maximal bite force	–0.001	0.043

Ln: logarithmic transformation; *b*: broadness/distribution of particles in the different sieves.

Normality test (Shapiro–Wilk): $p = 0.226$. Constant variance test: $p = 0.166$.

they observed that boys presented better masticatory efficiency than girls. In adults, this distinction seems to be clearer due to gender-related differences in occlusal force,[31,32] which may become significant after puberty.[33] In the pre-pubertal children enrolled in the present study, either no significant difference in BF was observed, corroborating previous studies.[5]

A weak relation between MP (“*b*”) and maximum BF was observed. The present results corroborate the previous findings which showed that MP improves with greater BF,[11,31] although the model accounted for only 7% of the variation of “*b*”. Since food breakage may require other attributes beyond maximum force, other variables not included in this study may also influence MP variation, such as diet, craniofacial morphology, occlusal contact area and masticatory behaviour.[34] It is important to consider that the properties of the food being chewed, in terms of its hardness, fat content, size and structure, may influence the evaluations. Natural foods have the advantage of being well-accepted and more pleasant taste and texture than test materials, which may be considered the limitation of the present study.

In conclusion, the concentration of some analytes was associated with saliva flow and pH in healthy children. In addition, age, salivary flow and AMY concentrations were related significantly with gustatory sensitivity. Exploratory studies are important to better understand the masticatory and salivary functions, obtaining parameters from healthy children to help the early detection of alterations.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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