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Characteristics of the saliva flow rates of minor salivary glands in healthy people

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ABSTRACT

Objectives: To investigate the normal range and characteristics of saliva secretion in the minor salivary glands (MSGs).

Design: The flow rates of MSGs were measured in 4 anatomical locations of oral mucosa, and the relationship between MSG flow rates and whole saliva flow rates were assessed in 300 healthy subjects stratified by age and sex. An additional 30 young females were further evaluated for flow symmetry, effects of stimulation, circadian effects in MSGs, and the relationship with the flow rates of major salivary glands.

Results: (1) The mean saliva flow rates were 2.10 ± 0.66 (lower labial glands), 2.14 ± 0.62 (upper labial glands), 2.88 ± 0.72 (buccal glands) and 2.15 ± 0.51 (palatal glands) $\mu\text{l}/\text{min}/\text{cm}^2$, respectively. The flow rate of buccal glands was significantly higher than the rates of SMGs in other locations ($P < 0.01$). (2) 5-year-old children had the lowest MSG flow rates ($P < 0.0001$) while the 10–14-year-old group had the highest ($P < 0.001$). (3) MSG flow rates were independent of sex ($P > 0.05$), right vs. left ($P > 0.05$), and citric acid (2.5%) stimulation ($P > 0.05$). (4) Only labial MSG displayed a significant secretory circadian rhythm with the highest rate in the evening ($P < 0.05$). (5) A weak correlation was found between the flow rate of palatal glands and that of unstimulated whole saliva ($r = 0.195$, $P = 0.007$).

Conclusions: Our findings provide a reference for functional evaluation of MSGs and for donor site selection of MSG transplantation for treatment of severe dry eye syndrome.

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1. Introduction

The surface of oral tissues is covered by a thin coat of saliva. This film is mainly composed of small amounts of mucous secretions from the minor salivary glands (MSGs). MSGs are distributed throughout the oral mucosa, and according to their

locations, they can be divided into labial, buccal, palatal, lingual and retromolar glands.¹ There are 600–1000 MSGs in the human oral cavity, and they contribute to less than 10% of the volume of whole saliva.² Despite the small volume of saliva secreted, MSGs have long been considered to be of significant importance for oral tissue protection, lubrication, maintenance of local immunity and the sense of taste. These

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Abbreviations: MSGs, Minor salivary glands; SM, Submandibular gland; PG, Parotid gland.

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functions are supported by their unique anatomical distribution, their proximity to oral tissue surfaces, and the relatively large amounts of mucins and immunoglobulins compared with the major salivary glands. MSGs are thought to secrete spontaneously, which makes these functions possible when people are at rest, such as during sleep.³ However, our knowledge about the saliva secreted from MSGs is much less compared to that about whole saliva or saliva from major salivary glands. Furthermore, reported data on the saliva flow rates of MSGs are conflicting because of various uncontrollable factors.³

Saliva flow rates vary across MSGs in different locations. It is widely accepted that the flow rate of the buccal glands is relatively high, followed by the lower labial and palatal glands.⁴ However, the normal values of saliva flow rates in MSGs in different locations, which is very important to evaluate the secretory function of MSGs, are of great variation. Other factors, such as age and sex, are known to influence the flow rate of whole saliva. Stimulation with citric acid increases secretion of the major salivary glands. Saliva secretion follows the circadian rhythm, with the lowest secretion rate during sleep and the highest rate in late afternoon.⁵ However, most of these flow rates were measured for whole saliva or saliva from major salivary glands, and it is not clear whether the saliva flow rate of MSGs has the same characteristics as that of whole saliva or saliva from major salivary glands. Therefore, a well-controlled systemic study is critical to reveal the characteristics of the saliva flow rates of MSGs in healthy people.

Several features of MSGs may cause the difference in secretion from major salivary glands. Firstly, the structure of MSGs is not as complex as that of major salivary glands. MSGs consist of small clusters of secretory cells with a short excretory duct that transports the saliva to the surface of the mucosa.⁶ Secondly, apart from the lingual von Ebner's glands, which secrete serous saliva, MSGs are predominantly mucous, with a varying amount of sero-mucous cells in their structure. Thirdly, the MSGs have little or no sympathetic innervations. In addition to other innervations that include neuropeptide-containing (vasoactive intestinal peptide, substance P, neuropeptide Y) and nitric oxide synthase-positive nerve fibres, MSGs are mainly controlled via the parasympathetic nervous system with cholinergic transmission.^{7,8} It is supported by the studies that parasympathetic agonist (carbachol) activates and antagonist (atropine) blocks secretion from the cells of human labial glands.^{9,10} Whether other factors influencing the secretion of the major salivary glands are also related to the secretion of MSGs remains unclear.

The aim of this study was to investigate the normal range of saliva flow rates from MSGs and the characteristics of MSGs in different locations in healthy subjects.

2. Materials and methods

2.1. Subjects

Three hundred and thirty subjects (150 males and 180 females; aged 5–89 years) were enrolled in this study. They are divided into two groups. In the first group, 300 subjects were enrolled for measurement of the saliva flow rates from

MSGs. The subjects were further divided into five age groups: 5, 10–14, 15–44, 45–59, and 60–89 years, with 30 males and 30 females in each group. In the other group, 30 females aged 21–25 years were enrolled to observe the flow rates of MSGs at symmetric sites and after stimulation with 2.5% citric acid, and the possible circadian rhythm of saliva secretion. All subjects were in good health, had good oral hygiene, and were free of caries and salivary gland diseases. None of them had received any medication that could cause dry mouth. This project was approved by the Ethics Committee of the Peking University School of Stomatology (IRB00001052-080-48). All subjects signed their informed consent for participation.

2.2. Saliva collection

To reduce possible influence of circadian and seasonal variations on saliva secretion, saliva samples were collected at the same time of day, at 09:00 AM and 11:00 AM, in an air-conditioned room, where room temperature was kept 20–24 °C and humidity was kept 40–70%.¹¹ Subjects were asked to refrain from eating, drinking, smoking, and brushing their teeth for at least 90 min before collection. Saliva collection was performed by an experienced researcher.

2.3. Collection of whole saliva

Before collection, the subjects were instructed to rinse their mouths with water and then rest for 5 min with their eyes open and head tilted slightly forward. By using the spitting method,¹² whole saliva at rest was collected for 5 min into a pre-weighed cup. After 5 min of rest, the stimulated whole saliva was collected by smearing 2.5% citric acid solution on the lateral side of the tongue with a swab every 30 s for another 5 min. By defining specific gravity of saliva as 1, flow rates were calculated and recorded in ml/min.

2.4. Collection of saliva from MSGs

MSG saliva was collected from the following sites of oral mucosa (Fig. 1): the upper labial mucosa (left to the midline, halfway between the vermilion border and the labial fraenum), the lower labial mucosa (left to the midline, halfway between the vermilion border and the labial fraenum), the buccal mucosa (halfway between Stensen's duct and the angle of the mouth) and the palatal mucosa (5 mm left to the midline, medially at the border of the soft and hard palate). Saliva from the palatal gland was not collected in 5-year group and 10–14-year group considering their inability to cooperate.

During collection of labial gland saliva, the labial mucosa was everted gently. During collection of buccal or palatal gland saliva, the subjects were asked to keep their mouths wide open. After the mucosa was carefully dried with gauze, a strip of filter paper (Whatman No. 41, 1 × 2 cm² in size) was immediately placed onto the mucosa and then light pressure was applied. The saliva was collected for 30 s. The strip of filter paper was removed and placed in an air-tight container to protect the collected saliva from evaporation. The container and the strip were weighed before and after

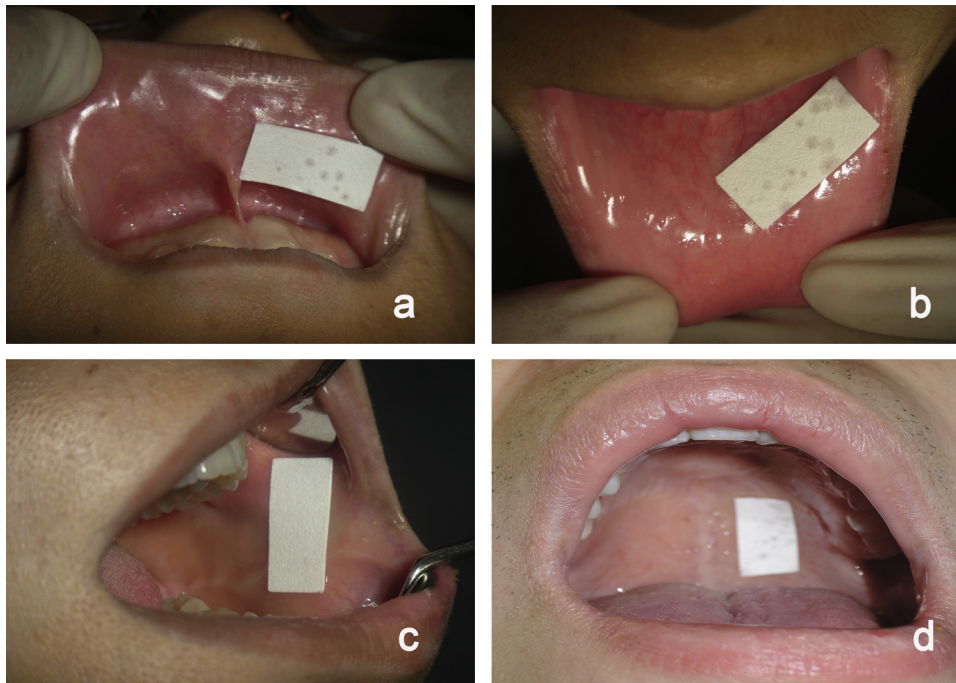


Fig. 1 – Saliva collecting sites. (a) Upper labial glands (b) Lower labial glands (c) Buccal glands (d) Palatal glands

collection using an analytical balance (Denver Instrument Co. Ltd., Beijing; TP-114 Max 110 g $d = 0.1$ mg). By defining specific gravity of MSG saliva as 1, flow rates were calculated and recorded in $\mu\text{l}/\text{min}/\text{cm}^2$.

2.5. Influence of location, stimulation, and biorhythm on the flow rates of MSGs and collection of major salivary glands saliva

In the extra group of 30 females aged 21–25 years, we further measured the resting flow rates of MSGs on the right side, the stimulated (with 2.5% citric acid) flow rates of MSGs on the left side, and the resting flow rates of MSGs at two extra time points on the same day (afternoon, between 15:00 and 17:00; evening, between 19:00 and 21:00), following a previously described procedure. With modified Lashley cup and the Wolff apparatus,^{12,13} saliva from the parotid gland (PG) on the left and the submandibular gland (SMG) at rest as well as after citric acid stimulation was collected for 5 min.

2.6. Statistical analysis

The results of flow rate were presented as mean \pm SD. SPSS 16.0 was used for the statistical analyses. The difference in saliva flow rates between male and female was analysed by independent samples t-test. The variation of saliva flow rates at different locations and ages were analysed by one-way analysis of variance. The effects of acid stimulation and symmetrical location were analysed by paired sample t-test. The circadian rhythm of MSG secretion was analysed by analysis of variance. The correlation of flow rates between MSGs and major salivary glands or whole saliva was analysed by partial correlation. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Saliva flow rates of MSGs

In the 300 healthy subjects, the mean saliva flow rates were 2.10 ± 0.66 (lower labial glands), 2.14 ± 0.62 (upper labial glands), 2.88 ± 0.72 (buccal glands) and 2.15 ± 0.51 (palatal glands) $\mu\text{l}/\text{min}/\text{cm}^2$, respectively (Table 1). Among MSGs, the buccal glands had the highest secretion rate ($P < 0.01$), followed by the palatal glands and labial glands. No significant difference was found between saliva flow rates of the palatal, upper labial and lower labial glands ($P > 0.05$).

3.2. Saliva flow rates according to age and sex

The mean flow rates of MSGs in different locations in healthy subjects of different age and sex are shown in Table 1. No significant difference was found according to sex in the saliva flow rates of MSGs in different locations ($P > 0.05$). A significant difference was found between the five age groups ($P < 0.0001$, Fig. 2). The highest rate was found in 10–14-year group ($P < 0.001$), followed by 15–44-year group ($P < 0.001$), and then 45–59-year group and 60–89-year group ($P > 0.05$), with 5-year group having the lowest value.

3.3. Effect of symmetrical location, stimulation with citric acid and biorhythm on the saliva flow rate

In the 30 female subjects aged 21–25 years, the mean saliva flow rate of MSGs at symmetrical sites is shown in Table 2. No significant difference was found between MSGs at symmetrical sites ($P > 0.05$), including lower labial, upper labial, and buccal glands.

Table 1 – Flow rates of whole saliva and MSGs according to age and sex.

Age (year)	Sex	N	Resting whole saliva	Stimulated whole saliva	Lower labial glands	Upper labial glands	Buccal glands	Palatal glands
5	M	30	0.31 ± 0.20	1.57 ± 0.78	1.58 ± 0.50	1.85 ± 0.58	2.65 ± 0.68	
	F	30	0.39 ± 0.26	1.65 ± 0.56	1.60 ± 0.42	1.81 ± 0.52	2.53 ± 0.32	
10–14	M	30	0.44 ± 0.37	1.93 ± 1.07	2.49 ± 0.64	2.44 ± 0.54	3.40 ± 0.62	
	F	30	0.37 ± 0.34	2.10 ± 1.03	2.37 ± 0.60	2.31 ± 0.76	3.14 ± 0.62	
15–44	M	30	0.57 ± 0.30	3.52 ± 1.26	2.40 ± 0.62	2.26 ± 0.54	2.83 ± 0.66	2.24 ± 0.42
	F	30	0.41 ± 0.27	2.52 ± 1.15	2.49 ± 0.63	2.12 ± 0.47	2.96 ± 0.49	2.22 ± 0.34
45–59	M	30	0.44 ± 0.41	2.70 ± 1.73	2.06 ± 0.55	2.16 ± 0.52	2.81 ± 1.11	2.25 ± 0.58
	F	30	0.27 ± 0.19	2.53 ± 0.99	2.00 ± 0.45	2.01 ± 0.63	2.82 ± 0.51	1.97 ± 0.47
60–89	M	30	0.54 ± 0.36	2.22 ± 1.12	2.07 ± 0.60	2.27 ± 0.67	2.95 ± 0.84	2.08 ± 0.59
	F	30	0.36 ± 0.34	1.93 ± 1.20	1.83 ± 0.68	2.17 ± 0.72	2.64 ± 0.80	2.10 ± 0.62
Total	M + F	300	0.41 ± 0.32	2.27 ± 1.24	2.10 ± 0.66	2.14 ± 0.62	2.88 ± 0.83**	2.15 ± 0.51
	M	150	0.45 ± 0.34	2.36 ± 1.38	2.09 ± 0.66	2.18 ± 0.60	2.91 ± 0.83	2.19 ± 0.53
	F	150	0.36 ± 0.28	2.19 ± 1.07	2.10 ± 0.66	2.09 ± 0.63	2.84 ± 0.60	2.11 ± 0.48

ml/min for whole saliva and $\mu\text{l}/\text{min}/\text{cm}^2$ for MSGs. Values represent mean \pm SD.

** $P < 0.01$ compared with labial and palatal glands.

After stimulation with 2.5% citric acid, the saliva flow rates of MSGs did not show any obvious increase ($P > 0.05$), including lower labial, upper labial, and buccal glands.

The mean saliva flow rates of MSGs and whole saliva in the morning (9:00–11:00), afternoon (15:00–17:00), and evening (19:00–21:00) on the same day are shown in Tables 2 and 3. A significant difference in the flow rate of resting whole saliva was found at different times. The flow rates in the afternoon ($P = 0.01$) and in the evening ($P = 0.004$) were higher than in the morning. However, the flow rates of whole saliva stimulated with 2.5% citric acid did not show any time-based difference ($P > 0.05$). The saliva flow rates of the lower labial glands ($P < 0.05$) and upper labial glands ($P < 0.001$) showed time-based variations, which were different from those observed for resting whole saliva (Fig. 3). The saliva flow rate of the buccal glands did not show any time-based variations ($P = 0.198$).

3.4. Correlation between the flow rates of whole saliva and MSGs

There was a weak correlation between the resting flow rate of whole saliva and the palatal glands ($r = 0.195$, $P = 0.007$, $n = 180$

as lack of data for 5-year group and 10–14-year group). No correlation was found between flow rates of other MSGs and major salivary glands or whole saliva. The flow rates of MSGs in 4 locations correlated with each other ($P < 0.01$). Details are shown in Table 4.

4. Discussion

In this study, we measured the flow rate of MSGs to determine the normal range; to understand the influence of age, sex, location, biorhythm and stimulation with 2.5% citric acid; and to elucidate the relationship between the flow rate of MSGs and that of major salivary glands or whole saliva.

There are no standard protocols for the collection of saliva from MSGs. Nonetheless, various studies with subjects of different ages and both sexes using different collecting and measuring methods have been carried out. The results of these studies are therefore understandably incongruous. Even so, some concordances have been found among the reported secretion rates. The majority of the data indicates that salivary secretion is significantly higher in the buccal glands than in the labial glands, while the palatal flow rate is the lowest (see

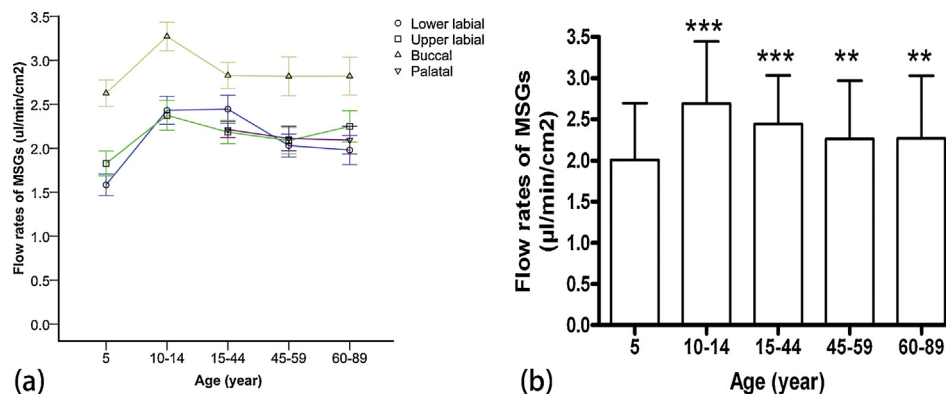


Fig. 2 – (a) Saliva flow rates of different MSGs in different age groups. Error bars: 95% CI. (b) Average saliva flow rate of MSGs in different age groups 10–14-year group had the highest flow rate and the 5-year group had the lowest flow rate. Error bars: SD * $P < 0.001$ compared with 5-year group; ** $P < 0.01$ compared with 5-year group**

Table 2 – Saliva flow rates of MSGs 30 young females.

Time	n	Flow rate (µl/min/cm ²)					
		Lower labial glands		Upper labial glands		Buccal glands	
		Left	Right	Left	Right	Left	Right
9:00–11:00	30	2.55 ± 0.75	2.49 ± 0.59	02.11±.45	2.11 ± 0.46	2.88 ± 0.61	2.91 ± 0.55
15:00–17:00	30	2.31 ± 0.64	2.27 ± 0.51	2.77 ± 0.78			
19:00–21:00	30	2.73 ± 0.65 [#]	2.64 ± 0.48 ^{**}	2.68 ± 0.51			

Values represent mean ± SD.
^{**} P < 0.01 compared with 9:00–11:00; [#] P < 0.05 compared with 15:00–17:00.

Table 3 – Flow rates of whole saliva, PG and SM of 30 young females.

Time	n	Whole saliva (ml/min)			Major Salivary Glands (ml/min)		
		Resting	Stimulated	Resting PG	Stimulated PG	Resting SM	Stimulated SM
9:00–11:00	30	0.33 ± 0.21	2.44 ± 1.16	0.02 ± 0.01	0.42 ± 0.42	0.14 ± 0.11	0.60 ± 0.56
15:00–17:00	30	0.43 ± 0.25 ^{**}	2.28 ± 1.10				
19:00–21:00	30	0.44 ± 0.17 ^{**}	2.47 ± 0.75				

Values represent mean ± SD.
^{**} P < 0.01 compared with 9:00–11:00.

review by Eliasson and Carlen, 2010).³ In our study, based on a large sample of 300 healthy subjects, the buccal glands have the highest flow rate, which is consistent with other observations. However, the flow rates of the palatal glands are similar to the rates of the upper and lower labial glands, which are different from the results in previous reports. The factors with the potential to influence saliva secretion, such as certain diseases, medications, smoking, drinking alcohol, wearing denture, stress, or any emotional disturbance, were under control in the present study. However, the influence of the race or ethnicity of the subjects is worth for further investigation.¹⁴ Our subjects are from an Asian population, which might play a role in the difference in flow rates from other studies whose subjects were mainly Caucasians.

As the surface area of the oral cavity is about 215 cm², the number of MSGs has been estimated to be 600–1000, and they are distributed in different locations.¹⁵ Even in the same

region, the distribution of the MSGs may vary in different areas. For example, there is a much smaller number of MSGs in the middle than in the lateral parts of the labial glands.¹⁶ Therefore, site selection is critical for the evaluation of saliva flow rates of MSGs. In our study, we tried to measure mucosa sites where the density of MSGs is higher. As a result, we chose a lateral site instead of a midline site for measuring the saliva flow rate of both the lower and upper labial glands.

A recent study¹⁷ found that the buccal saliva flow rate is higher in more lateral sites than in sites close to the angle of the mouth. This result might be due to the presence of parotid saliva. The measurements in this area over a time period of 5 s varied considerably and non-systematically.³ In our study, we tried to minimize the variation by measuring the rate for 30 s. We also kept our subjects in a tilt backward position so that the influence from the saliva secreted from parotid glands could be ruled out.

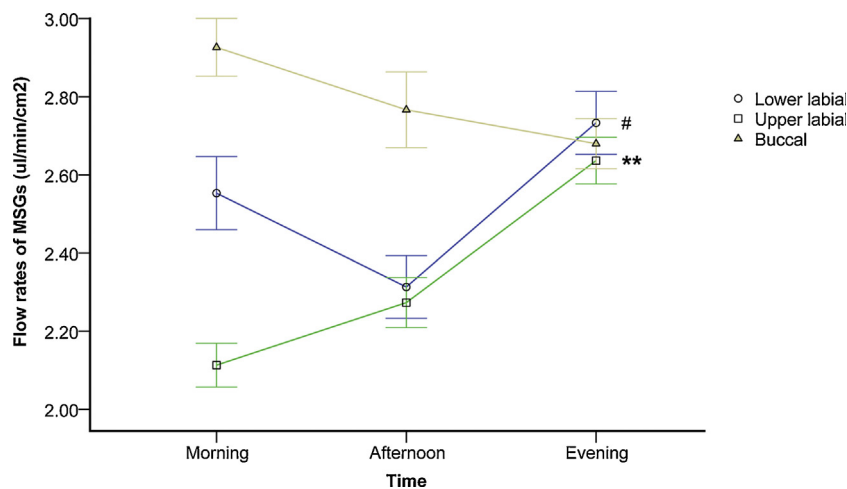


Fig. 3 – The pattern of biorhythm in MSG secretion. Morning: 09:00–11:00 AM; Afternoon: 15:00–17:00 PM; Evening: 19:00–21:00 PM; Error bars: 50% CI ^{} P < 0.01 compared to the flow rate in the morning; [#] P < 0.05 compared to the flow rate in the afternoon.**

Table 4 – Correlation among flow rates of MSGs, major salivary glands and whole saliva.

		Stimulated whole saliva	Lower labial glands	Upper labial glands	Buccal glands	Palatal glands	Resting SMG	Stimulated SMG	Resting PG	Stimulated PG
Resting whole saliva	Correlation coefficient	0.564**	0.041	−0.026	0.057	0.195**	0.546**	0.054	0.252	−0.351
	P	0.000	0.446	0.625	0.289	0.007	0.002	0.776	0.052	0.057
	N	330	330	330	330	180	30	30	30	30
Stimulated whole saliva	Correlation coefficient		0.089	0.005	0.094	0.098	0.665**	0.401*	0.076	−0.042
	P		0.096	0.921	0.079	0.171	0.000	0.028	0.104	0.824
	N		330	330	330	180	30	30	30	30
Lower labial glands	Correlation coefficient			0.375**	0.357**	0.223**	−0.333	−0.165	0.134	−0.046
	P			0.000	0.000	0.002	0.073	0.384	0.354	0.809
	N			330	330	180	30	30	30	30
Upper labial glands	Correlation coefficient				0.318**	0.198**	0.265	0.191	0.251	−0.432
	P				0.000	0.005	0.157	0.311	0.435	0.217
	N				330	180	30	30	30	30
Buccal glands	Correlation coefficient					0.202**	−0.285	−0.252	−0.731	0.465
	P					0.004	0.127	0.180	0.710	0.810
	N					180	30	30	30	30
Resting SMG	Correlation coefficient							0.760**	0.102	−0.102
	P							0.000	0.061	0.590
	N							30	30	30
Stimulated SMG	Correlation coefficient								0.329	0.092
	P								0.595	0.629
	N								30	30
Resting PG	Correlation coefficient									0.57*
	P									0.043
	N									30

* $P < 0.05$, ** $P < 0.01$.

N: the number of subjects.

Higher values of palatal flow rate have been reported in the posterior and medial sites.¹⁸ In our study, we measured in more posterior sites and found higher flow rates. Thus, unlike previous studies, no difference was noticed between palatal flow and labial flow.

A large degree of similarity has been reported between the flow rates of bilateral parotid glands in healthy subjects as well as in patients with Sjögren's syndrome.¹⁹ In our study, in a group of 30 female subjects aged 21–25 years, no significant difference was found in the saliva flow rates of MSGs at symmetrical sites. This result indicates that it is reasonable to use unilateral MSGs when measuring the flow rates of MSGs.

It is well known that whole saliva flow rates are lower in women than in men.²⁰ However, the results on sex-based differences in the saliva flow rates of MSGs is inconsistent. A higher palatal secretion rate in men compared with women was found in Ostlund's study,²¹ but no similar differences were found in the series of studies reported by Niedermeier et al.^{22,23} Similarly, no sex-based difference was found in the labial secretion rate,^{24,25} whereas other studies showed lower, but not statistically significant, secretion in the labial, buccal, and palatal glands in women.^{18,26} Ono et al. reported that significant gender differences in chewing-stimulated whole saliva flow rates disappeared after the standardisation with

gland sizes and weight. They concluded the gender difference of chewing-stimulated whole saliva flow rates is due partly to different gland sizes in healthy young subjects.²⁷ For MSGs, situations were similar. Our research showed no statistical difference in the flow rates between male and female subjects.

As age grows, saliva secretion from major salivary glands decreases.²⁰ However, whether a similar tendency exists in the function of minor salivary glands remains controversial. Although reduced function of the labial minor glands due to structural degenerative changes has been reported,^{28,29} measurements of labial, palatal, and buccal saliva secretion have shown both decreased and unperturbed flow rates with increasing age.³ Becks et al. observed that the flow rate of resting whole saliva in the 5–9-year group lower than that of the 10–14-year group, while after 14 years until 95, the flow rate plateaus. Although the flow rate of the 50–95-year group is slightly lower than that of the 5–49-year group, the difference is not significant.³⁰ In our study, the highest flow rate was observed in the 10–14-year group, followed by the 15–44-year group, 45–59-year group, 60–89-year group, and finally 5-year group. Similar to the major salivary glands, the MSGs undergo a process of maturation in their function and morphology. Thus, the lowest saliva flow rate observed in the 5-year-old children is likely due to their immature glands, which is

consistent with the results reported by Sonesson.^{31,32} As children grow, the function of MSGs increases. This explains why the 10–14-year group had a higher flow rate of MSGs. Afterwards, though the function of major salivary glands and MSGs plateaus, the surface of their oral cavity might increase. Sparse openings of MSGs per area might cause decreased flow rates of MSGs per mucosa area. Unlike the findings reported for the major salivary glands, the decrease in the saliva flow rate of MSGs in the aged subjects was not obvious in this series. This indicates that the degeneration that occurs as a result of aging is not so obvious in the MSGs as in the major salivary glands.

The major salivary glands have been reported to have increased flow rates in response to gustatory stimulation with citric acid.^{13,33,34} Our study showed similar results for the major salivary glands and whole saliva. However, results on the effect of acid stimulation on the flow rate of MSGs are controversial. Shern et al. could not confirm the difference in flow rates after 2% citric acid stimulation.¹⁸ Speirs reported that the percentage increase in flow rate from the parotid gland was much greater than that in labial glands with acid stimulation.³⁵ Some investigators suggested that stronger stimuli, such as lemon drop, may increase the secretion of MSGs.²⁴ We studied the effect of stimulation by applying 2.5% citric acid to the surface of the tongue. Our results showed that although there was a slight increase after acid stimulation, the difference was not significant ($P > 0.05$), suggesting that the regulation of the secretion in MSGs is different from that of the major salivary glands.

Biorhythm is a well-known factor that affects flow rates and the composition of whole saliva and saliva of the major salivary glands.⁵ This is why it is important to collect whole saliva and saliva secreted from major salivary glands at a fixed time, usually between 9:00 AM and 11:00 AM in the morning. In our study, we further explored the biorhythm pattern of the saliva secretion in MSGs. The saliva flow rates of the lower and upper labial glands showed a clear pattern of biorhythm. However, the rhythm was different from that of resting whole saliva. The saliva flow rates of the lower and upper labial glands showed differences with time. These results suggest that the collection of samples for determining the saliva flow rates of MSGs should also be conducted at a fixed time of the day due to changes associated with its biorhythm.

The positive correlation between the saliva flow rates of MSGs and whole saliva has been reported.^{36,37} However, our results did not show a correlation between the flow rates of MSGs and whole saliva or saliva from major salivary glands except for the palatal glands in healthy people, which is consistent with previous studies.^{18,22} Because the flow rate of the whole saliva primarily reflects the secretion from major salivary glands such as submandibular glands and sublingual glands, MSG secretion may present a small portion of the whole saliva.² It is understandable that MSG secretion may not be able to change the flow rate of whole saliva. Our data also indicate that MSGs secrete constantly, which may have a significant biological importance. The oral mucosa needs constant lubrication, and MSGs may play a critical role in providing continuous supply of small amount of saliva.

5. Conclusion

The present study determined the normal range of saliva flow rates of MSGs at different oral locations and indicated that fewer factors, including location, age, and biorhythm, affect MSG secretion. The normal range makes this noninvasive technique possible in evaluating dry mouth caused by Sjögren's syndrome or radiation therapy against head and neck tumour, or aids in choosing better donor site before transplantation of minor salivary glands to treat severe dry eye syndrome.³⁸ Considering all influencing factors and the relationship between MSGs and major salivary glands, a standard protocol for collection of MSGs needs to be established.

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Conflict of interests

There is no conflict of interest.

Ethics approval

This study "Characteristics of the saliva flow rates of minor salivary glands in healthy people" was conducted with the approval of Peking University Health Science Center and Beijing Bureau of Health on July 31, 2008.

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REFERENCES

- Carlson E, Ord R. *Textbook and Color Atlas of Salivary Gland Pathology: Diagnosis and Management*. John Wiley & Sons; 2009.
- Dawes C, Wood CM. The contribution of oral minor mucous gland secretions to the volume of whole saliva in man. *Arch Oral Biol* 1973;18(3):337–42.
- Eliasson L, Carlen A. An update on minor salivary gland secretions. *Eur J Oral Sci* 2010;118(5):435–42.
- Schmid G, Kivovics P, Marton K. Flow rate of minor salivary glands in elderly patients wearing complete dentures. *Fogorv Sz* 2007;100(4):153–8.
- Dawes C. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *J Dent Res* 1987;66:648–53SpecNo:-L.

6. Ozawa N, Sumi Y, Shimozato K, Chong C, Kurabayashi T. In vivo imaging of human labial glands using advanced optical coherence tomography. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;108(3):425–9.
7. Feher E, Zelles T, Nagy G. Immunocytochemical localisation of neuropeptide-containing nerve fibres in human labial glands. *Arch Oral Biol* 1999;44(Suppl 1):S33–7.
8. Rossoni RB, Machado AB, Machado CR. A histochemical study of catecholamines and cholinesterases in the autonomic nerves of the human minor salivary glands. *Histochem J* 1979;11(6):661–8.
9. Turner RJ, Paulais M, Valdez II, Evans RL, Fox PC. Ion transport and signalling in human labial glands. *Arch Oral Biol* 1999;44(Suppl 1):S15–9.
10. Izutsu K, Ensign W, Ramsey B, Schubert M, Allan B, Truelove E. Autonomic regulation of potassium release from human labial salivary glands in vitro. *Arch Oral Biol* 1988;33(7):519–23.
11. Elishoov H, Wolff A, Kravel LS, Shiperman A, Gorsky M. Association between season and temperature and unstimulated parotid and submandibular/sublingual secretion rates. *Arch Oral Biol* 2008;53(1):75–8.
12. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci* 1993;694:72–7.
13. Wolff A, Begleiter A, Moskona D. A novel system of human submandibular/sublingual saliva collection. *J Dent Res* 1997;76(11):1782–6.
14. Bjornstad L, Crossner CG. Stimulated salivary flow rate and buffer effect in schoolchildren from Greenland and Sweden: a comparative study. *Acta Odontol Scand* 2007;65(3):162–6.
15. Collins LM, Dawes C. The surface area of the adult human mouth and thickness of the salivary film covering the teeth and oral mucosa. *J Dent Res* 1987;66(8):1300–2.
16. Sumi M, Yamada T, Takagi Y, Nakamura T. MR imaging of labial glands. *AJNR Am J Neuroradiol* 2007;28(8):1552–6.
17. Eliasson L, Birkhed D, Carlen A. Feeling of dry mouth in relation to whole and minor gland saliva secretion rate. *Arch Oral Biol* 2009;54(3):263–7.
18. Shern RJ, Fox PC, Li SH. Influence of age on the secretory rates of the human minor salivary glands and whole saliva. *Arch Oral Biol* 1993;38(9):755–61.
19. Burlage FR, Pijpe J, Coppes RP, Hemels ME, Meertens H, Canrinus A, et al. Variability of flow rate when collecting stimulated human parotid saliva. *Eur J Oral Sci* 2005;113(5):386–90.
20. Srivastava A, Wang J, Zhou H, Melvin JE, Wong DT. Age and gender related differences in human parotid gland gene expression. *Arch Oral Biol* 2008;53(11):1058–70.
21. Ostlund SG. Palatine glands and mucin; factors influencing the retention of complete dentures. *Odontol Tidskr* 1954;62(1):1–128.
22. Niedermeier WH, Kramer R. Salivary secretion and denture retention. *J Prosthet Dent* 1992;67(2):211–6.
23. Niedermeier W, Huber M, Fischer D, Beier K, Muller N, Schuler R, et al. Significance of saliva for the denture-wearing population. *Gerodontology* 2000;17(2):104–18.
24. Smith DJ, Joshipura K, Kent R, Taubman MA. Effect of age on immunoglobulin content and volume of human labial gland saliva. *J Dent Res* 1992;71(12):1891–4.
25. Boros I, Keszler P, Zelles T. Study of saliva secretion and the salivary fluoride concentration of the human minor labial glands by a new method. *Arch Oral Biol* 1999;44(Suppl 1):S59–62.
26. Sivarajasingam V, Drummond JR. Measurements of human minor salivary gland secretions from different oral sites. *Arch Oral Biol* 1995;40(8):723–9.
27. Ono K, Inoue H, Masuda W, Morimoto Y, Tanaka T, Yokota M, et al. Relationship of chewing-stimulated whole saliva flow rate and salivary gland size. *Arch Oral Biol* 2007;52(5):427–31.
28. Drummond JR, Chisholm DM. A qualitative and quantitative study of the ageing human labial salivary glands. *Arch Oral Biol* 1984;29(2):151–5.
29. Syrjanen S. Age-related changes in structure of labial minor salivary glands. *Age Ageing* 1984;13(3):159–65.
30. Becks H. Rate of flow of resting saliva of healthy individuals. *J Dent Res* 1943;22:391–414.
31. Sonesson M, Eliasson L, Matsson L. Minor salivary gland secretion in children and adults. *Arch Oral Biol* 2003;48(7):535–9.
32. Sonesson M. On minor salivary gland secretion in children, adolescents and adults. *Swed Dent J Suppl* 2011;215:9–64.
33. Fischer D, Ship JA. Effect of age on variability of parotid salivary gland flow rates over time. *Age Ageing* 1999;28(6):557–61.
34. Engelen L, van den Keybus PA, de Wijk RA, Veerman EC, Amerongen AV, Bosman F, et al. The effect of saliva composition on texture perception of semi-solids. *Arch Oral Biol* 2007;52(6):518–25.
35. Speirs RL. Secretion of saliva by human lip mucous glands and parotid glands in response to gustatory stimuli and chewing. *Arch Oral Biol* 1984;29(11):945–8.
36. Eliasson L, Almstahl A, Lingstrom P, Wikstrom M, Carlen A. Minor gland saliva flow rate and proteins in subjects with hyposalivation due to Sjogren's syndrome and radiation therapy. *Arch Oral Biol* 2005;50(3):293–9.
37. Eliasson L, Birkhed D, Osterberg T, Carlen A. Minor salivary gland secretion rates and immunoglobulin A in adults and the elderly. *Eur J Oral Sci* 2006;114(6):494–9.
38. Geerling G, Raus P, Murube J. Minor salivary gland transplantation. *Dev Ophthalmol* 2008;41:243–54.