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Daily and annually variation of unstimulated whole saliva flow rate and pH and their relation with body profile in healthy young adults

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Abstract. – AIM: To analyse pH and flow rate (FR) of unstimulated whole saliva (UWS), detecting their possible correlations both among themselves and with body profile; in addition to identify daily, annually and gender differences.

MATERIALS AND METHODS: Eighty-one (47 ♀; 34 ♂) healthy young adults (mean age 22.7±4.09 years old) were enrolled. Saliva was sampled using spitting method. The data were statistically analysed using Pearson's coefficient, ANOVA or Kruskal-Wallis test, Student's *t* test or the Wilcoxon-Mann-Whitney test.

RESULTS: The mean UWS/FR was 0.643 ml/min (range 0.164-1.656 ml/min; percentile 25 = 0.400 ml/min; percentile 50 = 0.643 ml/min, percentile 75 = 0.832 ml/min; median = 0.590 ml/min) and no significant differences were found in gender. The mean UWS/pH was 6.95 (range 6.06-7.91, S.D. 0.28, RSD % 4.08): pH was higher in males (7.02) than females (6.92; $p = 0.009$). The UWS/FR increased almost steadily during the day: from 0.593 ml/min at 9:00 to 0.669 ml/min at 17:00 ($p = 0.04$), the greatest increase was found between 9:00 and 11:00. Through the seasons the UWS/FR decreased from summer to spring with a difference of 0.048 ml/min ($p < 0.05$). The UWS/pH showed a slight increase between 9:00 and 17:00 ($p < 0.05$). There were little differences in UWS/pH among the seasons (max. 0.09; $p < 0.05$). Only a significant correlation between UWS/FR and pH was found (R = 0.20; $p = 0.008$).

CONCLUSIONS: We did not find correlations between body profile vs UWS/FR or pH. UWS/FR varies more widely than UWS/pH: maintaining a proper acid/base balance is an essential factor for the homeostasis of the oral cavity and probably this would explain the reason for the lack of the variables evaluated influencing UWS/pH.

Key Words:
Saliva, pH, Flow rate.

Abbreviations

BH = body height; BMI = body mass index; BSA = body surface area; BW = body weight; FR = flow rate; RSD% = relative standard deviation; SD = standard deviation; UWS = unstimulated whole saliva; R = correlation coefficients; UWS/FR = unstimulated whole saliva flow rate; UWS/pH unstimulated whole saliva pH.

Introduction

The salivary flow rate is the amount of saliva produced by salivary glands, expressed in ml/min or g/min. It can be divided into unstimulated (basal secretion) which is independent from the presence of stimuli (food, chewing, etc.) and stimulated, secreted in response to sensory stimulation, gustatory and masticatory mainly[1]. Saliva can be divided into "Duct saliva" that is the serous, mucous, or mixed fluid, produced by glands[2,3] and "Whole saliva", the fluid composed by duct saliva with the addition of the secretions of oral, nasal and pharynx mucous; this fluid also contains microorganisms, desquamated epithelial cells, blood cells, food debris, e.g.[2,3].

Saliva chemical and physical properties play an important role in maintaining the health and functions of the oral cavity. Lubrication of alimentary bolus, protection against virus, bacteria and fungi, buffer capacity, protection and reparation of oral mucosa and dental remineralisation are some of the functions of saliva[1,3,4]. The buffer capacity depends on the acids and buffer base contained in the secreted saliva[5,6]. Bicarbonate is the main buffer that opposes acid, but is completely effective only at high salivary flow rates because its concentration increases markedly with FR[6,7]. It is well know that patients with quantitative and/or qualitative alterations in saliva may complain about subjective oral dryness, suffering from eating and difficulties speaking and swallowing; furthermore dental caries and opportunistic infections of the oral cavity may increase[1-4,8-12]. About 25% of the general population suffer from dry mouth[8,11,13].

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There are conflicting data in literature concerning FR (Table I)[4,6,8,9,14-16] and pH (Table II)[4,6,12,17-19] of unstimulated whole saliva and very few publications about their correlation[12,20]. Moreover, the effects of factors such as gender, body profile and salivary gland size on the FR and pH of saliva are controversial[9,12,14,22,] .

The aims of our observational prospective study were:

1. to determine the FR and pH of UWS in a sample of healthy young volunteers and to verify whether there are any gender-based differences;
2. to assess the daily and annually changes of FR and pH value;
3. to investigate possible correlations between the two variables (FR, pH) and body profile.

In our research we have chosen to measure UWS, as it is an easy, non-invasive and comfortable procedure, which favours its use in population studies. UWS/FR is the basal rate of saliva flow and it is the greatest contributor to total salivary output during the diurnal cycle[23,24]. UWS reflects basal salivary FR, it is present in our mouths for about 14 hours a day and its secretion provides protection to oral tissues[4]. Stimulated saliva represents the secretion during food intake, and it is present in our mouths for up to 2 hours[4,25]. Furthermore stimulating the flow of saliva can alter its composition; for example the concentration of bicarbonate which increases progressively with the duration of stimulation[23,24]. These features reflect a greater variability in stimulated saliva rather than UWS, thus, to deem UWS has a more clinically reliable parameter.

However, several factors could influence UWS/FR and pH: oral and systemic diseases, drugs, age (UWS/FR decreases with increasing age), nutrition, bite-force, stress, sport activity, e.g.[1-3,8-11,13,24].

To reduce this variability we used a very select sample of young adult, student of our Dental School, under strict annually medical supervision.

Materials and Methods

The study initially involved 89 Dental School students: 52 females and 37 males with a mean age of 23.8 years old, range 18.02-34.7. All subjects were selected in a homogeneous (Caucasian) population. They were informed of the purpose of the study, approved by our local Ethics Committee (N.

Table I. Comparison between UWS/FR values from references and our research.

Authors	N° Subjects (Gender)	Age years old	FR ml/min
Navazesh M, et al (1992)[8]	21 (♂ = 14 ♀ = 7)	25.4	0.383 ± 0.043
Bergdahl M (2000)[9]	843 (♂ = 441 ♀ = 402)	20-69	♂ = 0.35 ± 0.27 ♀ = 0.27 ± 0.20
Fenoll-Palomares C, et al (2004)[4]	159 (♂ = 52 ♀ = 107)	44.16 (♂ = 44.58 ♀ = 43.95)	0.48 (♂ = 0.57 ♀ = 0.42)
Gaviao MBD, Van der Bilt A (2004)[14]	16 (♂ = 8 ♀ = 8)	35 ± 13 16 - 60	0.53 ± 0.28
Kariyawasam AP, Dawes C (2005)[15]	46 (♂ = 26 ♀ = 20)	20	0.528
Yamamoto K, et al (2009)[16]	200 (♂ = 100 ♀ = 100)	24	0.53 ± 0.32 (♂ = 0.65 ± 0.34 ♀ = 0.42 ± 0.25)
Wang P, et al (2011)[6]	60	12-13	0.46 ± 0.22
Actual Research	69 (♀ = 41 ♂ = 28)	22.8 (♀= 23.0 ♂= 22.4)	0.643 (♂ = 0.632 ♀ = 0.649)

Table II. Comparison between UWS/pH values from references and our research.

Authors	N° Subjects (Gender)	Age years old	pH
Larsen MJ, et al (1999)[17]	11	n.d.	5.76 - 7.96
Fenoll-Palomares C, et al (2004)[4]	159 (♂ = 52 ♀ = 107)	44.16 (♂ = 44.58 ♀ = 43.95)	6.79 ± 0.29 (♂ = 6.84 ± 0.32 ♀ = 6.77 ± 0.27)
Rockembach MI, et al (2006)[18]	22 (♀ = 22)	29.5	7.5 ± 0.4
Wu KP, et al (2008)[12]	16	12-14	7.24 ± 0.22
Moreira AR, et al (2009)[20]	30 (♀ = 15 ♂ = 15)	7-18	7.0 ± 0.6
Wang P, et al (2011)[6]	60	12-13	7.42 ± 0.20
Actual Research	69 (♀ = 41 ♂ = 28)	22.8 (♀ = 23.0 ♂ = 22.4)	6.95 ± 0.28 (♀ = 6.92 ± 0.28 ♂ = 7.02 ± 0.29)

RQ3010), and enrolled after giving their signed informed consent. All subjects answered an anamnestic questionnaire in order to exclude those with systemic diseases, that could lead to decrease saliva productions, or symptoms such as dry mouth or oral burning syndrome, those taking drugs (except estrogens contraceptives) and women that could be pregnant. All subjects enrolled each year undergo a medical examination, including electrocardiogram, blood and urine tests for admission to the school attendance. Their body profiles were evaluated using the parameters body weight (BW), body height (BH), body mass index (BMI) calculated as BW in kilograms divided by BH in metres squared, and body surface area (BSA) calculated using the method of Du Bois and Du Bois[16,26] BSA = (W 0.425 x H 0.725) x 0.007184 (W = weight and H = height of the subject). To each subject was assigned an identification code consisting of a letter and a number, and was referred to an oral examination during which particular attention was given to the condition of the mucous membrane in order to exclude subjects with oral diseases, wearing any intraoral appliances and having a poor oral hygiene. The oral examinations were performed by two stomatologists expert in oral medicine and trained in salivary testing. Eight subjects were excluded during the preliminary selection: four, because had oral mucosa pathologies and four because taking drugs that may induce hyposialia; so that the final sample consisted of 81 subjects. The enrolled subjects were submitted to a rigid protocol of behavioural norms: in the two preceding weeks they had to avoid consumption of sugar-free chewing gum; in the day before saliva collection they had to be relaxed and not to practice sport activity. In the sampling days participants had to be free from symptom of fever and/or cold; if they were hungry or thirsty they could eat or drink water, but later immediately they had to clean their teeth with a provided toothpaste; during the last hour before the salivary collection, it was not permitted them to eat, to drink or to smoke.

The sample of 81 subjects underwent salivary samples in different months (July 2010, October 2010, January 2011 and March 2011) to assess a possible seasonal variation and in different hours (9:00, 11:00, 13:00, 17:00) to evaluate the possible existence of a circadian rhythm. A total of 1296 samples were collected; on these pH and FR were immediately detected and the samples were marked with date (year, month and season) and working hours. All subjects were experienced, during the test, in the Province of Novara (Italy) or surrounding areas. The UWS was detected under controlled temperature (22-24°C) and humidity conditions (75% ± 5%), in order to minimize variations induced by these two variables, using the spitting method[22].

UWS was collected for a 5 min time span. The undisturbed subject, sitting in a comfortable position, swallowed residual saliva present in the mouth

before the beginning of the collection and then, with the head down and mouth slightly open, saliva was allowed to drip from the lower lip into a weighed, dried and deionised sterile plastic test tube. In the last few seconds of the 5 min, saliva accumulated in the mouth was spat out into the plastic funnel. No other conscious movements of the oral musculature were made during the collection. The salivary samples were weighed using Precisa Balances, Series Bj (Dietikon, Switzerland) in order to determine the FR, which was calculated by dividing the net weight of saliva by the five minutes of the collection period. The FR was calculated as g/min, which is nearly equivalent to ml/min; in fact pilot studies revealed that weight and volume determinations of FR were very highly correlated, but that volume measures were less reliable[2,8,27].

A portable pH meter (HI 9026, Hanna Instruments, Burlington, VT, USA) with a special 5 mm diameter electrode was used to measure pH.

Statistical Analysis

The data were statistically analysed using R software 2.12.1. The variables were descriptively analysed with mean, maximum, minimum, standard deviation (SD), including their relative standard deviation (RSD %) and distribution (Shapiro-Wilk normality test). Those with a normal distribution were compared by means of a Student's *t* test for independent samples or ANOVA test (when comparing more than two groups, e.g. seasons); the abnormally distributed variables were compared using Wilcoxon's non-parametric test or Kruskal-Wallis test. We also calculated the correlation coefficients (R) between FR, pH and body profiles in the samples as a whole, and in the two gender groups. A p value of ≤ 0.05 was considered statistically significant in all of the tests.

Results

From statistical analysis we have excluded 12 subjects with a measurement of FR ≤ 0,16 ml/min, that can be defined as condition of hyposialia[4,28]. In conclusion, the final sample statistically analysed was composed of 69 subjects 41 females and 28 males with a mean age of 22.8 ± 4.19 years old (range 18.00-35.00).

Flow Rate

The values of UWS/FR ranged from 0.164 to 1.656 ml/min (percentile 25 = 0.400 ml/min; percentile 50 = 0.643 ml/min, percentile 75 = 0.832 ml/min; median = 0.590 ml/min) and they were not normally distributed ($p < 0.05$). The corresponding data in the male and female groups were respectively 0.168 - 1.656 ml/min (percentile 25 = 0.404 ml/min; percentile 50 = 0.632 ml/min, percentile 75 = 0.806 ml/min; median = 0.586 ml/min) and 0.164-1.602 ml/min (percentile 25 = 0.399 ml/min; percentile 50 = 0.649 ml/min, percentile 75 = 0.838 ml/min; median = 0.605 ml/min); the difference between gender was not statistically significant ($p = 0.488$). The UWS/FR increased almost steadily during the day with a difference of 0.076 ml/min about 12.00% from 9:00 to 17:00 ($p = 0.04$). The greatest increase (+0.060 ml/min) was found between 9:00 and 11:00 (Figure 1). The UWS/FR decreased during the seasons from summer to spring with a difference of 0.048 ml/min, representing about 7.6 % of the average value of the total sample ($p < 0.05$) (Figure 2).

pH

The values of UWS/pH ranged from 6.06 to 7.91 (mean 6.95, S.D. 0.28, RSD % 4.08) and they were normally distributed ($p = 0.793$). The corresponding data in the female and male groups were respectively 6.06 - 7.91 (mean 6.92, S.D. 0.28, RSD % 6.06) and 6.27-7.71 (mean 7.02, S.D. 0.29, RSD % 4.08); the difference between the two groups was statistically significant ($p < 0.05$).

The UWS/pH showed a trend to increase (+0.08) from morning to afternoon like FR ($p < 0.05$) (Figure 1). The greatest variation of pH was found between 9:00 and 11:00 (+0.06 points, 0.82%). The UWS/pH decreased during the seasons from summer to spring with a difference of 0.09 points, representing 1.24 % of the average value of the total sample ($p < 0.05$) (Figure 2).

Body Profile

The results relating to body profile are summarized in Table III.

We found significant differences in BW ($p < 0.05$), BH ($p < 0.05$), BMI ($p = 0.04$) and BSA ($p < 0.05$) between male and female groups.

Correlations

Among all the samples there was only one positive correlation with p-values below the predetermined limit of 0.05 (pH/FR, R = 0.20, $p = 0.008$). Neither UWS/FR nor UWS/pH were correlated with body profile. When correlations were separately analyzed in male and female groups, only one significant positive correlation was found in female group (pH/FR, R = 0.24) (Figure 3).

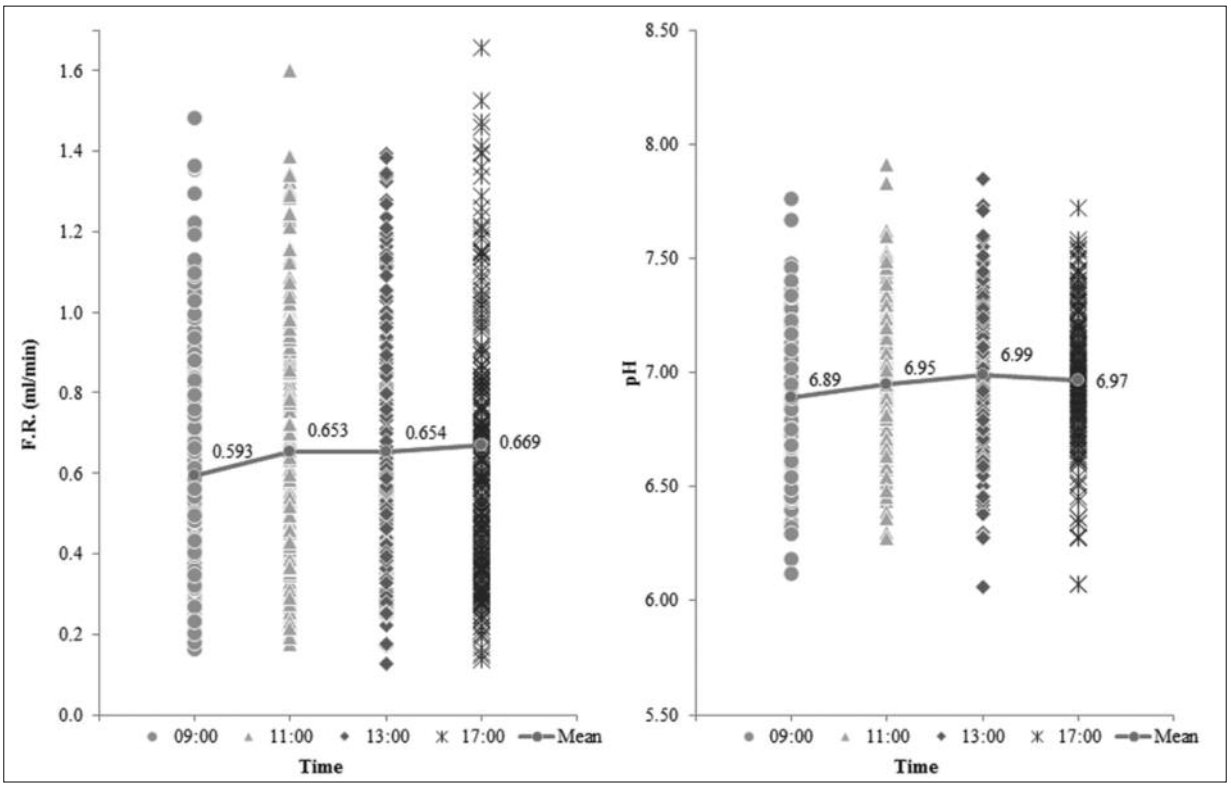

Figure 1. Daily variation of UWS/FR and UWS/pH.

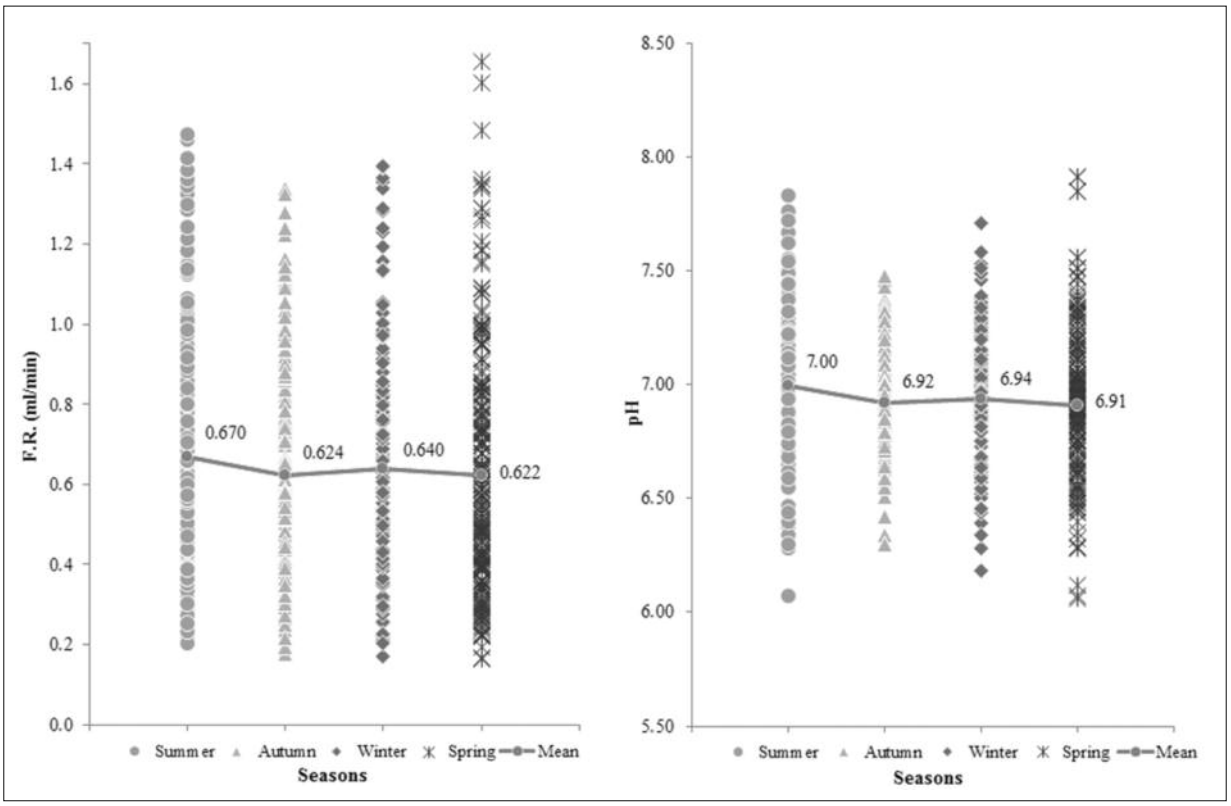

Figure 2. Annually variation of UWS/FR and UWS/pH.

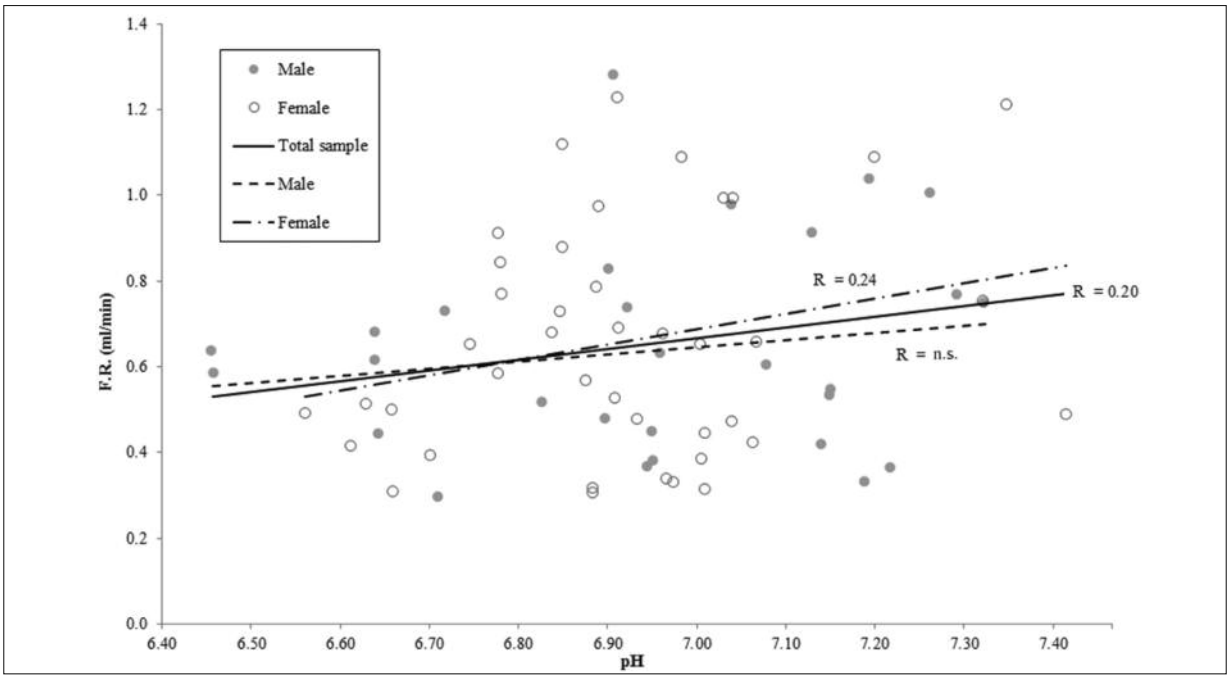

Figure 3. UWS/pH versus UWS/FR in analysed population (n.s. = not significant).

Table III. Body profile of subjects.

	Total sample (69)			Female (41)			Male (28)		
	Mean ± DS	Range	RSD (%)	Mean ± DS	Range	RSD (%)	Mean ± DS	Range	RSD (%)
BW (kg)	64.01 ± 8.66	89.00-47.00	13.61	59.14 ± 5.95	70.00-47.00	10.07	70.86 ± 7.05	89.00-56.00	9.94
BH (m)	1.71 ± 0.08	1.96-1.57	4.44	1.66 ± 0.05	1.78-1.57	3.27	1.77 ± 0.05	1.96-1.67	2.96
BMI (kg/m^2)	22.05 ± 2.38	29.18-16.90	10.08	21.54 ± 2.32	25.79-16.90	10.79	22.82 ± 2.45	29.18-19.14	10.72
BSA (m^2)	1.74 ± 0.15	2.08-1.44	8.37	1.65 ± 0.09	1.79-1.44	5.56	1.86 ± 0.10	2.08-1.63	5.53

Discussion

It is known from International Literature (Table I) that there is high variability in the value of UWS/FR (e.g. Fenoll-Palomares et al[4] range = 0.1-2 ml/min, Yamamoto et al[16] range = 0.05-1.68 ml/min, Bergdahl et al[9] range 0-2.07 ml/min, our research 0.164-1.656 ml/min). Based on the results of our study we can say that this wide variability (nearly 50%) is also present in a highly selected sample of healthy young adults. The factors currently considered (gender, age, body profile, collection method, daily and annually changes) are not sufficient to explain the high variability of the FR, so we can assume that there are other variables that affect the values of FR.

Mean UWS/FR found in our work (0.643 ml/min) was higher than those found in the literature. Fenoll-Palomares et al[4] revealed an average value of 0.48 ml/min, but the average age of their sample was 44.16 years old. We remember that the cross-sectional study of Navazesh et al[8] demonstrated an age-dependent decrease in UWS/FR in Caucasian subjects. Yamamoto et al[16] revealed an average value of UWS/FR of 0.53 ml/min in a sample with a mean age similar to our study, but in the statistical analysis they did not exclude subjects with hyposialia, in addition the sample was not Caucasian. Also Bergdal et al[9] considered in the statistical analysis the subject affected by hyposialia. UWS/FR did not have a normal distribution, as previously reported by Fenoll-Palomares

et al[4]. Our study has also underlined a daily and annual circadian rhythm of the FR, with meaningful differences even if not remarkable in comparison to the individual variability.

The mean UWS/pH value was similar to that reported by some authors[4] but not to others[6,10,15,17]: the difference may be due to subjects' age and to the time intervening between saliva collection and the measurement of pH. We found a significant difference between genders.

Our research shows that the pH variation (RSD % 4.04) is lower than the one found in FR (RSD% 48.96). Our data as those taken from the literature review reach values around neutrality (Table II). This fact leads us to say that maintaining a proper acid/base balance is an essential factor for proper homeostasis of the oral cavity and this would explain the reason for the lack of influence of the variables we have evaluated on the pH.

We did not find any correlations between the two variables (FR, pH) and body profiles like Fenoll-Palomares et al[4]. The study of Yamamoto et al[16] showed that UWS/FR is not correlated with males and females body profiles when analysed separately: they found a correlation only in the whole sample, and their results therefore indicated that the FR is not influenced by gender. Also our study show that there are no significant differences between gender in FR, therefore more authors founded significant differences; it is possible that a more strict selection of the cohort could reduce the difference between gender.

We found a significant correlation between FR and pH, like Fenoll-Palomares et al[4] and Wu et al[12]. This correlation was evident in daily and annually variations, in which were possible to notice the similar trend of FR and pH, but with a more wide relative variation of FR in comparison to pH. Kteusser et al[7] show a markedly increasing concentration of bicarbonate with high FR, this could be a valid clinical verification of the correlation pH/FR. Salivary pH and FR play important roles in the oral mucosa defense and their role is constantly supported even in adverse conditions[23,24,25,29]. Some authors consider FR and pH the salivary conditions mainly associated with caries risk[24,28]; therefore, it can be considered important to investigate their physiological aspects. Studying saliva is very complicated without standard procedures. Many authors have often used different times and methods for the collection of saliva. If we consider these factors as well as biological variations, it is clear that is impossible to make a direct comparison between the results of different studies.

Conclusions

The standardization of the methods and the times to collect saliva are fundamental for the study of UWS and for the clinic applications[30]. Therefore, is necessary to create a protocol that standardize the collection and analysis of FR and pH to be able to analyze the different possible variants of a larger cohort.

Conflict of Interest

The authors declare that they have no conflict of interests.

Ethical approval

The study was approved by our Faculty Ethics Committee N. RQ3010

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