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Measurement of unstimulated salivary flow rate in healthy children aged 6 to 15 years

KEYWORDS
Saliva
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SUMMARY

The aim of the present study was to measure unstimulated saliva flow rate (uSFR), pH value and buffer capacity in healthy children aged 6 to 15 years to serve as reference values for subsequent investigations, in particular to define threshold values for abnormality. Our basic data will power the limited amount of currently available data on salivary flow rate in healthy children. The uSFR was evaluated for correlations with pH value and buffer capacity. The unstimulated saliva of 274 children aged 6 to 15 years was collected (n: 154 ♂; 120 ♀) by the spitting method within three minutes. The samples were examined immediately after saliva collection in terms of uSFR, pH value and buffer capacity. From the 274 participating children 18 were excluded due to the intake of medication.

The medians (IQR) of uSFR of the tested children were 0.87 (0.54, 1.11) ml/min for boys, 0.65 (0.37, 0.98) ml/min for girls and 0.76 (0.49, 1.05) ml/min in total. The uSFR correlated with the pH value and the buffer capacity ($p < 0.001$). For different genders there was a statistically significant difference regarding uSFR ($p = 0.008$) and pH value ($p = 0.016$). Based on the available data, the pH value and the buffer capacity were closely related to the uSFR. Boys seemed to have a higher uSFR than girls.

Introduction

Whole saliva is a mucoserous fluid formed by exocrine glands as well as sulcular fluid in the oral cavity, which is mixed with remnants of food, bacteria, sloughed-off epithelial cells and mucus from nasal cavities and the pharynx and assists in maintaining oral health and the formation of the food bolus (Humphrey & Williamson 2001; Sreebny & Vissink 2010; Falcão et al. 2013). Saliva consists of > 99% water (Humphrey & Williamson 2001; Falcão et al. 2013). It contains various organic molecules and inorganic electrolytes (Sreebny & Vissink 2010; Falcão et al. 2013). Substances such as sodium, calcium, bicarbonate, phosphate etc. are among the inorganic molecules and electrolytes of saliva (Humphrey & Williamson 2001; Dodds et al. 2005; Falcão et al. 2013). Proteins like amylase, lysozyme, lactoferrin, immoglobuline (IgM, IgG, IgA), mucin etc. are part of the organic components (Dodds et al. 2005; Wisner et al. 2006; Sreebny & Vissink 2010; Falcão et al. 2013).The functions of these and other proteins are buffering of acids, immune defence, enzymatic digestion of the food pulp, elimination of free radicals, healing of the oral and oropharyngeal mucosa as well as the gastric mucosa, pain modulation and forming of the pellicle (Humphrey & Williamson 2001; Dodds et al. 2005; Wisner et al. 2006; Sreebny & Vissink 2010; Falcão et al. 2013; Gordan 2016). Primarily, salivary secretion originates from the three great salivary glands, the parotid gland, submandibular gland and sublingual gland as well as from the minor salivary glands (Sreebny & Vissink 2010). The parotid gland is the largest salivary gland (Silvers & Som 1998) and produces around 20–30% of the total amount of saliva under unstimulated conditions (Sreebny & Vissink 2010). When stimulated, this value increases markedly up to about 53% (Humphrey & Williamson 2001; Gordan 2016). This gland produces no or hardly any mucin-containing secretion and, thus, is a purely serous exocrine salivary gland (Gordan 2016). The submandibular gland is the second largest salivary gland (Silvers & Som 1998) and produces about 65% of the total amount of saliva under unstimulated condition (Sreebny & Vissink 2010). The submandibular gland is a mixed gland, so part of the acinus cells can produce and secrete mucin (Gordan 2016). The sublingual gland is the third largest salivary gland and produces under unstimulated condition about 7–8% of the entire amount of saliva (Silvers & Som 1998; Humphrey & Williamson 2001; Sreebny & Vissink 2010). The acinus cells of the sublingual gland mainly release mucin-containing secretion. Under unstimulated condition the 400–500 minor salivary glands produce less than 10% of the entire amount of saliva and secrete mucin (Sreebny & Vissink 2010; Gordan 2016).

Saliva can be collected by stimulating or not stimulating the salivary flow. Likewise, the saliva of single salivary glands or the hole saliva can be collected (Sreebny & Vissink 2010). Additionally, the amount and composition of the saliva depends on the collection method (Navazesh 1993).

Human saliva has three buffer systems: the bicarbonate, the phosphate and the protein buffer system (Bardow et al. 2000; Sreebny & Vissink 2010). Buffer capacity of the unstimulated whole amount of saliva is less than that of the stimulated one (Bardow et al. 2000). Both the bicarbonate and the phosphate concentration are higher in stimulated than in unstimulated saliva. Thus, the pH value of the saliva is lower in the unstimulated than in the stimulated saliva (Bardow et al. 2000). The pH value of saliva cited in literature is in the range of 6 to 7, making it slightly acidic, but when stimulated it may increase to about pH 7.8 (Humphrey & Williamson 2001).

In healthy adults the reported average uSFR is about 0.25–0.35 ml/min (Sreebny & Vissink 2010) and the stimulated SFR about 1–3 ml/min (Sreebny & Vissink 2010; Bardow et al. 2014; Gittings et al. 2015). In a healthy child the average uSFR is about 0.32–0.96 ml/min, the stimulated SFR about 1.05–2.5 ml/min (Gutman & Ben-Aryeh 1974; Rotteveel et al. 2004; Psoter et al. 2008; Moreira et al. 2009). The uSFR of the whole saliva decreases in the course of life (Gutman & Ben-Aryeh 1974; Sreebny 2000; Sreebny & Vissink 2010). The rate of the reduction increases by regular intake of medication. The reduction is due to parenchyma being slowly replaced by connective tissue and fat, which also occurs in the parenchyma of the minor salivary glands (Gutman & Ben-Aryeh 1974; Sreebny 2000).

Because there is a limited amount of currently available data on salivary flow rate in healthy children, the aim of the present study was to validate and strengthen this data in a larger population of volunteers by collecting samples to measure unstimulated saliva flow rate (uSFR), pH value and buffer capacity in healthy children aged 6 to 15 years. These data may serve as reference values for subsequent investigations, in particular for comparison to sick children, to show the influence of diseases on uSFR, pH value and buffer capacity.

Material and Methods

The present work was approved by the Ethics Committee of North-West and Central Switzerland (identification number: EKZN 2015–003). The study conforms to the principles of the revised version of the Declaration of Helsinki (World Medical Association 2013). Informed consent was obtained: in 26 randomly selected school classes in the cantons of Basel-Landschaft and Basel-Stadt (Switzerland) the parents and their children were given envelopes containing study information, consent forms and a questionnaire about the general health of each individual child. Children aged 6 to 15 years were allowed to participate in the study. The questionnaire included the questions listed in Table I. Although data from children taking medication were excluded from our results retrospectively, they were allowed to participate in the saliva test in order to prevent discrimination and to ensure anonymity. In the present work we focused on unstimulated whole saliva.

To ensure anonymity of the subjects, cups with number code were distributed to the children; the empty weight of each numbered cup was determined beforehand using a scale (111 g × 0.01 g,

Tab. I Questionnaire about the general health of each individual child

Did your child receive medical treatment in the past four weeks? If yes, what was the reason?
Was your child ill in the past four weeks (with a cold, fever etc.)? If yes, what kind of disease had your child been suffering from?
Does your child suffer from a diagnosed psychological or physical condition? If yes, what kind of condition?
Has your child been taking any medication in the past four weeks? If yes, what kind?

Proscale Europe, Germany). The children were instructed to swallow once before time measurements began, then to keep on spitting for three minutes, and not to swallow any saliva during that time. Thereafter, the cups were collected, weighed and analysed for pH value and buffer capacity. The amount of saliva was determined by weighing (111 g × 0.01 g, Proscale Europe, Germany): While the specific gravity of saliva and water is almost identical (Humphrey & Williamson 2001; Falcão et al. 2013), the weight of the full cup minus the cup's empty weight could be converted 1:1 from grams [g] to millilitres [ml]. The uSFR [ml/min] was calculated by dividing the amount of saliva by time (3 minutes). The pH value, measured by pH test strips (Saliva Check Buffer, GC), was determined by the colour change after dipping the strip into the saliva. Buffer capacity was determined by buffer test strips (Saliva Check Buffer, GC) by colour change two minutes after wetting the test surface. All tests of pH value and buffer capacity were conducted at room temperature (ca 20 °C) to comply with use instructions of the test strips manufacturer (15–30 °C). After determining uSFR, pH value and buffer capacity, the saliva samples were discarded. The participating children were assigned to three age groups (Tab. II): early mixed dentition (6–9 years, 115 children), late mixed dentition (10–12 years, 101 children), and permanent dentition (13–15 years, 40 children).

Descriptive statistics included means and SD for age and median (IQR) for uSFR, pH value, buffer capacity. Corresponding significance tests were performed like t-test and rank-sum tests, respectively. Multiple linear regression models were performed to predict log transformed uSFR with the other clinical parameters. The models were adjusted for age and gender. A p-value < 0.05 was considered as significant. Because of the descriptive nature of the study, adjustment of significance level for multiple comparisons was omitted. All analysis was performed with the statistical program R version 3.1.2. (www.r-project.org 2014).

Tab. II Number of participating children sorted according to age groups and gender

Dentition groups	Years of age	Number of boys	Number of girls	Total number
Early	6	2	0	2
	7	19	6	25
	8	27	25	52
	9	21	15	36
Late mixed	10	5	13	18
	11	14	10	24
	12	29	30	59
Permanent	13	13	8	21
	14	5	8	13
	15	3	3	6
		Total: 138	Total: 118	Total: 256

Tab. III Children, who had taken medication possibly having an effect on the saliva flow rate

Effect on the SFR	Medication	Number of children
Frequent to very frequent effect on the SFR (1/10–1/100)	Ritalin	5
	Xyzal	2
	Concerta	1
	Loratadine	1
	Toplexil	1
Rare effect on the SFR (1/1000–1/10,000)	Neocitran	2
	Zyrtec	1
	Aerius + Zatiden	1
	Immodium	1
	Feniallerg pills	1
	Arbid N drops	1
	Makatussin	1
Total exclusions		18

(http://compendium.ch 2016)

Results

Of the 519 distributed envelopes, 274 (52.8%) were returned (n: 154 ♂; 120 ♀). Retrospectively, eighteen children had to be excluded because of medicine intake (Tab. III). The average age of the included children did not significantly differ ($p = 0.11$) between boys , mean (SD) 10.0 (2.3) years and girls 10.5 (2.2) years.

For all the participating children aged 6 to 15 years, median (IQR) values were 0.76 (0.49, 1.05) ml/min for uSFR, 7.5 (7.4, 7.6) for pH value and 6.0 (4.0, 8.0) for buffer capacity, respectively. The results for each parameter for the children are shown in Table IV.

For different genders there was a statistically significant difference regarding uSFR ($p = 0.008$) and pH ($p = 0.016$): Boys produced significantly more saliva than girls (♂: 0.87 [0.54, 1.11] ml/min, ♀: 0.65 [0.37, 0.98] ml/min) and had a higher pH value (♂: 7.6 [7.4, 7.6], ♀: 7.4 [7.0, 7.6]). No statistically significant difference was detectable between different genders and buffer capacity ($p = 0.15$). The uSFR correlated significantly ($p < 0.001$) with the pH value and the buffer capacity of the participating children (Fig. 1 and 2). The age of the children had no influence on the uSFR ($p = 0.27$) nor the pH value ($p = 0.11$), or the buffer capacity ($p = 0.63$). Table V shows an overview of the statistically significant correlations in this study.

Discussion

The aim of the present study was to assess unstimulated saliva flow rate (uSFR), pH value and buffer capacity in healthy children aged 6 to 15 years to serve as reference values for subsequent investigations, in particular for comparison to sick children. Our basic data will augment the limited power of currently available data on salivary flow rate in healthy children. Our results presented above have shown that the average uSFR (median and IQR) of children aged 6 to 15 years was 0.87 (0.54, 1.11) ml/min for boys, 0.65 (0.37, 0.98) ml/min for girls and 0.76 (0.49, 1.05) ml/min in total. This value is higher than the average uSFR in adults (Sreebny & Vissink 2010), which is

Tab. IV Overview of the descriptive statistics (mean and SD for age, median and IQR for clinical parameters)

Defined parameters	Mean (SD)/median (IQR)		
Gender	♂	♀	♂ + ♀
Age	10.0 (2.3)	10.5 (2.2)	10.3 (2.3)
uSFR (ml/min)	0.87 (0.54, 1.11)	0.65 (0.37, 0.98)	0.76 (0.49, 1.05)
pH value	7.6 (7.4, 7.6)	7.4 (7.0, 7.6)	7.5 (7.4, 7.6)
Buffer capacity	6.5 (4.0, 8.0)	6.0 (4.0, 8.0)	6.0 (4.0, 8.0)

Tab. V Overview of the statistically significant correlations: X = $p < 0.05$; – = $p > 0.05$

Parameter	uSFR	Age	pH	Buffer capacity	Gender
uSFR		–	X	X	X
Age			–	–	
Gender			X	–	

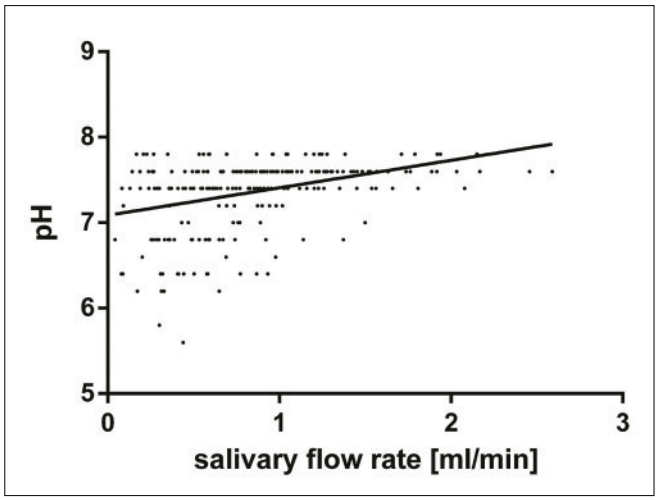

Fig. 1 The uSFR correlated with the pH value of the saliva ($p < 0.001$)

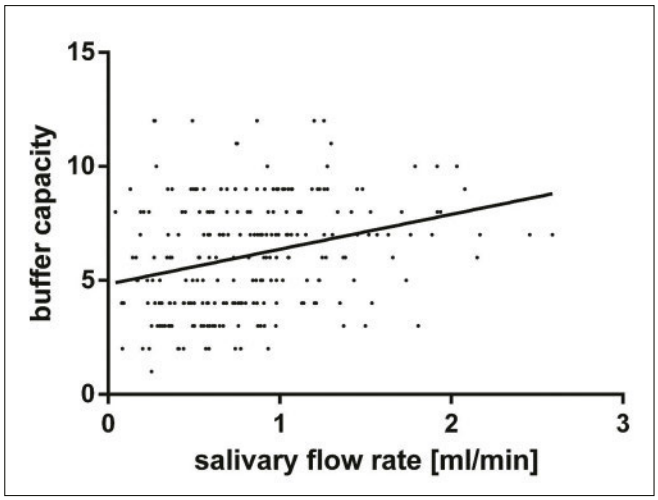

Fig. 2 The uSFR correlated with the buffer capacity of the saliva ($p < 0.001$)

likely due to the age-related reduction in uSFR (Gutman & Ben-Aryeh 1974; Sreebny 2000; Sreebny & Vissink 2010), driven by the slow conversion of the salivary parenchyma into connective tissue and fat (Gutman & Ben-Aryeh 1974; Sreebny 2000). Our results are in agreement with the average uSFR of children from studies published previously (Gutman & Ben-Aryeh 1974; Rotteveel et al. 2004; Psoter et al. 2008; Moreira et al. 2009). The uSFR correlated positively with the pH value and the buffer capacity of the saliva (Fig. 1 and 2; $p < 0.001$). This observation is in agreement with previously published studies (Bardow et al. 2000; Humphrey & Williamson 2001). The pH value and buffer capacity were measured by colometric test strips. These measuring methods are less precise than measurements by pH meter and titration. However this was the more practical approach given the number of schoolchildren sampled and then measured immediately in the classrooms. A slightly higher uSFR was detected in boys than in girls (♂: 0.87 ml/min, ♀: 0.65 ml/min; $p = 0.008$) as well as an increased pH value (♂: 7.6, ♀: 7.4; $p = 0.016$). There are other studies showing that men have a higher uSFR than women (Sreebny & Vissink 2010). Age did not have an influence on the parameters uSFR ($p = 0.27$), pH value ($p = 0.11$) and buffer capacity ($p = 0.63$). This, however, was not to be expected with the narrowly restricted age group that was chosen. Envelopes containing study information, consent forms and a questionnaire about the general health of each individual child were given to the parents and their children. By this means possible illnesses and medications were evaluated, which may have an influence on salivary flow rate. Eighteen children had to be excluded due to medication (Tab. III). Clinical examinations have not been performed because the sample collection had to be made during regular school hours. Therefore, differences in caries activity and other oral diseases could not be considered. Because there are many factors that can influence salivary flow and the composition of saliva, it is important to standardize the saliva collection: Therefore, the so-called spitting method was chosen for saliva collection because it is the easiest method for children to handle and it provides reliable results (Jones et al. 2000; Sreebny & Vissink 2010). This method requires the subjects to spit in a cup for a defined period of time (Sreebny

& Vissink 2010). The advantages of the spitting method are its reproducibility and reliability of the SFR. The disadvantages include a certain evaporation of the saliva until the time of measurement and a certain stimulation of the salivary flow caused by the spitting (Falcão et al. 2013).

It is acknowledged that these conditions may have influenced the study results (Bardow et al. 2014). The participating children were assigned to three age groups: early mixed dentition (6–9 years, 115 children), late mixed dentition (10–12 years, 101 children), and permanent dentition (13–15 years, 40 children). This grouping is relevant because each phase (early mixed dentition, late mixed dentition, permanent dentition) can be investigated separately and because the development of the salivary glands is completed during puberty (Tucker 2007).

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Zusammenfassung

Einleitung

Ziel der vorliegenden Studie war es, Referenzdaten im Sinne der Grundlagenforschung zur unstimulierten Speichelfliessrate (uSFR), zum pH-Wert und zur Pufferkapazität gesunder Kinder im Alter von 6 bis 15 Jahren zu ermitteln. Diese Referenzdaten können zukünftig mit den Speichelwerten erkrankter Kinder anderer Studien verglichen werden. Die in der Literatur vorhandenen Zahlen zur Speichelfliessrate betragen beim gesunden Erwachsenen ca. 0,25–0,35 ml/min (uSFR) bzw. ca. 1–3 ml/min (stimulierte SFR). Beim gesunden Kind beträgt die durchschnittliche uSFR ca. 0,32–0,96 ml/min, die stimulierte SFR ca. 1,05–2,5 ml/min.

Materialien und Methoden

In 26 zufällig ausgewählten Schulklassen wurden Couverts an Eltern und deren Kinder mit Studieninformationen, Einverständniserklärungen und einem Fragebogen zur Gesundheit des Kindes verteilt. Die teilnehmenden Kinder wurden in drei Altersgruppen eingeteilt: frühes (6–9 Jahre, 115 Kinder) und spätes Wechselgebiss (10–12 Jahre, 101 Kinder) und bleibende Dentition (13–15 Jahre, 40 Kinder). Die Kinder wurden instruiert, vor Messbeginn noch einmal zu schlucken und dann während drei Minuten in einen Becher zu spucken, ohne in dieser Zeit Speichel zu schlucken. Danach wurden die Becher eingesammelt und Speichelmenge, pH-Wert und Pufferkapazität untersucht. Die Speichelmenge wurde durch Wiegen bestimmt, der pH-Wert und die Pufferkapazität durch Farbumschlag von Teststreifen (Saliva Check Buffer, GC) ermittelt.

Resultate

Von den 519 ausgeteilten Couverts wurden 274 (52,8%) retourniert (n: 154 ♂; 120 ♀). 18 Kinder mussten aufgrund relevanter Medikamenteneinnahmen nachträglich ausgeschlossen werden. Die durchschnittliche uSFR (Median) der teilnehmenden Kinder lag bei 0,76 ml/min (♂: 0,87 ml/min, ♀: 0,65 ml/min), der durchschnittliche pH-Wert (Median) bei 7,5 (♂: 7,6, ♀: 7,4) und die durchschnittliche Pufferkapazität (Median) bei 6,0 (♂: 6,5, ♀: 6,0). Zwischen den verschiedenen Geschlechtern wurde ein statistisch signifikanter Unterschied bezüglich uSFR ($p = 0{,}008$) und pH-Wert ($p = 0{,}016$) gefunden: Knaben produzierten mehr Speichel als Mädchen und hatten einen höheren pH-Wert. Kein statistisch signifikanter Unterschied wurde zwischen den verschiedenen Geschlechtern bezüglich Pufferkapazität ($p = 0{,}15$) gefunden. Die uSFR korrelierte mit dem pH-Wert und der Pufferkapazität der teilnehmenden Kinder ($p < 0{,}001$). Das Alter der Kinder hatte weder Einfluss auf die uSFR ($p = 0{,}27$) noch auf den pH-Wert ($p = 0{,}11$) und die Pufferkapazität ($p = 0{,}63$).

Diskussion

Die durchschnittliche uSFR (Median) der teilnehmenden Kinder ist höher als die durchschnittliche uSFR von Erwachsenen und liegt im Bereich der Ergebnisse der durchschnittlichen uSFR von Kindern anderer Studien.

Die uSFR korrelierte mit dem pH-Wert und der Pufferkapazität der teilnehmenden Kinder (Abb. 1 und 2). Diese Beobachtung stimmt mit Resultaten anderer Studien überein, die den Zusammenhang zwischen zunehmender Speichelfliessrate und steigendem pH sowie Pufferkapazität beschreiben. Die fehlende klinische Untersuchung der Kinder vor dem Speicheltest und die Messung des pH-Wertes und der Pufferkapazität mittels Farbumschlag anstelle eines pH-Meters und Titration könnten die Genauigkeit der Daten der vorliegenden Studie beeinflusst haben. Aus Praktikabilitätsgründen wurde darauf verzichtet, weil die Speicheltests zu regulären Unterrichtszeiten durchgeführt und direkt im Anschluss noch im Klassenzimmer ausgewertet wurden.

Résumé

Introduction

Le but de cette étude a été d'établir des données de références en recherche fondamentale pour la valeur du pH et la capacité tampon du flux salivaire non stimulé (uSFR) chez des enfants en bonne santé âgés de 6 à 15 ans. Ces données de référence pourront par la suite être utilisées pour établir des comparaisons avec la salive d'enfants malades. Les valeurs disponibles dans la littérature mentionnent les chiffres suivants pour le flux salivaire non stimulé, de 0,25 à 0,35 ml/min chez des adultes en bonnes santé et de 1 à 3 ml/min pour le flux salivaire stimulé (SFR). Chez des enfants en bonne santé, ces valeurs atteignent une moyenne de 0,32 à 0,96 ml/min pour l'uSFR et 1,05 à 2,5 ml/min pour le SFR.

Matériel et Méthodes

Dans 26 classes d'écoles choisies aléatoirement, des courriers contenant les informations sur l'étude, les déclarations de consentement ainsi qu'un questionnaire sur la santé de l'enfant furent envoyés aux parents et aux enfants concernés. Les enfants participants ont été classés dans trois groupes en fonction de leur tranche d'âge: dentition mixte précoce (6 à 9 ans, 115 enfants), dentition mixte tardive (10 à 12 ans, 101 enfants),

dentition permanente (13 à 15 ans, 40 enfants). Il a été demandé aux enfants d'avaler leur salive avant le début des mesures, puis de cracher dans un gobelet durant 3 minutes sans en avaler. Après l'écoulement des trois minutes, les gobelets ont été récoltés et directement étudiés pour les valeurs de pH et capacité tampon. La quantité de salive a été mesurée par pesée des échantillons, le pH ainsi que la capacité tampon par virage de couleur avec des bandelettes de mesures (Saliva Check Buffer, GC).

Résultats

Sur les 519 courriers distribués, 274 (52,8%) ont été renvoyés (n: 154 ♂; 120 ♀). 18 enfants ont dû être retirés de l'étude par la suite, pour prise de médicaments significative.

L'uSFR moyen (médiane) des enfants participants était de 0,76 ml/min (♂: 0,87 ml/min, ♀: 0,65 ml/min), le pH moyen (médiane) de 7,5 (♂: 7,6, ♀: 7,4) et la capacité tampon moyenne (médiane) de 6,0 (♂: 6,5, ♀: 6,0). Une différence entre sexes a été observée pour l'uSFR ($p = 0{,}008$), ainsi que pour le pH ($p = 0{,}016$): les garçons produisaient plus de salive que les filles et avaient une salive d'un pH plus élevé. Aucune différence entre sexes n'a pu être observée pour la capacité tampon ($p = 0{,}15$). L'uSFR montre une corrélation avec la valeur du pH et la capacité tampon chez les enfants participants ($p < 0{,}001$). L'âge des enfants n'a eu d'influence ni sur l'uSFR ($p = 0{,}27$) ni sur la valeur du pH ($p = 0{,}11$) et la capacité tampon ($p = 0{,}63$).

Discussion

L'uSFR moyen (médiane) des enfants participants est plus élevé que l'uSFR moyen des adultes et se trouve dans le même domaine que l'uSFR moyen d'enfants dans d'autres études sur le sujet.

L'uSFR a montré une corrélation avec la valeur pH et la capacité tampon des enfants participants (fig. 1 et 2). Cette observation coïncide avec les résultats d'autres études sur le sujet, qui décrivent aussi une relation entre le SFR augmenté et le pH augmenté, tout comme la capacité tampon augmentée. L'absence d'examen clinique avant l'examen de la salive ainsi que la mesure par virage d'un colorant au lieu d'un pH-mètre ou d'une titration pourraient avoir eu une influence sur l'exactitude des données de cette étude. Ces mesures n'ont pas été réalisées ainsi pour des raisons pratiques, car les prélèvements ont été réalisés durant des heures de cours normales et leur examen a été réalisé directement après, en classe.

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