Assessment of salivary parameters and oral microbiota in amateur swimmers

Sandra Regina Santos Meyfarth[1], Mariana Gouvêa Latini Abreu[1], Pedro Antônio da Silva Moura[2], Rodrigo Von Held Marques[1], Helvécio Cardoso Corrêa Póvoa[3], Natalia Lopes Pontes Póvoa Iorio[3,4], Lívia Azeredo Alves Antunes[1,2,4,5], Leonardo Santos Antunes[1,2,4,5]

[1]Postgraduate Program. School of Dentistry. Fluminense Federal University. Niterói. Rio de Janeiro. Brazil. [2]Health Institute of Nova Friburgo. School of Dentistry. Fluminense Federal University. Nova Friburgo. Rio de Janeiro. Brazil. [3]Department of Basic Science. Fluminense Federal University. Nova Friburgo. Rio de Janeiro. Brazil. [4]Postgraduate Program. School of Dentistry. Fluminense Federal University. Nova Friburgo. Rio de Janeiro. Brazil. [5]Department of Specific. Fluminense Federal University. Nova Friburgo. Rio de Janeiro. Brazil.

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Summary

Objectives: The growing diffusion of sports activities is centering attention on the development of diseases correlated with sports performance. The most common diseases reported by sswimmers are dental stains. They are also exposed to the onset of erosive tooth wear and harbor cariogenic bacteria. Considering that the oral cavity of swimmers is in close contact with the swimming pool water in their daily training environment, this study aimed to evaluate whether swimming can change salivary parameters and oral microbiota of amateur athletes.
Material and method: This before-after study included 18 amateur athletes between 10 to 18 years old from a Swimming Team who practiced the sport at least three times a week. The swimmers were interviewed by a questionnaire and clinically evaluated. Unstimulated saliva was collected before and immediately after swimming. The salivary flow, pH, and buffer capacity were evaluated. The microbiological analysis included: total microorganisms, mutans *streptococci* group, *Lactobacillus* spp., and *Candida* spp. Wilcoxon test was applied before and after swimming with 5% level of significance.
Results: A total of 18 subjects participated in this study. All of the pool parameters were under acceptable limit. There was no statistical difference in the salivary parameters: salivary flow ($P = 0.264$), pH ($P = 0.132$); buffer capacity ($P = 0.067$). Regarding the oral microbiota, no significant differences were found before and after swimming for mutans *streptococci* group ($P = 0.950$), *Lactobacillus* spp. ($P = 0.432$), *Candida* spp. ($P = 0.386$), and total microorganisms ($P = 0.332$).
Conclusion: No change was observed in the salivary parameters and the oral microbiota before and after swimming in the evaluated group.

Key words:
Swimmer. Saliva. Swimming. Microbiota.

Evaluación de los parámetros salivales y de la microbiota oral de nadadores aficionados

Resumen

Objetivos: La creciente difusión de las actividades deportivas está centrando la atención en el desarrollo de enfermedades relacionadas con el rendimiento deportivo. Las enfermedades más comunes reportadas por los nadadores son las manchas dentales. También están expuestos a la aparición de desgaste dental erosivo y albergan bacterias cariogénicas. Teniendo en cuenta que la cavidad bucal de los nadadores está en estrecho contacto con el agua de la piscina en su entorno de entrenamiento diario, este estudio tuvo como objetivo evaluar si la natación puede cambiar los parámetros salivales y la microbiota oral de los atletas aficionados.
Material y método: Este estudio antes-después incluyó 18 deportistas aficionados de entre 10 y 18 años de un Equipo de Natación que practicaban este deporte al menos tres veces por semana. Los nadadores fueron entrevistados mediante un cuestionario y evaluados clínicamente. La saliva no estimulada se recogió antes e inmediatamente después de nadar. Se evaluó el flujo salival, el pH y la capacidad amortiguadora. El análisis microbiológico incluyó: microorganismos totales, grupo *Streptococcus mutans*, *Lactobacillus* spp. y *Candida* spp. Se aplicó la prueba de Wilcoxon antes y después de nadar con un nivel de significancia del 5%.
Resultados: Un total de 18 sujetos participaron en este estudio. Todos los parámetros del pool estuvieron por debajo del límite aceptable. No hubo diferencia estadística en los parámetros salivales: flujo salival ($p = 0{,}264$), pH ($p = 0{,}132$); capacidad amortiguadora ($p = 0{,}067$). En cuanto a la microbiota oral, no se encontraron diferencias significativas antes y después de nadar para el grupo *Streptococcus mutans* ($p = 0{,}950$), *Lactobacillus* spp. ($p = 0{,}432$), *Candida* spp. ($p = 0{,}386$), y microorganismos totales ($p = 0{,}332$).
Conclusión: No se observó cambio en los parámetros salivales y en la microbiota oral antes y después de nadar en el grupo evaluado.

Palabras clave:
Nadadores. Saliva. Natación. Microbiota.

Correspondencia: Leonardo Santos Antunes
E-mail: leonardoantunes@id.uff.br

Introduction

Sport is a double-edged sword with positive and negative effects on health[1]. On the one hand, sports and physical activity can improve the quality of life through the prevention of diseases and the maintenance and recovery of the individual's health[2-4]. On the other hand, negative effects include the risk of failure leading to poor mental health[5,6], risk of injuries[7,8], eating disorders[9], burnout[10], oral cavity alterations[11-14], and exercise-induced gastrointestinal tract discomfort[15].

The growing diffusion of sports activities is centering attention on the development of diseases correlated with sports performance[16]. The main disorders of the athletes' oral cavity are dentofacial trauma, caries, erosions, and periodontal disease[11-14]. Such oral diseases harm quality of life and have a negative impact on self-esteem, eating ability, and health, causing pain, anxiety, and impaired social functioning[17,18].

Regarding the swimmers, the most common diseases reported are dental stains. In addition, they are exposed to the onset of erosive tooth wear (ETW), a chemical-mechanical process characterized by a painful and irreversible cumulative loss of the enamel of a non-bacterial nature[18,19]. Swimmers can also harbor cariogenic bacteria, such as *Streptococcus mutans, Streptococcus sobrinus,* and *Lactobacillus* spp. and favor their growth[11]. There is little information available about the correlation between the performance of swimming and tooth decay occurrence[20]. Dental caries is a lifetime disease that depends on biological factors present within the saliva and dental plaque. Dental plaque favors the emergence of *Streptococcus mutans* and *Lactobacillus* spp., which are capable of rapidly fermenting dietary carbohydrates and lowering the pH causing tooth demineralization[21,22]. The concentration of cariogenic bacteria levels within saliva and plaque will be determinant for the occurrence of caries[23].

Saliva is a biological oral fluid, involved in several functions of oral health and homeostasis, and plays an active role in maintaining oral health itself[24,25]. The saliva components have several vital roles in the oral mucosa immunity (immunoglobulin A [IgA], mucins and cystatins), in the protection of teeth against the action of microorganisms (lysozyme, lactoferrin, histamine)[26], in food digestion (alpha-amylase and protease)[27], and in the buffering of acidic substances (bicarbonate, phosphate and proteins)[28,29]. The performance of sports activities influences the main characteristics of saliva, such as consistency, flow, pH, and buffer capacity[25]. Exercising may also alter saliva protein content. Physical activity activates the autonomic nervous system which impacts the secretion and content of saliva[30]. The stimulation of the sympathetic system results in the secretion of low volumes of saliva which is high in protein, whereas the stimulation of the parasympathetic system causes increased secretion of water and mucin[31]. The stimulation of the sympathetic system may result in changes in the salivary flow, reabsorption and the secretion of electrolytes in the secretory cells by modifying the ion concentration of the saliva[32].

Among the most abundant salivary proteins in the sports area are the salivary α-amylase and IgA, which play a key role in oral mucosa immunity[27,33]. Some factors are responsible to regulate the oral ecology, such as salivary pH, flow rate, buffering capability, total bacterial count, cariogenic bacterial load, and IgA levels[34,35]. Considering that swimmers are in close contact with the swimming pool water on their daily basis, this study aimed to evaluate whether swimming can change salivary parameters and oral microbiota of amateur athletes. The hypothesis is that the exposure to pool water changes the salivary parameters and the oral microbiota.

Material and method

Ethical approval

The study was approved by the local Ethics and Research Committee (#830.318). Appropriate written informed consent was obtained from all participants and legal guardians in case of underage athletes.

Study design

This before and after study consisted of 18 amateur swimmers, aged between 10 and 18 years, from a swimming team in the city of Nova Friburgo, Rio de Janeiro state, Brazil.

Subjects were invited and participated voluntarily in the study. The following inclusion criteria were considered: amateurs athletes practicing swimming at least three times a week for at least one year and that participate in competitions. Exclusion criteria were: swimmers who were not willing to participate in all parts of the survey; syndromes or chronic systemic diseases; systemic antibiotics, or local antimicrobials within the previous 3 months; patients under medication affecting the saliva flow rate; partial or total removal prosthesis or orthodontic appliances.

A self-administered questionnaire was used to obtain data concerning hours and frequency of weekly training, complete pathological history, history of hard and soft tissues of the oral cavity, family history, oral hygiene practices, and eating habits (supplements consumed and dietary information such as intake of drinks, fruit juices, and soda)[11].

The selection of the participants was done through convenience sampling.

Clinical monitoring and saliva collection

Each patient was clinically evaluated and intraoral examination was performed and the presence/absence of bad habits and/or parafunctional habits, and dental erosions were assessed.

The unstimulated saliva from each swimmer was collected before and immediately after the training, which averaged two hours (13:00-15:00). The volunteers were instructed to not brush their teeth, eat or drink excepting for water, at least 1 hour prior to the saliva collection. During the sample collection, the subjects were comfortably seated in a ventilated and lighted environment with head slightly down. They were instructed not to swallow or move their tongue or lips during the collection process. With the use of a chronometer, over a period of 6 minutes, the swimmers were asked to spit the accumulated saliva on a tube type Falcon calibrated. Abnormal values were less than 0.1 mL/min without stimulation.

The saliva samples were immediately placed on ice and transferred to the laboratories for processing. The pH, buffer capacity, salivary flow at rest, and counts of mutans *streptococci* group, *Lactobacillus* spp., and *Candida* spp., expressed in Colony Forming Unit (CFU)/mL were analyzed.

The saliva pH was measured using a pH indicator strip (0 to 14) (Macherey-Nagel GmbH & Co, Düren, Nordrhein-Westfalen, Germany) according to the manufacturer.

For buffer capacity analysis, 500 µL of saliva samples were mixed with 1.5 mL of 5 mM HCl (hydrochloric acid) in a sterile microtube. Then, the microtubes were agitated for 1 min and opened for 5 min to allow the CO2 to escape. After that, 10 µL of the solution was pipetted onto pH indicator strip (0 to 14) (Macherey-Nagel GmbH & Co).

Microbiological evaluation

For counting total microorganisms, mutans *streptococci* group, *Lactobacillus* spp., and *Candida* spp., the saliva was vortexed for 1 min. Afterwards, an aliquot of 100 µL of each sample was transferred to sterile microtube containing 900 µL of sterile saline (0.9% NaCl). Serial decimal dilutions were made up to 10-8 and 50 µL of each dilution were inoculated into Petri dishes containing the culture media to be tested. The medium used for counting the mutans *streptococci* group was the commercial *Mitis Salivarius agar* (HiMedia Laboratories, LLC, Mumbai, India). The culture medium used for counting *Lactobacillus* was *Rogosa agar* (HiMedia Laboratories, LLC, Mumbai, India). The culture medium used for counting *Candida* spp. was the Sabouraud dextrose agar (Becton, Dickinson and Company, Sparks, USA). The Mitis Salivarius and Rogosa plates were incubated in a candle jar, while Sabouraud dextrose plates were aerobically incubated, for 48 h at 36 °C. The colonies with morphological characteristics of the respective microorganism were considered for counting. The results were expressed in CFU/mL of saliva.

Swimming pool water collection

Samples of the swimming pool water (10 mL) were also collected in test tubes at regular intervals throughout the day and sent to the laboratory for evaluation.

Free Residual Chlorine, pH, Bicarbonate Alkalinity, Calcium Severity and salinity were evaluated by the LabAgua Environmental Laboratory, Niterói, RJ, Brazil.

Statistical Analysis

Data were analyzed through the Statistical Package for the Social Science program (SPSS® for Windows; version 16.0; Chicago, IL, USA). The Shapiro-Wilk test was used to verify the normal distribution of the microbiological results. The principle of normality of the sample was not confirmed. The Wilcoxon test was applied to establish a comparison between variables of interest (P <0.05).

Results

A total of 18 subjects participated in this study (11 females and 7 males). The mean age was 13.44 ± 2.14 years. The athletes practice swimming at least 3 times a week and two hours a day. The demographic and clinical characteristics of the studied population are presented in Table 1.

Table 1. Questionnaire with the prevalence (%) of clinical characteristics and habits.

Questions	Non-competitive swimmers (n=19)	%
Gender		
Male	7	39
Female	11	61
How many times per week do you swim?		
3	1	5
4	1	5
5 or more	16	88
Dental erosion		
Have you observed erosive effects on your teeth after drinking isotonic or energy drinks?		
Yes	1	6
No	17	94
"Yellow teeth"		
What color do you think your teeth are?		
Yellow	12	67
White	6	33
Oral habits		
Bruxism (Clench or grind your teeth?)		
Yes	4	22
No	14	78
Mouth or nose breathing?		
Mouth	8	44
Nose	10	56
Hygine habits		
Do you brush your teeth after meals?		
Yes	12	67
No	6	33
How many times a day do you use dental floss?		
Never	5	28
Once a day	8	44
2 or more times a day	5	28
Does your gum usually bleed when brushing your teeth or flossing?		
Yes	6	33
No	12	67
What is the best way for you to hydrate yourself? (Water, Isotonic, Energetic, natural juice?)		
Water	18	100
Isotonic drinks intake?		
Yes	10	56
No	8	44
Energetic drinks intake?		
Yes	3	17
No	15	83
Acid drinks intake?		
Soda		
Yes	1	6
No	17	94
Juice fruit		
Yes	5	28
No	13	72

Pool parameters at the time of swimming and saliva collection are shown in Table 2. All of the parameters were under acceptable limit.

There was no statistical difference between salivary parameters, such as sialometry, pH and buffer capacity before and after swimming (P >0.05). The salivary parameters before and after swimming are shown in Table 3.

Regarding the oral microbiota, no significant differences were found before and after swimming for mutans *streptococci* group (P = 0.950), *Lactobacillus* spp. (P = 0.432), and *Candida* spp. (P = 0.386) (Table 4).

Discussion

Swimming is often a recommended sport due to its benefits on the whole body. With the increasing number of amateurs swimmers regularly attending swimming pools, this study aimed to evaluate whether exposure to chlorine derivatives may change salivary parameters and oral microbiota of amateur swimmers. The authors hypothesized that the exposure to pool water could influence the salivary parameters and the oral microbiota of amateur swimmers. This hypothesis was rejected.

Table 2. Pool parameters at the time of swimming.

Test	Result	Unit	Acceptable limit
pH	7.92	-	7.0 – 7.6
Free Residual Chlorine	1.53	mg/L	1.0 – 3.0
Bicarbonate Alkalinity	89	mg/L CaCO3	120
Calcium Severity	126	mg/L CaCO3	200 – 400
Salinity	10.5	%	---

The results of this study showed that all amateur swimmers did not experience a significant alteration of their salivary parameters and oral microbiota. Contrastingly, Bretz *et al.*[36] found that competitor athletes who had swum at a gas-chlorinated swimming pool experienced a significant alteration in their salivary parameters. This difference between amateur and competitor swimmers may be explained by the time spent inside the swimming pools and performing a more vigorous physical exercise. Consequently, having more contact between water and oral cavity. In this sense, amateur swimmers seem to be much less exposed than competitive swimmers[18]. Chlorine-based disinfectants are used for microbial disinfection of the swimming pools[37,38]. They provide rapid and long-lasting disinfection effects in the water[39]. The use of ozone and ultraviolet disinfectants has been adopted in some cases, although generally they are used together with either chlorine or bromine for the provision of a residual disinfectant[37]. However, chlorination has the potential to produce a wide range of disinfection by-products (DBPs)[40]. The American Public Health Association recommends that proper pool maintenance records be kept, including thrice-daily chlorine level measurements and pH readings as well as the daily consumption of chlorine gas and soda ash[41].

Swimmers in improperly maintained swimming pools may be susceptible to acid erosion of the enamel[42]. Some authors believe that if the pH of the pool water decreases below that of saliva, erosions on dental hard tissue may occur[36,43]. In this study, the pool water showed a daily pH of 7.9, thereby meeting the required pH values (from 7.20 to 9.0) and having no effect on swimmers in relation to the development of erosions, which is in accordance with other studies[11,43]. A tendency for a decrease in average salivary pH after the swimming session has also been reported[36]. However, other data pointed to an opposite trend with no significant alteration in pH, before and after training sessions[25,44]. Similar results were observed in this study.

Table 3. Salivary parameters before and after swimming.

	Sialometry		pH		Buffer capacity	
	Before	After	Before	After	Before	After
Mean	3.05	2.80	6.86	7.00	4.25	3.73
(SD)	(2.30)	(2.28)	(0.59)	(0.51)	(0.84)	(0.81)
Median	2.10	1.70	7.00	7.00	4.00	3.50
(Q1-Q3)	(1.25- 4.35)	(1.18-4.50)	(6.37-7.12)	(6.87-7.50)	(3.50-5.00)	(3.00-4.50)
Significance	P = 0.264		P = 0.132		P = 0.067	

Wilcoxon test; P <0.05 indicates statistical significance; Q1-Q3: 1st and 3rd quartiles (25%, 75%, respectively).

Table 4. Oral microbiota before and after swimming.

Microorganism	Total microorganisms		*Streptococcus* spp.		*Lactobacillus* spp.		*Candida* spp.	
log10 CFU/mL)	Before	After	Before	After	Before	After	Before	After
Mean	7.236	7.120	2.731	2.443	2.637	2.307	2.248	2.103
Standard deviation	1.017	0.920	0.842	1.089	0.496	0.893	0.990	1.026
Median	7.477	7.031	2.929	2.812	2.656	2.477	2.964	2.280
Quartiles (Q3-Q1)	6.577-7.700	6.705-7.594	2.508-3.000	2.088-3.000	2.306-3.000	2.299-2.930	1.434-3000	1.516-3.000
Significance	P = 0.332		P = 0.950		P = 0.432		P = 0.386	

Wilcoxon test; P <0.05 indicates statistical significance; Q1-Q3: 1st and 3rd quartiles (25%, 75%, respectively).

During swimming, energy consumption is high and prolonged. This condition may alter the salivary flow rate[24]. The loss of electrolytes and water through sweat may contribute to decrease the salivary flow rate[45]. In this study, it was observed a decrease in salivary flow rate and buffer capacity before and after training session, but this decrease was not statistically significant (P >0.05). Previous studies also found a decrease in the salivary flow rate[36,46], which has been explained by an increase in sympathetic activity during intense exercise, since sympathetic innervations cause a marked vasoconstriction, resulting in reduced salivary volume[29]. Another explanation is the loss of electrolytes and water through sweat and restricted fluid intake during exercise that may contribute to decrease the salivary flow rate[32].

Athletes require high performance standards, especially swimmers, who must be totally healthy. Thereby, it is of paramount importance to evaluate the oral health of hard and soft tissues and the prevalence of caries in swimmers by assessing salivary cariogenic bacteria. *Streptococcus* spp. are related to dental caries[47]. *S. mutans* and *S. sobrinus* have greater cariogenic potential in humans[48,49]. *Lactobacillus* spp. are also secondary pathogens[50]. Besides these traditional culprits for dental caries, it has been discovered that *Candida albicans* has a synergistic interaction with *S. mutans* which may influence early childhood caries (ECC), since children with *C. albicans* present five times greater odds of experience ECC[51]. Therefore, this study also assessed salivary cariogenic bacteria (*Streptococcus* spp., *Lactobacillus* spp.), and *C. albicans* before and after swimming and found no statistical difference in the athletes' oral microbiota. Contrastingly, D'Ercole *et al*.[25] found that the swimmers total bacterial count and the load of *S. mutans, S. sanguis, L. fermentum* and *A. gerencseriae* underwent a statistically significant increase after swimming. *S. mutans, S. mitis* and *L. acidophilus* mean values were significantly higher in swimmers than in controls. A possible explanation is that the frequency and duration of swimming may increase the risk, since athletes are exposed to adverse conditions, such as pH and salivary flow changes during sports practice.

Despite not being the focus of this study, the eating habits and the use of isotonic and/or supplements need some attention since there are evidences, they can be responsible for alteration of the salivary pH[52,53]. In vitro studies have shown that acidic beverages cause dental erosion, for example cola drinks, energy drinks, sports drink and acidic juices[54,55]. Regarding isotonic drinks, marketing strategies emphasize performance improvement, and replacement of fluids and electrolytes lost in sweat during and after exercise[56]. Many athletes regularly consume these beverages[57,58]. This is in accordance with the present study, where 56% of swimmers informed the habit of isotonic drinks intake. Water was the most frequently consumed liquid (100%), and in most cases, it is the appropriate choice to maintain hydration before, during, and after physical exercise[56]. There was no association between consumption of isotonic drinks and dental erosion in the present study, and the number of athletes presenting dental erosion was very low (6%). A systematic review[59] also reported no association between isotonic drink consumption and the prevalence of dental erosion. This lack of association may be due to the fact that isotonic drinks have varied concentrations of calcium and phosphate in their formulations which keep high concentrations of these salts in salivary fluids, thereby inhibiting tooth demineralization[60,61].

This study has some limitations. First, it included only amateur swimmers. Second, the athletes were asked to self-report their training habits, and it is thus possible that some athletes had under- or over-reported their weekly training habits. Even so, our findings contribute to provide new information about the influence of swimming on salivary and microbiological parameters of athletes. This study also highlights the importance of studying salivary flow and composition since these parameters are useful tools in prevention programs or individualized treatment in several clinical situations.

Conclusion

Under the limitations of this study, it can be concluded that the salivary parameters and oral microbiota of amateur athletes had no significant differences before and after swimming. Additional studies are needed to observe and compare these parameters between amateurs and competitive swimmers.

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Author's contribution statement

All authors equally contributed to this research.

Conflict of interest

The authors declare no conflict of interest.

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