

Does Oral Hygiene Influence Salivary pH, Lactate, and IL-1 β of Basketball Players During Intense Exercise?

La Higiene Oral Influye en el pH Salival, el Lactato y la IL-1 β
de los Jugadores de Baloncesto Durante el Ejercicio Intenso?

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ABSTRACT: This study examined the salivary pH, salivary lactate, and salivary IL-1 β responses from a high-intensity intermittent running test, and the influence of hygiene oral status on these biomarkers in elite adolescent basketball players. Forty-six adolescent players participated. Saliva sampling was taken before and 3 min after a high-intensity exercise (Yo-Yo Intermittent Recovery Test Level 1; Yo-Yo IR1). In order to quantify and classify the oral hygiene level, the athletes were submitted to a dental examination, and an adapted Simplified Oral Hygiene Index was applied. After the dental examination, the whole group was divided into good oral hygiene group (GHG) and poor oral hygiene group (PHG). The results of a two-way analysis of variance showed a significant interaction effect ($P = 0.0003$), group effect ($P < 0.0001$), and time effect (pre to post Yo-yo IR1; $P < 0.0001$) for salivary pH and for salivary lactate (interaction effect, $P = 0.008$; group effect, $P < 0.0001$; time effect, $P < 0.0001$) with a lower salivary pH and a higher salivary lactate at pre and post-Yo-Yo IR1 for PHG, but no difference was observed for IL-1 β . The data demonstrated that the high-intensity exercise led to a significant change in salivary pH and salivary lactate concentration of the basketball players, and that the oral hygiene status influenced these responses, with a greater change for those players showing a poor oral hygiene.

KEY WORDS: saliva; cytokine; oral health; oral flora; youth athletes.

INTRODUCTION

Oral health is thought to be closely linked to athletes' general health (de Souza *et al.*, 2011; Needleman *et al.*, 2013). The alteration in the balance of the oral flora can increase the risk and occurrence of oral problems. Indeed, eating habits and oral hygiene may deleteriously influence the oral environment causing a decrease in salivary pH, which in turn may affect the oral biofilm formation. Such deleterious changes can lead to oral and systemic diseases, due to the migration of oral bacteria to a variety of sites such as smooth muscle, heart and joints (Forner *et al.*, 2006; Ljungqvist *et al.*, 2009; Borgnakke, 2015).

The salivary pH is susceptible to changes in the balance of the oral cavity; both the quality and the periodicity of dental brushing are related to the occurrence of oral diseases, such as dentin

hypersensitivity, non-carious cervical lesions and proper caries (Needleman *et al.*, 2014). Additionally, it has been suggested that the combination of physical exercise with the consumption of a diet rich in carbohydrates and acidic substances might lead to a decrease of salivary pH, increasing the dental erosion and caries risk (Frese *et al.*, 2015). The lactic acid concentration and pH present a strong relationship; the higher the lactic acid level, the lower would be the pH, which in turn would negatively impact the athlete oral health (Frese *et al.*).

Together with salivary pH and salivary lactate, other biomarkers have been proposed to be associated with oral health (Zhou *et al.*, 2012; Lee *et al.*, 2012). Among them, the IL-1 β isoform has been proposed as a potent stimulating bone resorption and it seems to

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occur more frequently in periodontitis compared to IL-1 α isoform (Kaushik *et al.*, 2011). Despite these assumptions on the usefulness of adopting salivary pH, salivary lactate, and salivary IL-1 β as biomarkers of oral health in athletes, it is still unknown whether the change of these salivary biomarkers during intense exercise could be influenced by hygiene oral level of elite adolescent athletes. Specifically, there is no scientific information on how hygiene oral level could affect changes in these markers in elite adolescent basketball players, who are involved daily in high-intensity training sessions (Miloski *et al.*, 2015; de Arruda *et al.*, 2018) and might be not aware about the appropriate oral habits to avoid poor oral health.

Therefore, the aim of the present study was to examine the responses of salivary pH, salivary lactate, and salivary IL-1 β in a high-intensity intermittent running test, and the influence of hygiene oral level on changes of these biomarkers in elite adolescent basketball players. It was hypothesized that the high-intensity exercise, taxing the anaerobic metabolism, would lead to changes in these biomarkers, and that the players' hygiene oral level could influence these responses.

MATERIAL AND METHOD

Participants. Forty-six male basketball players (mean \pm SD: age, 16.1 \pm 1.5 years; stature, 190 \pm 8 cm; body mass, 89 \pm 15 kg) volunteered to participate in this investigation. Players were from Under 15, Under 17 and Under 19 teams from the same club regularly competing in the major State Basketball Championship in São Paulo, Brazil. During the experimental period, the teams were ranked 1st or 2nd in their respective State Championship age-category. Each team was training five days (once or twice a day with sessions lasting 90-120 min) and playing one official match per week. Training sessions consisted of basketball drills aiming to enhance players' tactical and physical skills (using sprints, intermittent running exercises, specific conditioning drills) and resistance training and plyometrics. Since no relevant differences between age-categories in training contents and training schedules, players were considered as a whole group and data were therefore pooled for analysis. The participants were not taking any medication and were being exposed to the same environmental conditions, including eating the same food at the club and experiencing comparable psychophysiological stress (training load). Indeed, all the players were familiarized

with the sampling and testing procedures [i.e. the Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1)] and regularly training in the 6 months before the beginning of the study. Furthermore, to include the participants' data in the final analysis, the following criteria were adopted: (a) providing saliva samples for the experimental training session; (b) not presenting any injury or illness during the investigation period that could impair their performance during training and testing session; (c) not consuming any supplements. Six players were excluded from the analysis because of a problem arising from saliva collection, or due to a high intra-assay coefficient of variation (CV). Therefore, data from 40 players were used for the final analysis. Participants and their guardians (for U15 and U17) provided written informed consent before the study commenced. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, and all procedures received the University Ethics Committee approval (CAAE: 43023815.0.0000.0075).

Saliva sampling and analysis. Saliva sampling was taken by passive drool before and three min after the Yo-Yo Intermittent Recovery Test Level 1 (Yo-Yo IR1). No food was taken 1.5 hours before the first saliva sample (Gibson *et al.*, 1999). Saliva was collected in a sterile 15-ml centrifuge tube over a five-minute period and stored at -80°C until assay. After thawing and centrifugation (1500 g for 15 min), salivary pH and salivary lactate were analyzed on the pH meter (pH policontrol 150®) and biochemistry analyzer YSI 1500 (Yellow Springs, Ohio, USA), respectively. Saliva samples were then tested for IL1 β concentration, using enzyme-linked immunosorbent assays (ELISA, Salimetrics™ expanded range kit), in accordance to previously described procedures (de Arruda *et al.*).

Yo-Yo Intermittent Recovery Test Level 1 (Yo-Yo IR1). In the present study, the Yo-Yo IR1 test was used as a controlled high-intensity exercise. Yo-Yo IR1 has been shown to elicit maximal aerobic responses while highly taxing the anaerobic system (Castagna *et al.*, 2008). In this test, the players should perform a repeated 20-m shuttle runs, back and forth between the starting line and finish line marked by cones, at progressively increasing speeds dictated by an audio bleep emitting from a CD player. Between each shuttle, the players had a 10-s period of walking around a cone placed five m from the starting line. Failure to achieve the shuttle run on two successive occasions resulted in termination of the test.

Perception of oral hygiene level. All subjects underwent a dental examination to evaluate the oral hygiene level. The adapted Simplified Oral Hygiene Index (IHO-S) (Greene & Vermillion, 1964; Mashima *et al.*, 2017) was applied. In order to quantify and classify the oral hygiene level, according to the Simplified Oral Hygiene Index, the following approach was used: A value of 0, means clean tooth and total absence of biofilm; 1, means tooth that presents 1/3 of its surface with biofilm; 2, tooth that presents biofilm up to 1/2 of its surface, and 3, tooth presents biofilm beyond 1/2 of its surface. The values were then summed and divided by the number of counted dental surfaces, thus establishing a final score. The score between 0 and 2.5 represented a good oral hygiene, while a score greater than 2.5 represented poor oral hygiene.

Statistical analysis. The whole group was divided a posteriori into good oral hygiene group (GHG; score ≤ 2.5) and poor oral hygiene group (PHG; score > 2.5). A two-way analysis of variance (ANOVA two-way; condition [GHG and PHG] and time-point assessments [pre-Yo-Yo R1 and post - Yo-Yo R1]) with repeated measures in the second factor were used after checking for data normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test), for analysing salivary pH, salivary lactate and IL-1 β concentrations. The sphericity of data was assumed, according to the Mauchly's test results. Statistical significance was set at $p < 0.05$.

RESULTS

A significant interaction effect ($F = 15.33$; $P = < 0.0001$), group effect ($F = 22.94$; $P < 0.0001$), and time effect (pre to post Yo-yo IR1; $F = 1164$; $P < 0.0001$) was observed for salivary pH. The PHG presented a lower value at pre and post-Yo-Yo IR1 compared to GHG, despite the decrease in pH concentration for both groups after performing the Yo-Yo IR1. (Fig. 1A). A similar result was observed for salivary lactate. A significant interaction effect ($F = 7.60$; $P = 0.008$), group effect ($F = 21.91$; $P < 0.0001$), and time effect (pre to post Yo-yo IR1; $F = 391$; $P < 0.0001$) was also observed for salivary lactate. The PHG group presented a higher salivary lactate concentration at pre and post-Yo-Yo IR1 compared to GHG group, despite the increase in salivary lactate concentration for both groups after the Yo-Yo IR1 (Fig. 1B).

The results of IL-1 β showed no effect of interaction ($F = 0.0008$; $P = 0.97$), group ($F = 0.40$; $P = 0.52$), or time ($F = 0.07$; $P = 0.78$).

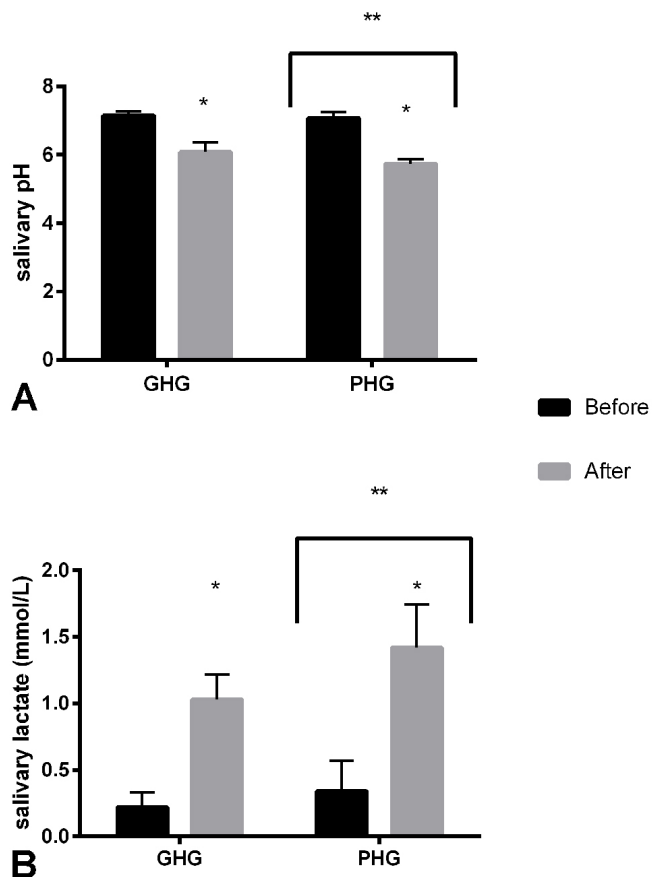


Fig. 1. Salivary pH (A) and salivary lactate concentration (B) at pre (before) and post (after) Yo-Yo IR1 for poor oral hygiene group (PHG) and good oral hygiene group (GHG). * = difference to before ($P < 0.05$); ** = Significantly different from GHG.

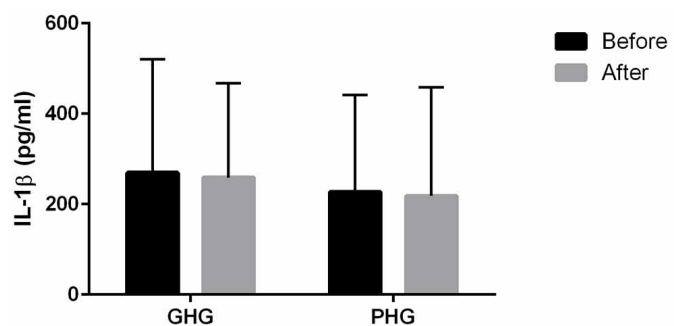


Fig. 2. Salivary IL-1 β at pre (before) and post (after) Yo-Yo IR1 for poor oral hygiene group (PHG) and good oral hygiene group (GHG).

DISCUSSION

This study aimed to examine the response of salivary pH, salivary lactate, and salivary IL-1 β in elite adolescent basketball players after a high-intensity intermittent running test (Yo-Yo IR1). In addition, the influence of oral hygiene on these biomarkers was addressed. The findings of the study corroborate the hypothesis that the high-intensity exercise would tax the anaerobic metabolism, leading to a change in salivary pH and salivary lactate, with a decrease (pH) and an increase (lactate concentration) of these biomarkers from pre to post-exercise, respectively. Nevertheless, contrary to the expectation the IL-1 β concentration was not affected by the exercise, nor by the oral hygiene level. As hypothesized, however, the oral hygiene status influenced the salivary pH and salivary lactate responses. The change of salivary pH and salivary lactate were less pronounced in the GHG compared to PHG.

The present findings indicate that a high-intensity exercise can lead to a modification in salivary pH and salivary lactate in elite adolescent basketball players. Indeed, the present data corroborate earlier assumptions that the combination of physical exercise with the eating habits may lead to a decrease in salivary pH, which in turn might contribute to the increase in dental erosion and caries risk (Frese *et al.*). Moreover, it is worth mentioning that the present results for salivary pH, and the fact that it may be influenced by the level of oral hygiene, should be viewed as an important information to practitioners in sports setting and dentistry community in general working with adolescent players, as it has been proposed that a decreased salivary pH, as observed in the present study, might be associated with tooth wear (Horswill *et al.*, 2006; Mulic *et al.*, 2012).

The findings of the present study are novel and add important information to the literature. The data suggest that the oral hygiene status is associated with decreased salivary pH in elite adolescent basketball players. The players group with poor oral hygiene (PHG) demonstrated a greater decrease in salivary pH compared to the good oral hygiene group (GHG). Taking these results into account, it could be speculated that due to repeated training sessions taxed the anaerobic metabolism, which are inherent to the youth elite basketball players training program (Miloski *et al.*; Moraes *et al.*, 2017), the player who presents poor oral hygiene might be more susceptible to present oral biofilm formation, and might be therefore exposed to a greater risk of local oral and systemic diseases. These alterations

could not only compromise his oral health, but might also affect performance.

Moreover, together with the acidification of salivary pH, the higher salivary lactate concentration was observed for PHG. An interaction effect, group effect, and also time effect (pre to post Yo-Yo IR1) was observed. The PHG presented a higher salivary lactate concentration at pre (before) and post (after) Yo-Yo IR1 compared to GHG; despite the observed increase in salivary lactate concentration in both groups after (vs before) the Yo-Yo IR1. These results are aligned with the hypothesis that the poorer the oral hygiene, the higher the change in these biomarkers. In addition, the increase in salivary lactate for both groups reveals that an exercise that tax the anaerobic metabolism could elevate salivary lactate, likely because of the intensity of the performed exercise; in conjunction, these results suggest that a higher acidic oral environment might be associated with a poor oral hygiene status in youth basketball players. These results add to previous findings demonstrating the usefulness of adopting salivary lactate as an alternative to blood lactate to examine the intensity of the performed exercise (Bardon' *et al.*, 1983; Segura *et al.*, 1996; Bocanegra *et al.*, 2012; Tékus *et al.*, 2012) while suggesting that the analysis and interpretation of salivary response should include the assessment of the oral hygiene status, as it seems to play a key role in the responses of both salivary lactate and salivary pH.

In the present study, contrary to the hypothesis, there was no significant change in IL-1 β from pre-to-post exercise, as well as, no difference between groups in IL-1 β concentration was observed. The increase in IL-1 β was expected as the high-intensity exercise could induce to elevated inflammatory response (Cooper *et al.*, 1985). Indeed, pro-inflammatory cytokines has been proposed to play a role in regulating the bidirectional communication between neuro-endocrine and immune systems (Besedovsky & Del Rey, 2007; O'Connor *et al.*, 2009).

Due to the increased sympathetic activity and the elevated energetic demand from the intense exercise, the IL-1 β increase was hypothesized. The lack of change in IL-1 β from pre to post-exercise, might be in part explained by the high fitness level of the assessed players, which might reduce the inflammatory responses from the performed exercise. Interestingly, de Arruda *et al.* did not observe changes in IL-1 β in a sample of similar level of that examined in the present study. They investigated the effects of competition stage on some salivary biomarkers including IL-1 β cytokine in elite

adolescent basketball players, and reported no change in IL-1 β from pre-to-post matches regardless the competition stage, and despite the increase in salivary cortisol testosterone and alpha-amylase. The authors suggested that one possible reason for the results would be the high-level fitness of the players. Another possible explanation was the increased salivary cortisol which might have blunted the IL-1 β response (pre-vs post-matches). This negative feedback loop is suggested to play a critical role in regulating inflammation and maintaining health (Irwin & Cole, 2011; Bilbo & Schwarz, 2012). Despite no examination of the salivary cortisol responses in the present study, it might be suggested that the high-intensity exercise performed by players, which induced to an increase in salivary lactate concentration from pre to post exercise, might contributed to such physiological response.

Taking into account the results of the present study and those reported by de Arruda *et al.*, it is reasonable to note that both the high-intensity exercise with a short-duration and basketball match-play did not promote signals of tissue damage and destruction and inflammatory response (Cooper *et al.*, 2017) which in turn could lead to changes in IL-1 β concentration. Moreover, the current results also suggest that the IL-1 β concentration and responses from an intense exercise might not be a sensitive indicator of oral hygiene status. However, as previous studies have shown that IL-1 β could be potentially useful in early diagnosis and management of periodontal disease in adults (Rangbulla *et al.*, 2017), further studies with distinct populations and conditions (athletes vs non-athletes, sedentary vs trained individuals, males vs females, resting vs exercising conditions, and so on) should be conducted to extend the knowledge on this issue.

Despite the novel and important results presented in this investigation, some limitations should be highlighted. The present study evaluated elite adolescent basketball players from only one club. The results might be influenced by the local training environment, including the habitual food consumption, the training program, the high-fitness level of the players, as well as the professional structure in which they were involved. Therefore, caution is required to make inferences regarding the results for other adolescents. Moreover, this study used a cross-sectional experimental design which limits the generalization of the results for long-term responses. Future studies adopting the analysis used in the present investigation, assessing other team sports adolescent players, with different fitness and expertise levels, and conducted longitudinally, may aid

in advancing the knowledge regarding the research question addressed in the current study.

In summary, the results of the present study demonstrated that the high-intensity exercise taxed the anaerobic metabolism, and led to a change in salivary pH and salivary lactate. A decrease was observed in salivary pH and an increase in salivary lactate concentration from pre to post-exercise. Moreover, the findings suggest that the level of the oral hygiene can influence the salivary pH and salivary lactate responses, with a less pronounced response for those who present good oral hygiene compared to peers showing poor oral hygiene. The present results, therefore, should be viewed as important information for both, practitioners in sports setting and the dental community working with adolescent players, in order to monitor players' oral habits by means of biological and behavioral measures, while educating them to be aware about the appropriate oral habits to avoid poor oral health.

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RESUMEN: Este estudio examinó las respuestas de pH salival, lactato salival e IL-1 β salival de una prueba de carrera intermitente de alta intensidad, y la influencia del estado de higiene oral en los biomarcadores en jugadores adolescentes de baloncesto de élite. En el análisis participaron 46 jugadores adolescentes. Se tomó una muestra de saliva antes y 3 minutos después de un ejercicio de alta intensidad (Prueba de recuperación intermitente Yo-Yo Nivel 1; Yo-Yo IR1). Para cuantificar y clasificar el nivel de higiene oral, los atletas fueron sometidos a un examen dental y se aplicó un índice adaptado de higiene oral simplificado. Después del examen dental, el grupo se dividió en un grupo de buena higiene oral (GHG) y un grupo de mala higiene oral (PHG). Los resultados de un análisis de varianza mostraron un efecto de interacción significativo ($P = 0.0003$), efecto de grupo ($P < 0.0001$) y efecto de tiempo (antes y después de Yo-yo IR1; $P < 0.0001$) para el pH salival y para lactato salival (efecto de interacción, $P = 0.008$; efecto de grupo, $P < 0.0001$; efecto de tiempo, $P < 0.0001$) con pH salival más bajo y lactato salival más alto en IR1 pre y post YoY para PHG, pero no se observó una diferencia para IL-1 β . Los datos demostraron que el ejercicio de alta intensidad genera un cambio significativo en el pH salival y el lactato de los jugadores de baloncesto, y que el estado de higiene oral influyó en estas respuestas, con un cambio mayor para aquellos jugadores que mostraron una mala higiene oral.

PALABRAS CLAVE: saliva, citosina, salud bucal, flora oral; atletas juveniles.

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