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Monitoring Salivary Immunoglobulin A Responses to Official and Simulated Matches In Elite Young Soccer Players

by

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The purpose of the present study was to examine SIgA responses (concentration [SIgAabs] and a secretion rate [SIgArate]) to official and simulated competitive matches in young soccer players. The sample was composed of 26 male soccer players (age 15.6 ± 1.1 yrs, stature 177.0 ± 6.1 cm, body mass 70.5 ± 5.7 kg). Four soccer matches (two simulated matches [SM] and two official matches [OM]) were conducted. The matches consisted of two halves of 35 min with a 10 min rest interval. Each assessed player participated in only one SM and one OM. All matches were performed in the same week, during the competitive season, and at the same time of the day (9:00 am), separated by 48 h. Saliva samples were collected before and after every match. The session rating of perceived exertion was reported 30 min after each match in order to determine the internal training load (ITL). A significant decrease in SIgAabs and SIgArate after OM was observed when compared to the pre-match value. In addition, the SIgArate was higher at pre-OM when compared to pre-SM. A higher ITL for OM was observed compared to SM. The current findings indicate that OM may lead to a decrease in the main mucosal immunity function parameter of young soccer players that could increase the risk of URTI. Coaches should be aware of it in order to plan appropriate training loads and recovery procedures to avoid or minimize the likelihood of upper respiratory tract infection occurrences.

***Key words**: SIgA, mucosal immunity, athletic performance, sports, adolescents.*

Introduction

The mucosal surfaces are normally protected by a network of organized structures located in the gut, urogenital tract, oral cavity and respiratory system, collectively known as the mucosal immune system (MIS) (Gleeson and Pyne, 2000). A major component of the MIS is salivary immunoglobulin A (SIgA). In fact, a low level of SIgA has been associated with an increased risk of upper respiratory tract infection (URTI) in athletes (Bishop and Gleeson, 2009; Fahlman and Engels, 2005; Gleeson et al., 2012). Moreover, a transient fall in SIgA has been shown to be a good predictor for increased risk of URTI (Neville et al., 2008). The role of SIgA is to prevent viral replication and to inhibit the attachment of bacteria and viruses at mucosal epithelium in the mouth, throat and upper respiratory tract (Mackinnon, 1996). Therefore, a decrease in the production and/or concentration of this antibody would represent a risk factor for subsequent URTI episodes in athletes (Fahlman and Engels, 2005).

With regard to studies on endurance athletes, the SIgA acute responses in team sport athletes have been less investigated (He et al., 2010; Koch et al., 2007; Moreira et al., 2011a; Moreira et al., 2013a). Results from these

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investigations may be considered somewhat inconclusive as some showed an increment in SIgA concentration after the assessed matches while others demonstrated neither change nor decrease in SIgA concentration. However, recently, Owen et al. (2014) demonstrated a significant reduction in SIgA after completion of a high intensity training session when compared to a low intensity unit in a sample of 10 elite male professional soccer players. This result suggests that training intensity plays a key role in SIgA changes. In addition, some studies have demonstrated that the psychological stress can increase SIgA concentration during tasks that do not involve physical effort (Benham et al., 2009; Bosch et al., 2001; Zeier et al., 1996).

However, most of the aforementioned studies were conducted on young adults or professional players. Only a few studies that have examined the SIgA responses to training or competition in young athletes (Filaire et al., 2004; Li et al., 2012; Mortatti et al., 2012; Nieman et al., 2000). Nowadays, like adult athletes, young team sport athletes are often submitted to intense training loads and high competition demands (Hartwig et al., 2009). This population is also under continued pressure to obtain good results in their age category competitions and has to cope with the uncertainty of achieving the high-level professional setting. This scenario, therefore, imposes a great level of psychological and physiological stress to young soccer players which might not be prepared to deal appropriately with all these demands.

Among the few studies conducted with young team players, focused on SIgA response and its association with stress (Filaire et al., 2004; Li et al., 2012; Nieman et al., 2000), Mortatti et al. (2012) monitored young soccer players through an official competition which included 7 matches played over 20 days, and reported a significant relationship between the SIgA decrease and the incidence of URTI symptoms, as the team advanced towards the final stages of the competition. Indeed, Moreira et al. (2014) examined the effect of a 21 week competitive season divided into a pre-season (12 weeks), a competitive season (7 weeks) and detraining (2 weeks) on salivary cortisol, SIgA and upper respiratory tract infection (URTI) symptoms in 34 pre-adolescent male soccer players. The results of the study showed a significant increase in a SIgA secretion rate and a decrease in URTI symptoms after the 2 week detraining period, suggesting that the accumulated training loads and competitive demands negatively affected the mucosal immunity of youth players and that a short-prophylactic period may attenuate mucosal immunosuppression related to URTI. Additionally, Nazem et al. (2011) showed a significant decrement in SIgA concentration after the most difficult matches in young female handball athletes assessed over successive matches.

Collectively, these findings suggest that intense training loads and high competition demands, as well the importance and difficulty of the match, may affect the responses of mucosal immunity. However, up to now, no previous investigations have compared the SIgA responses to simulated matches (SM) and official matches (OM) in elite young soccer players. The advance of this knowledge could aid coaches and staff members to prescribe appropriate training loads and recovery periods in order to minimize the risk of the reduced mucosal immune function. These data would also contribute to a deeper understanding of players' preparedness for training, competition and performance. Therefore, the purpose of the present investigation was to examine SIgA responses (absolute concentration and secretion rate) to OM and SM in young soccer players. It was hypothesized that OM would lead to a greater decrement in SIgA responses due to its higher psychophysiological stress.

Material and Methods

Participants

Forty-four soccer players initially participated in the study and completed two SM. However, only data from 26 players who participated in both OM and SM were retained for analysis. The sample was composed of 26 male soccer players (age 15.6 ± 1.1 yrs, body height 177.0 ± 6.1 cm, body mass 70.5 ± 5.7 kg). Usually, the assessed players were involved in a schedule training program which consisted of 10 to 12 sessions per week, with duration of 90-120 min, including strength and conditioning sessions, sprints and repeated-sprint bouts as well as intermittent running exercises, soccer-specific drills and small-sided games.

The investigated players were living at the training facilities of the club during the period of the investigation. Therefore, they shared accommodations, had the same meals being served at the same time, and were also submitted to similar recovery times after daily training sessions. This situation provided an appropriate environment control, therefore, reducing factors which could influence the acute SIgA responses from the investigated matches, such as nutrition status, exposition to pathogens, recovery from training and OM, and awakening time. After approval of the Research Ethics Committee of the School of Physical Education and Sport, University of São Paulo, the experimental protocols were explained in detail. Written informed consent was obtained from each participant and their respective parents or guardians.

Procedures

Four soccer matches (two SM and two OM) were conducted. The matches were composed of two halves of 35 min with a 10 min rest interval. Each assessed player participated in only one SM and one OM. All matches were performed in the same week, during the competitive season and at the same time of the day (9:00 am), separated by 48 h. Data from the two SM and the two OM were pooled for analysis. Saliva samples were collected before and after each match. The session rating of perceived exertion (session-RPE) was reported 30 min after each match in order to monitor the internal training load (ITL).

Measures

Internal Training Load (ITL)

A session-RPE score was assessed after each match, in order to quantify the ITL, as proposed by Foster (1998). Briefly, each player rated the training session using the CR-10 sliding scale, where 0 = nothing at all, and 10 = very, very hard (maximal). These data were collected 30 min after each match to ensure that the perceived effort was based on the entire game rather than the last effort intensity. In order to determine the ITL expressed in arbitrary units (AU), the product of session duration (min) and the RPE score (CR-10) rated by the player was used. All assessed players were familiarized with the CR-10 scale, as it had been used routinely during their training program.

Saliva collection

Athletes provided saliva samples approximately 10 min before the pre-match warm-up; post-match saliva samples were collected within 10-15 min of the end of the SM and OM. The participants abstained from consuming food and caffeine-containing products for at least 2 h before the collection of saliva. After rinsing their mouth with distilled water, athletes were seated with eyes open, the head tilted slightly forward and making minimal orofacial movement. Unstimulated saliva was collected into sterile 15 ml centrifuge tubes over a 5 min period and then stored at -80ºC until assayed for SIgA concentration. The tubes were reweighed before analysis so that saliva volume could be estimated in accordance with the procedure adopted by Moreira et al. (2013b). Saliva density was assumed to be 1.00 $g \cdot mL^{-1}$. The recovery interval from the last bout of exercise was 24 h.

SIgA assays

SIgA concentration was measured in duplicate by an enzyme-linked immunosorbent assay (ELISA; s-IgA EIA kit, ALPCO Diagnostics, Salem, MA, USA) USA). Saliva samples were thawed, centrifuged at 1,630 g for 10 min, and the supernatant was diluted (1: 2,000) in the ELISA wash buffer. Subsequently, 100 mL of calibrators and diluted saliva samples were added to microtiter wells (precoated with polyclonal rabbit anti-human IgA) and incubated for 1 h, with constant shaking, at room temperature. After incubation, the plate was aspirated and washed 5 times with 250 mL of ELISA wash buffer to remove all unbound substances. Then, 100 mL of peroxidase-labeled mouse anti-IgA conjugated was added to each well on the microtiter plate. After incubating the plate for 1 h, with constant shaking at room temperature, the contents of the plate were decanted and washed 5 times with 250 mL of the ELISA wash buffer to remove all unbound substances. After washing, 100 mL of tetramethylbenzidine (TMB) substrate solution was added and incubated for 5 min at room temperature with no mixing. This enzyme acted on the substrate and caused a blue color to appear in proportion to the amount of the peroxidase present. Finally, 50 mL of the stop solution was added to the wells and the optical density was read on the plate reader at 450 nm. A yellow color was formed after the stop solution was added.

The amount of color detected was directly proportional to the amount of SIgA present. From a calibration curve (optical density vs. IgA concentration of the calibrators), the concentration of SIgA ($SIgA_{abs}$ - micrograms per milliliter) in each sample was interpolated. The SIgA secretion rate ($SIgA_{rate}$ - micrograms per minute) was calculated by multiplying the absolute $SIgA_{abs}$ by a salivary flow rate (milliliters per minute), in accordance with the procedure adopted in previous studies (Moreira et al., 2012b, 2013b).

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software (IBM SPSS Statistics 18, Inc., Chicago, IL). Descriptive data are reported as means and standard deviation (SD). Data normality was confirmed by the Shapiro-Wilk test. A two-way analysis of variance with repeated measures (time factor; pre-match and post-match) was used to compare the conditions (OM and SM) and time-points (pre and post). The Bonferroni test was used as a post hoc test. The level of significance was set at $p \leq 0.05$.

Results

SIgA responses to the SM and OM are presented in Figure 1. A significant decrease in $SIgA_{abs}$ (Figure 1A) was observed after OM when compared to pre-match value. In addition, a significant reduction in $SIgA_{rate}$ (Figure 1B) was detected after OM when compared to pre-match value. There was no alteration for these variables when compared pre- and post-SM values. A significant increase in $SIgA_{rate}$ was observed at pre-OM compared to pre-SM.

Figure 2 presents the magnitude of the ITL, which was assessed by a session RPE method and determined for SM and OM. A significantly higher ITL was observed for OM compared to SM.

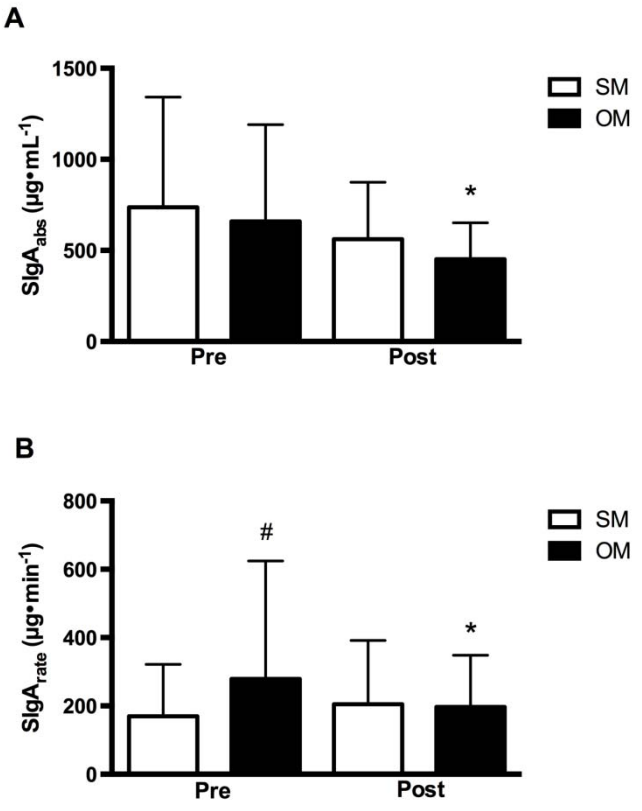

Figure 1

*Salivary IgA (SIgA) responses (concentration 1A and rate 1B) to simulated matches (SM) and official matches (OM). Note: # - indicates significant difference from SM ($p < 0.05$). * - indicates significant difference from pre-match ($p < 0.05$).*

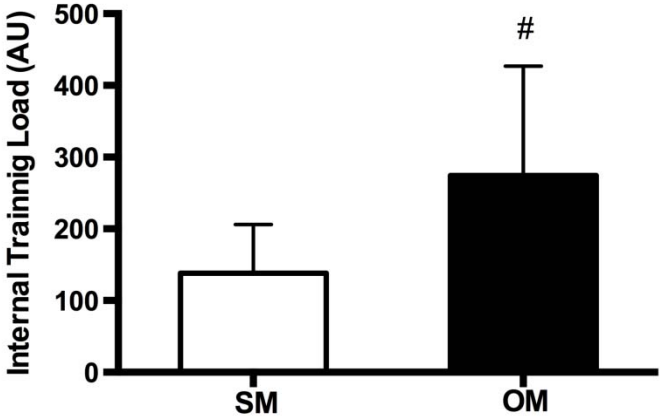

Figure 2

Internal training load for simulated (SM) and official (OM) matches expressed by arbitrary units (AU; mean ± SD).
 # - indicates significant difference from SM ($p < 0.05$).

Discussion

The key findings of this study were: 1) a significant decrease in SIgA responses ($SIgA_{abs}$ and $SIgA_{rate}$) from pre to post-OM, and 2) a higher pre-OM $SIgA_{rate}$. The decrease in SIgA responses for OM is in line with the study's hypothesis which postulated that such a decrease from pre to post-OM would be observed. However, a higher pre-OM $SIgA_{rate}$ was not expected. It is reasonable to speculate that the elevated psychological stress experienced before an official competition may influence the responses of mucosal immunity measures.

The absence of change in SIgA concentration from pre to post SM is in line with previously reported data on professional soccer players (Moreira et al., 2009), but differ from observations of Moreira et al. (2011b), Fredericks et al. (2012) and Owen et al. (2014). Moreira et al. (2009) demonstrated that both $SIgA_{abs}$ and $SIgA_{rate}$ were not altered from pre to post soccer SM (two halves of 35 min with a 10 min interval). The authors suggested that some factors, such as withdrawal from the real competitive environment and the characteristics of the sport (intermittent character) might affect the responses. Together, the present results and findings reported by Moreira et al. (2009) indicate that SM do not represent a significant source of stress to modulate the mucosal immunity function of soccer players. The novelty of the present study is that its findings suggest that the same acute SIgA responses from SM may be expected regardless of the soccer player's age (adults or young soccer players). This result adds to the literature by advancing understanding of the effect of SM on mucosal immunity trends.

Contrary to the aforementioned findings, however, Fredericks et al. (2012) investigated the SIgA responses in English Premier League professional soccer players during the first training session of the season, which was completed after the summer off-season. They observed a decrease of SIgA/protein measure after 20 min of the end of the activity (Fredericks et al., 2012). One possible explanation for the differences between findings from that study and the results of the present study could be the fact that in the Fredericks et al.'s study the investigated players were returning from an off-training period, and the players in the present study and in the study

of Moreira et al. (2009) were assessed during the competitive period. These discrepancies, therefore, could suggest that stress induced by SM, training sessions or friendly matches, could be higher when players are not in their optimal fitness preparation state and could negatively affect the mucosal immune function.

Besides the fitness level, the characteristics and specific demands of the different team sports also appear to influence the acute SIgA responses. For instance, Moreira et al. (2011a) described a reduction in SIgA variables after SM in adult futsal players. The difference between the findings of this study and the results of the present investigation could be attributed to the distinct characteristics of the assessed team sports (soccer vs. futsal). Collectively, the findings of the aforementioned studies and the results of the present investigation suggest that the fitness level as well as the characteristics and demands of team sports should be taken into account for comparing results from different studies that have investigated acute SIgA responses.

Differentially from the responses to SM, in the present study, OM represented a sufficient source of stress that resulted in a significant decrement in SIgA concentration. This result suggests that the additional stress provided by participating in an official competition may lead to changes in the mucosal immunity function in young soccer players. These findings are in agreement with data from the study conducted on young female handball players (Nazem et al., 2011) that reported a decrement in SIgA concentration after matches perceived as "more difficult". Considering the results from the present study and the findings reported by Nazem et al. (2011), it is plausible to suggest that additional factors such as match intensity, competitive anxiety, pressure to perform, associated with the participation in OM (more important or even more difficult matches), may impose a higher level of stress on young team sport players, leading, therefore, to alteration in their mucosal immunity and then increasing the risk of URTI.

An inherent drawback of the present study could be the fact that the sampling period undertaken was not long enough to assess the relationship between the SIgA acute responses from OM and SM and the URTI incidence. While it could be viewed as a limitation of the study, it is noteworthy that the main aim of the present investigation was not to examine such a relationship, but to examine if mucosal immunity of youth players would be differently affected due to their participation in OM and SM. One could speculate that OM would impose a greater psychophysiological stress on players, leading to a decrease in SIgA. Since SIgA concentration and its change are used to predict susceptibility to infection, notably to URTI (Gleeson et al., 2012; Neville et al., 2008), and considering the fact that previous studies with young team sport athletes have demonstrated this relationship (He et al., 2010; Moreira et al., 2011b; Mortatti et al., 2012), it might be assumed from the findings of the present study that OM may negatively affect mucosal immunity of young soccer players leading to an elevated risk of URTI. On the other hand, it is important to highlight that while SIgA may be used as a useful and valid indicator of the mucosal immune function related to training loads or competitive demands, the relationship between this salivary marker and URTI symptoms might be not direct, mainly due to factors other than salivary antibody, which may contribute to infection development (Diamond et al., 2008).

It is noteworthy that a greater session-RPE score was observed for OM when compared to SM. The session-RPE is a reliable method to assess exercise intensity in team sports (Impellizzeri et al., 2004; Manzi et al., 2010; Moreira et al., 2012c). Therefore, it is reasonable to assume that OM are played at higher intensity, probably as a result of a higher level of athletes' commitment. The effect of the intensity in SIgA responses was recently investigated by Owen et al. (2014). They compared 4 training sessions (low intensity, LI vs high intensity, HI) that were undertaken by 10 professional soccer players and showed that the percentage change from SIgA baseline concentration to post-training differed significantly between HI and LI, with a significant decrease in SIgA in the HI session (Owen et al., 2014). Indeed, previous studies reported a greater RPE score for real competition when compared to training simulations (Moreira et al., 2012a, 2012b, 2012c). The results from the present study suggest that the session-RPE method together with SIgA responses could be an insightful approach for coaches and coaching staff in order to assess the

magnitude of training loads and competition demands which in turn could aid these professionals to adjust the training plan for young soccer player.

Another important finding of the present investigation was a higher $SIgA_{rate}$ observed in pre-OM compared to pre-SM. Despite the difficulty to explain this result, it could be attributed to the increased sympathetic activity associated with the anticipatory stress of the official competition (Alix-Sy et al., 2008). This increased sympathetic activity would stimulate the SIgA production or even the translocation of this immunoglobulin through the epithelial cells (Bishop and Gleeson, 2009). Therefore, the higher $SIgA_{rate}$ value would be associated with the enhanced psychological stress experienced by young players in anticipation of OM condition which in turn could amplify the sympathetic activity, that ultimately might lead to the increase in the production or in the translocation of SIgA elevating the available SIgA in the mucosal surface (Bishop and Gleeson, 2009).

During the pre-OM, there was no physical effort, therefore, the psychological stress may better justify the higher $SIgA_{rate}$ value observed for this time-point. This phenomenon has been observed in many previous studies designed to observe the SIgA responses to different psychological sources of stress (Benham et al., 2009; Bosch et al., 2001; Willemsen et al., 2002; Zeier et al., 1996). In general, these studies have been developed to investigate the effect of psychological stress on SIgA without performance of physical effort. Previous findings indicate that an increase in SIgA concentration may be expected as a response to psychological challenges in the absence of physical effort (Benham et al., 2009; Bosch et al., 2001; Willemsen et al., 2002; Zeier et al., 1996). Collectively, these results strongly suggest that psychological stress may elevate SIgA concentration, and may explain, at least partially, the higher $SIgA_{rate}$ value observed for pre-OM in the present study.

In summary, the results of the present study confirm the hypothesis that a decrease in $SIgA_{abs}$ and $SIgA_{rate}$ after OM would be observed. Additionally, a greater internal training load was found for OM. Together, these results indicate that a higher psychophysiological stress would be expected for OM when compared to SM in young soccer players, which in turn may compromise the mucosal immunity of these athletes. Interestingly, a higher $SIgA_{rate}$ at pre-OM compared to pre-SM was also noted. This is a novel result from the present study and it suggests that an increase in SIgA may be expected as a response to psychological challenges in the absence of physical effort. Coaches should be aware of the elevated psychophysiological stress induced by OM in young soccer players, as well as the post-match compromised mucosal immunity, which might increase the risk of URTI. Therefore, coaches should consider these findings when planning training loads and recovery procedures to avoid or minimize the likelihood of URTI occurrences.

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