DOI: 10.1519/JSC.0b013e3181b3dd7a

Salivary Immunoglobulin A Response to a Match in Top-Level Brazilian Soccer Players

Alexandre Moreira,[1] Franco Arsati,[2] Patrícia Ramos Cury,[2] Clóvis Franciscon,[3] Paulo Roberto de Oliveira,[4] and Vera Cavalcanti de Araújo[2]

[1]Department of Sport, School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil; [2]Department of Oral Pathology, São Leopoldo Mandic Dental Research Center, Campinas, Brazil; [3]Faculty of Physical Education, Metropolitan United Faculty – FMU, São Paulo, Brazil; and [4]Department of Sports Science, Faculty of Physical Education. State University of Campinas, Campinas, Brazil

Abstract

Moreira, A, Arsati, F, Cury, PR, Franciscon, C, Oliveira, PR, and Araújo, VC. Salivary immunoglobulin a response to a match in top-level brazilian soccer players. *J Strength Cond Res* 23(7): 1968–1973, 2009–It has been suggested that several parameters of mucosal immunity, including salivary immunoglobulin A (s-IgA), are affected by heavy exercise either in field sports or in the laboratory environment. Few observations have been made during a true sporting environment, particularly in professional soccer. We tested the hypothesis that salivary IgA levels will be decreased after a 70-minute regulation in a top-level professional soccer friendly match. Saliva samples from 24 male professional soccer players collected before and after the match were analyzed. Salivary immunoglobulin A concentration was measured by enzyme-linked immunosorbent assay and expressed as the absolute concentration (s-IgAabs), s-IgA relative to total protein concentration (IgA-Pro), and the secretion rate of IgA (s-IgArate). Rate of perceived exertion (RPE) was used to monitor the exercise intensity. The paired *t*-test showed no significant changes in s-IgAabs and s-IgArate ($p > 0.05$) from PRE to POST match. However, a significant ($p < 0.05$) increase in total protein concentration (1.46 ± 0.4 to 2.00 ± 07) and a decrease in IgA-Pro were observed. The best and most significant correlation was obtained with the RPE and changes in IgA-Pro ($rs = -0.43$) and could indicate that this expression may be an interesting marker of intensity in a soccer match. However, further investigation regarding exercise intensity, protein concentration, and immune suppression, particularly in team sports, is warranted. From a practical application, the variability of the responses among the players leads us to suggest that there is a need to individually analyze the results with team sports. Some athletes showed a decrease in s-IgA expressions, suggesting the need for taking protective actions to minimize contact with cold viruses or even reducing the training load.

Key Words immune function, sports, saliva, IgA

Introduction

In the past 2 decades there has been a substantial increase in research dedicated to determining the relationship between exercise and immune function. Before 1970 there were only a handful of papers describing the effects of exercise on the numbers of circulating white blood cells. Since the mid 1970s there has been an increasing number of papers published on this subject (6).

This is an important area of study because exercise may modulate the immune system's ability to monitor and protect the individual from disease and repair damage (30). A J-shaped relationship between the risk of contracting upper-respiratory tract infection (URTI) and the amount of regular exercise has been proposed (19). A J-shaped relationship between URTI incidence and training load suggests that a moderate level of regular exercise provides some protection against infectious episodes, whereas severe levels of chronic exercise seem to increase the incidence of infection above that of sedentary individuals (19,20). In most of these studies, endurance exercise has been the independent variable in attempts to establish the relationship of heavy exertion, immunity, and infection. The immune response to heavy endurance exertion has received much attention after the publication of several epidemiological studies indicating an increased risk of URTI during the 1-to 2-week period after marathon and ultramarathon race events (17,18,22,23,24).

Various authors have utilized the salivary concentrations of immunoglobulin A (s-IgA) to investigate the relationship of exercise training load, immune function, and URTI incidence. Salivary immunoglobulin A is the predominant immunoglobulin in mucosal fluids, serving to inhibit the attachment and replication of pathogens and neutralize viruses and toxins (36). Salivary immunoglobulin A has been show to correlate more closely with URTI incidence than serum antibodies or

Address correspondence to Alexandre Moreira, alemoreira@usp.br.

23(7)/1968–1973

Journal of Strength and Conditioning Research

other immune parameters (12). In addition, low resting levels of s-IgA have been correlated with an increased risk of URTI among competitive swimmers (4); it also has been suggested that decreases in the concentration of s-IgA may act as a marker of excessive training (13,29). A few studies have provided evidence for the potential of monitoring decreases in s-IgA concentration during training to predict an individual athlete's risk of URTI (5).

Studies of the acute response of s-IgA to heavy exercise have produced conflicting results. Several studies reported decreased s-IgA levels following various modes of intense exercise, including swimming (34), interval exercise (12), kayaking (11), running (21), and repeated sprint cycling (Wingate tests) (3), whereas some studies have reported no change in s-IgA levels after acute and chronic weight training (15) or after an 80-minute collegiate rugby match (9). Furthermore, several studies have reported increases in s-IgA following acute bouts of exercise (1,14,26,28,35); however, Sari-Sarraf et al. (26) did not report a statistically significant increase following an acute bout of exercise. The reason for these inconsistent and discrepant findings may be the different methods used to express IgA data; differences in the training and nutritional status of the subjects; and differences in the duration, intensity, and modality of exercise (e.g., cycling, running, swimming; intermittent vs. continuous exercise; team sports vs. individual sports).

Although the implications of an observed impact on s-IgA levels following heavy exercise are of great interest to sport scientists, relatively few data have been obtained in a true environment setting among team sports such as soccer, particularly in top-level professional teams. To our knowledge, no information is available in professional Brazilian soccer about acute changes in s-IgA during a soccer match.

The aim of the present study was to test the hypothesis that s-IgA would be depressed after a 70-minute regulation in a top-level professional soccer friendly match. To minimize discrepancies that may be attributed to the method of expressing IgA levels, we determined the absolute concentration of s-IgA (s-IgAabs), the IgA concentration relative to total protein concentration (IgA-Pro), and the secretion rate of IgA (s-IgArate; the multiple of saliva flow rate and s-IgA concentration). Rate of perceived exertion (RPE) was used to monitor the intensity of the exercise during the match.

Methods

Experimental Approach to the Problem

To test the hypothesis that s-IgA would be depressed after a professional soccer match, saliva samples were obtained from subjects before and after a regulation 70-minute friendly soccer match during the competitive season. Subjects provided resting saliva samples approximately 10 minutes before the presession warm-up (PRE), and postsession saliva samples were collected within 5 minutes after the conclusion of the match (POST). The aim was to model an actual match with specific characteristics. Two halves of 35 minutes with an interval of 10 minutes were carried out with 11 soccer players on each team (with 1 substitution for each team). At the interval the subjects were encouraged to drink water ad libitum. The session took place during the afternoon and the PRE saliva samples were obtained at 15:30. To quantify the intensity of the session, RPE was recorded for all subjects using Borg's 6-20 scale (8). The players were familiarized with this procedure during the regular training sessions.

Subjects

The casual, nonprobabilistic method was utilized for the selection of the sample of individuals from whom data were collected. Therefore, the sample belonged to a soccer team from the main São Paulo (Brazil) division (A) and was composed of 24 male professional soccer players (age 23 $\pm$ 4 years, height 179.6 $\pm$ 6.7 cm, body mass 76.3 $\pm$ 8.0 kg). This team achieved fourth place in the 2007 São Paulo State Championship, reaching the semi-final. This state championship can be considered the highest level of competition for Brazilian professional teams and players. At this level, for example, there are 2 teams that won the FIFA Club World Cup (2000 and 2005). Four members of the team support staff were selected as "environmental controls" by being exposed to the same environmental conditions, traveling during the season, and participating in the training sessions and on the sidelines at matches with potential exposure to similar pressures during the season as the soccer players. All subjects provided informed consent. The Ethics Research Committee of the School of Physical Education and Sport of University of São Paulo approved the research.

Saliva Collection

The subjects abstained from food and caffeine products for at least 2 hours prior to the PRE saliva collection. Initially, the subjects were required to rinse out their mouths with distilled water to remove potential sample contaminants that might affect salivary IgA levels. The subjects were in a seated position, with eyes open, head tilted slightly forward, and making minimal orofacial movement. Unstimulated saliva was collected into sterile 15-mL centrifuge tubes over a 5-minute period for each sample. Immediately after collection, the saliva samples were frozen and stored at -80°C until assayed for s-IgA concentration.

Assays

Salivary immunoglobulin A concentration was measured by enzyme-linked immunosorbent assay (ELISA; s-IgA EIA kit, ALPCO Diagnostics, Salem, Massachusetts, USA). Saliva samples were thawed and centrifuged at 3,000 rpm for 10 minutes, and supernatant was diluted (1:2000) in ELISA wash buffer. After that, 100 μL of calibrators and diluted saliva samples were added to microtiter wells (precoated with polyclonal rabbit anti-human IgA) and incubated for 1 hour, with constant shaking, at room temperature. After incubation, the plate was aspirated and washed 5 times with 250 μL of

ELISA wash buffer to remove all unbound substances. Then, 100 μL of peroxidase-labeled mouse anti-IgA conjugated was added to each well on the microtiter plate. After incubating the plate for 1 hour, with constant shaking at room temperature, the contents of the plate were decanted and washed 5 times with 250 μL of the ELISA wash buffer to remove all unbound substances. After washing, 100 μL of tetramethylbenzidine (TMB) substrate solution was added and incubated for 5 minutes 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 peroxidase present. Finally, 50 μL 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 stop solution was added. The amount of color detected was directly proportional to the amount of s-IgA present. From a calibration curve (optical density vs. IgA concentration of the calibrators) the concentration of s-IgA ($\mu g/mL^{-1}$) in each sample was interpolated. The s-IgA secretion rate ($\mu g/min^{-1}$) was calculated by multiplying the absolute s-IgA concentration by salivary flow rate (mL/min^{-1}). Salivary flow rate was determined by dividing the volume of saliva collected by the time of the sampling period. The relative amount of IgA to protein ($\mu g/mg^{-1}$) was determined by dividing the absolute concentration of s-IgA by the total protein concentration. Concentrations of total protein (mg/mL^{-1}) in the saliva were measured by using the BCA protein assay kit (Pierce Biotechnology, Rockford, Illinois, USA) using bovine serum albumin as a standard. The test–retest correlation of the s-IgA and total protein procedures in our laboratory are 0.98 and 0.99, respectively.

Statistical Analyses

All analyses were performed using the SPSS program (version 10.0, Chicago, Illinois, USA). Data are reported as means and *SD*. The distribution of the data was analyzed by the Shapiro-Wilk test. A paired *t*-test was used to compare PRE–POST patterns of change in each expression of s-IgA and to total protein. Pearson's product moment correlations were used to test the relationship among PRE–POST fluctuations using different methods of expressing IgA, and the Spearman correlation coefficient was used to test the correlations between change values (PRE–POST s-IgAabs, s-IgArate, IgA-Pro) and RPE.

Results

No changes in any expression of s-IgA were observed for the members of the team support staff. For this reason we presented only the results from soccer players.

RPE

The mean score obtained to RPE was 16.4 ($SD = 1.9$). The minimum score was 13. It is reasonable to admit that the mean soccer match intensity was above "hard" and it is not position (function) dependent. The minimum RPE among the players was "somewhat hard" (only 3 players and 1 was a goalkeeper). Another goalkeeper indicated that the intensity was hard. More than 45% of the subjects indicated that the exertion was "hard" and 25% said it was between "very hard" and "very, very hard."

Correlation Between Changes in Salivary IgA Expressions and RPE

The best and most significant correlation was obtained with the RPE and changes in IgA-Pro ($t = -2.26$; $p = 0.03$). The correlation ($rs = -0.43$) indicated that when the RPE increased, the decrements in IgA-Pro are greater.

PRE–POST s-IgA Expression Values, Total Protein, and Flow Rate

Table 1 gives s-IgA absolute concentration, s-IgA secretion rate, s-IgA expressed relative to total protein, and flow rate values from PRE to POST match. Significant differences are only to total protein and IgA-Pro. The values revealed increments to total protein and decrements to IgA-Pro. With other expressions of s-IgA (s-IgAabs, s-IgArate) and flow rate, no significant changes were observed.

Correlation Among PRE–POST Changes in s-IgA Measures

The correlation between changes in s-IgAabs and IgA-Pro was strongly and significantly correlated (0.78; $p = 0.00$), and a moderate correlation between s-IgAabs and s-IgArate was found (0.44; $p = 0.03$). The s-IgArate and IgA-Pro changes were also moderately correlated (0.44; $p = 0.03$).

Magnitude of the Individual Changes in s-IgA Expressions

Figure 1 depicts the magnitude of the individual changes in the various s-IgA expressions.

Table 1. Total protein, salivary IgA expressed relative to total protein, absolute concentration, secretion rate, and saliva flow rate for soccer players (mean + *SD*) ($n = 24$).

Variable	PRE	POST	*p*-value
Total protein (mg/mL^{-1})	1.46 ± 0.40	2.00 ± 0.7	0.00*
s-IgAabs ($\mu g/mL^{-1}$)	380 ± 22	384 ± 22	0.95
IgA-Pro ($\mu g/mg^{-1}$)	257 ± 11	196 ± 90	0.00*
s-IgArate ($\mu g/min^{-1}$)	172 ± 11	194 ± 14	0.38
Flow rate (mL/min^{-1})	0.50 ± 0.34	0.55 ± 0.36	0.25

PRE, resting saliva samples; POST, saliva samples within 5 minutes after the conclusion of the match; s-IgAabs, s-IgA absolute concentration; IgA–Pro, s-IgA relative to total protein; s-IgArate, s-IgA secretion rate.
*Significant at $p < 0.05$.

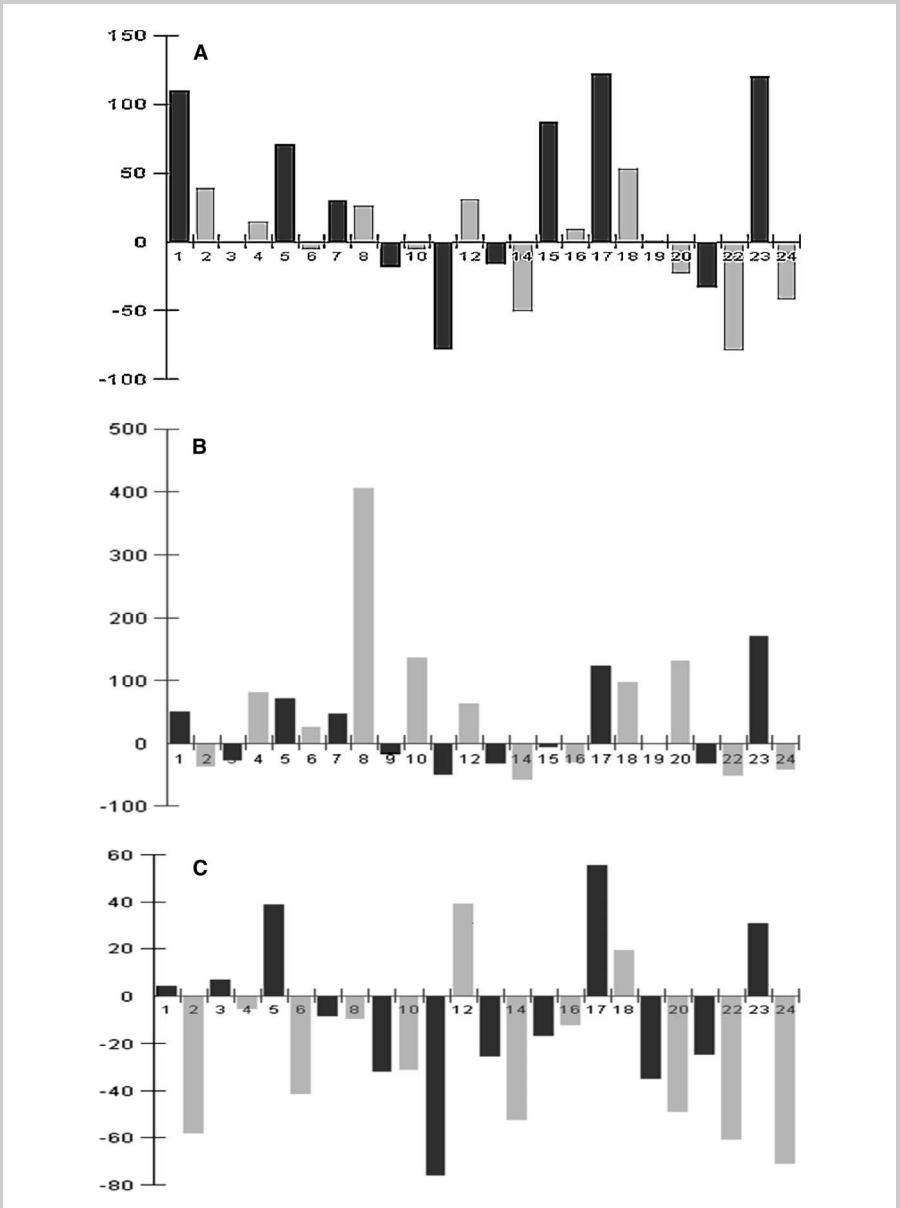

Figure 1. A, PRE–POST changes (%) in s-IgAabs for individual soccer players. B, PRE–POST changes (%) in s-IgArate for individual soccer players. C, PRE–POST changes (%) in s-IgA relative to protein for individual soccer players.

Discussion

The key finding of the present investigation was the observation of relative stability in mean IgA saliva content. This was observed when the s-IgA was expressed as absolute concentration or s-IgA secretion rate (difference was not significant) or as an increase in total protein and a decrease in s-IgA when expressed as relative to total protein (IgA-Pro). Furthermore, a significant correlation between RPE and changes in IgA-Pro was detected.

The ratio of IgA to total protein (IgA-Pro) has been investigated and discussed in previous exercise immunology research papers. Tomasi et al. (37) reported a 20% decrease in s-IgA concentration after 2 to 3 hours of competition in elite cross-country skiers that became a 40% decrease when expressed in relation to total saliva protein concentration. However, it has been suggested that the use of the ratio of IgA to total protein concentration can lead to a misinterpretation because the protein secretion rate itself can increase during exercise (1,38).

Bishop (2) has suggested that it may be more appropriate to express s-IgA concentration as a ratio to albumin because albumin is less affected by flow rate and is not actively secreted across the epithelial membrane. The total protein content of saliva is far more variable as a result of the high concentrations of enzymes such as amylase, which are induced by flow rate stimulation. Although this could be a limitation to analyze our results, in respect to mucosal immunity, in the present study the flow rate did not significantly change from PRE to POST. It is possible that the decrease in saliva flow rate was prevented or minimized by fluid intake during the half-time interval.

About total protein, Blannin et al. (1) observed a significant increase from 0.48 ± 0.10 mg/mL^{-1} of saliva protein concentration at preexercise to 1.80 ± 0.38 mg/mL^{-1} postexercise. Their subjects cycled on an electrically braked cycle ergometer at a work rate equivalent to 80% VO_2max until exhaustion. On another occasion the subjects cycled on the same ergometer at 55% VO_2max for 3 hours or to fatigue. The saliva protein concentration was higher for the 80% VO_2max condition at midexercise and postexercise. In this case, it is reasonable to assume that the increase in total protein could be a good marker of exercise intensity. The results of the present study also show that there was a significant increase in total protein from PRE (1.4 ± 0.4 mg/mL^{-1}) to POST (2.00 ± 0.7 mg/mL^{-1}) match. These changes appear to

result largely from the stimulation of amylase secretion by increased sympathetic nervous activity (2). The role of the total protein in immunological studies and the changes in protein levels under different physiological conditions are still poorly understood. These limitations are extensive for most of the salivary proteins functions (27). The main goal to search for the complete protein salivary composition will be its use as a diagnostic tool to monitor the physiological, health, or disease status of individuals (27).

In support of the speculation that the changes in total protein and likewise the IgA-Pro may be a useful marker of exercise intensity is the fact that the RPE (a marker of exercise intensity) was significantly correlated with changes in IgA-Pro. The results for IgA-Pro reflected the intensity of the exercise (RPE). Increases in the protein concentration caused the decrease in IgA-Pro from PRE to POST match, as s-IgAabs concentration remained relatively stable.

RPE has been shown to be a useful tool for prescribing exercise intensity based on its relationship with physiological indicators of exercise stress (8,31,33) and does not appear to be affected by exercise modality or training state (7,28). Besides that, the RPE has been accepted as a valuable tool for regulating intensity of exercise when designing prescribing exercise based, for example, on the blood lactate response (7) (an important marker of metabolic intensity).

Regarding IgA levels, in studies of elite or high-performance endurance athletes during their normal training programs or competitions, the agreement is that intense endurance exercise results in lower levels of IgA in saliva after each session (5). This consensus can be attributed by the fact that numerous studies have showed decreases in levels of IgA in saliva after intense exercise (3,4,11,12). The hypothesis that a rigorous soccer match would temporarily suppress mucosal immune function as assessed by s-IgA levels determined immediately after the match was not supported in the present study. Increases in s-IgA, which were observed in some players (Figure 1), also have been reported in other studies (1,9,26,35). The mechanism behind this phenomenon possibly includes mechanical stimulation of IgA secretion from drinking fluid (16). In an elite squad of squash players the response of s-IgA to exercise was an increase in healthy athletes and a decrease in those who developed URTI (10). An inherent drawback of the current study is the inability to control the occurrence of URTI and the association with s-IgA levels response.

Another confounding variable could be the fluid consumption during the half-time interval. Although it could potentially influence the observed results, differences were unavoidable. In the realm of sports immunology, especially in team sports, these variations may not be as accurate as in a laboratory because individual differences in fluid consumption and the individual workload may obscure the impact of exercise alone on postexercise s-IgA. Further research on the role of acute postexercise changes in s-IgA from top-level soccer players and the risks of URTI or even the use of IgA like a marker of excessive training is needed.

The present findings indicate some variation within each athlete's IgA levels and other previous studies (9,10,16). Kock et al. (9) have obtained saliva samples from subjects before and after an 80-minute game during the competitive season. The results are in part similar to the findings of the current investigation and also have an important external validity from a sports immunology standpoint because, as in our study, they have investigated an actual match situation in a team sport.

The present findings also have indicated that participation in a friendly soccer match does not produce uniform response in s-IgA expressions. There is a debate over the best method of expressing s-IgA changes during exercise. The correlation between changes in s-IgAabs and IgA-Pro in our study was strongly and significantly correlated, and a moderate correlation between s-IgAabs and s-IgArate was found. The s-IgArate and IgA-Pro changes also were moderately correlated. These findings reinforce the need to establish a uniform standard for s-IgA expression.

This study indicates that acute intense exercise does not seem to reduce saliva IgA. However, this does not mean that IgA should be ignored as a mechanism that may leave athletes more susceptible to infection, particularly during periods of overreaching or overtraining. Moreover, the nature of this exercise (team sports) and its demand is so different from the endurance exercise and the likelihood to obtain distinct effects could be taken into account.

Practical Applications

The major findings of our study are that participating in a 70-minute regulation soccer match does not significantly affect salivary IgA, and the total protein may be a good marker of match intensity. These findings are novel in that they were obtained from top-level professional Brazilian soccer players in the actual playing environment and thus may be more effective in being generalized and translated to practice. From a scientific point of view the lack of significant decrease in s-IgAabs and s-IgArate following the match is clear. However, from a practical application to coaches and athletes, the variability of the responses among the players leads us to suggest that there is a need to individually analyze the results with team sports. Some athletes showed substantial decrease in s-IgA expressions, suggesting the need for taking protective actions to minimize contact with cold viruses or even reducing the training load for these athletes, who may be in a overreaching state, or at least more susceptible to that, and suffering an increased risk of incidence of upper-respiratory illness. More research on the role of acute postexercise changes in salivary IgA expressions in elite professional soccer players is needed, especially in official soccer matches because the majority of research has been conducted in either endurance sports or in the laboratory environment.

Acknowledgments

The authors wish to acknowledge Michael Gleeson, PhD, of the School of Sports and Exercise Sciences, Loughborough

University, United Kingdom, for assistance with the English language and for reviewing the manuscript. We also thank all members of the Department of Oral Pathology from São Leopoldo Mandic Dental Research Center (Campinas–Brazil) for assistance with data analysis and the soccer players and team support staff involved in this study for their committed participation.

References

1. Blannin, AK, Robson, PJ, Walsh, NP, Clark, AM, Glennon, L, and Gleeson, M. The effects of exercising to exhaustion at different intensities on saliva Immunoglobulin A, protein and electrolyte secretion. *Int J Sports Med* 19: 547–552, 1998.
2. Bishop, NC. Acute exercise and acquired immune function. In: Gleeson, M (ed.). *Immune Function in Sports and Exercise*. London: Churchill Livingstone Elsevier, 2006: 91–113.
3. Fahlman, MM, Engels, HJ, Morgan, AL, and Kolokouri, I. Mucosal IgA response to repeated Wingate tests in females. *Int J Sports Med* 22: 127–131, 2001.
4. Gleeson, M, McDonald, WA, Pyne, DB, Cripps, AW, Francis, JL, and Fricker, PA. Salivary IgA levels and infection risk in elite swimmers. *Med Sci Sports Exerc* 31: 67–73, 1999.
5. Gleeson, M. Mucosal immunity and respiratory illness in elite athletes. *Int J Sports Med* 21(Suppl 1): S33–S43, 2000.
6. Gleeson, M. Preface. In: Gleeson, M (ed.). *Immune Function in Sports and Exercise*. London: Churchill Livingstone Elsevier, 2006: xiii–xv.
7. Hetzler, RK, Seip, RL, Boutcher, SH, Pierce, E, Snead, D, and Weltman, A. Effect of exercise modality on ratings of perceived exertion at various lactate concentration. *Med Sci Sports Exerc* 23: 88–92, 1991.
8. Irving, BA, Rutkowski, J, Brock, DW, Davis, CK, Barret, EJ, Gaesser, GA, and Weltman, A. Comparison of borg- and OMNI-RPE as markers of the blood lactate response to exercise. *Med Sci Sports Exerc* 38: 1348–1352, 2006.
9. Koch, AJ, Wherry, AD, Petersen, MC, Johnson, JC, Stuart, MK, and Sexton, WL. Salivary immunoglobulin A response to collegiate rugby game. *J Strength Cond Res* 21: 86–90, 2007.
10. Mackinnon, LT, Ginn, E, and Seymour, G. Effects of exercise during sports training and competition on salivary IgA levels. In: Husband, AJ (ed.). *Behaviour and Immunity*. Boca Raton, FL: CRC Press, 1992: 169–177.
11. Mackinnon, LT, Ginn, E, and Seymour, G. Decreased salivary immunoglobulin A secretion rate after intense interval exercise in elite kayakers. *Eur J Appl Physiol Occup Physiol* 67; 180–184, 1993.
12. Mackinnon, LT and Jenkins, DG. Decreased salivary immunoglobulins after intense interval exercise before and after training. *Med Sci Sports Exerc* 25: 678–683 1993.
13. Mackinnon, LT and Hooper, S. Mucosal (secretory) immune system responses to exercise of varying intensity and during overtraining. *Int J Sports Med* 15: S179–S183, 1994.
14. McDowell, SL, Chaloa, K, Housh, TJ, Tharp, GD, and Johnson, GO. The effect of exercise intensity and duration on salivary immunoglobulin A. *Eur J Appl Physiol Occup Physiol* 63: 108–111, 1991.
15. McDowell, SL, Weir, JP, Eckerson, JM, Wagner, LL, Housh, TJ, and Johnson, GO. A preliminary investigation of the effect of weight training on salivary immunoglobulin A. *Res Q Exerc Sport* 64: 384–351, 1993.
16. Nehlsen-Cannarella, SL, Nieman, DC, Fagoaga, OR, Kelln, WJ, Henson, DA, Shannon, M, and Davis, JM. Saliva immunoglobulins in elite women rowers. *Eur J Appl Physiol* 81: 222–228, 2000.
17. Nieman, DC. Prolonged aerobic exercise, immune response, and risk of infection. In: Hoffman-Goetz, L (ed.). *Exercise and Immune Function*. Boca Raton, FL: CRC Press, 1996: 143–161.
18. Nieman, DC, Johanssen, LM, Lee, JW, Cermak, J, and Arabatzis, K. Infectious episodes in runners before and after the Los Angeles Marathon. *J Sports Med Phys Fitness* 30: 316–328, 1990.
19. Nieman, DC. Exercise, infection and immunity. *Int J Sports Med* 15: S131–S141, 1994.
20. Nieman, DC. Immune response to heavy exertion. *J Appl Physiol* 82: 1385–1394, 1997.
21. Nieman, DC, Henson, DA, Fagoaga, OR, Utter, AC, Vinci, DM, Davis, JM, and Nehlsen-Cannarella, SL. Change in salivary IgA following a competitive marathon race. *Int J Sports Med* 23: 69–75, 2002.
22. Peters, EM and Bateman, EB. Ultramarathon running and upper respiratory tract infections. *SA Med J* 64: 582–584, 1983.
23. Peters, EM, Goetzsche, JM, Grobbelaar, B, and Noakes, TD. Vitamin C supplementation reduces the incidence of postrace symptoms of upper respiratory tract infection in ultramarathon runners. *Am J Clin Nutr* 57: 170–174, 1993.
24. Peters, EM, Goetzsche, JM, Joseph, LE, and Noakes, TD. Vitamin C as effective as combinations of anti-oxidant nutrients in reducing symptoms of upper respiratory tract infection in ultramarathon runners. *S Afr J Sports Med* 11: 23–27, 1996.
25. Reid, MR, Drummond, PD, and Mackinnon, LT. The effect of moderate aerobic exercise and relaxation on secretory Immunoglobulin A. *Int J Sports Med* 22: 132–137, 2001.
26. Sari-Sarraf, V, Reilly, T, and Doran, DA. Salivary IgA response to intermittent and continuous exercise. *Int J Sports Med* 27: 849–855, 2006.
27. Schipper, RG, Silletti, E, and Vingerhoeds, MH. Saliva as research material: Biochemical, physicochemical and practical aspects. *Arch Oral Biol* 52: 1114–1135, 2007.
28. Seip, RL, Snead, D, Pierce, F, Stein, P, and Weltman, A. Perceptual responses and blood lactate concentration: Effect of training state. *Med Sci Sports Exerc* 23: 80–87, 1991.
29. Shephard, RJ and Shek, PN. Acute and chronic over-exertion: Do depressed immune responses provide useful markers? *Int J Sports Med* 19: 159–171, 1998.
30. Simonson, SH. The immune response to resistance exercise. *J Strength Cond Res* 15: 378–384, 2001.
31. Steed, J, Gaesser, GA, and Weltman, A. Rating of perceived exertion and blood lactate concentration during submaximal running. *Med Sci Sports Exerc* 26: 797–803, 1994.
32. Steerenberg, PA, van Asperen, IA, van Nieuw Amerongen, A, Biewenga, J, Mol, D, and Medema, G. Salivary levels of Immunoglobulin A in triathletes. *Eur J Oral Sci* 105: 305–309, 1997.
33. Stoudemire, NM, Wideman, L, Pass, KA, McGinnes, CL, Gaesser, GA, and Weltman, A. The validity of regulation blood lactate concentration during running by ratings of perceived exertion. *Med Sci Sports Exerc* 28: 490–495, 1996.
34. Tharp, GD and Barnes, MW. Reduction of saliva immunoglobulin levels by swim training. *Eur J Appl Physiol Occup Physiol* 60: 61–64, 1990.
35. Tharp, GD. Basketball exercise and secretory immunoglobulin A. *Eur J Appl Physiol* 63: 312–314, 1991.
36. Tomasi, TB and Plaut, AG. Humoral aspects of mucosal immunity. In: Gallin, JI, Fauci, AS (eds.). *Advances in Host Defense Mechanisms*. New York: Raven Press, 1985: 31–61.
37. Tomasi, TB, Trudeau, FB, Czeirwinski, D, and Erredge, S. Immune parameters in athletes before and after strenuous exercise. *J Clin Immunol* 2: 173–178, 1982.
38. Walsh, NP, Blannin, AK, Clark, AM, Cook, L, Robson, PJ, and Gleeson M. The effects of high intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. *J Sports Sci* 17: 129–134, 1999.