ORIGINAL RESEARCH

Immune responses, upper respiratory illness symptoms, and load changes in young athletes during the preparatory period of the training periodization

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Objective: The aim of this study was to investigate the immunological responses and the association between variation in exercise load and self-reported occurrence of upper respiratory illness (URI) symptoms in young basketball athletes.

Materials and methods: The sample was composed of twelve young male athletes aged 12.7 ± 0.6 years, with a height of 170 ± 10 cm, body mass of 57.6 ± 12.6 kg, and fat-free mass of $18.7 \pm 5.9\%$. Daily training and occurrences of URI symptoms were recorded. Blood samples were collected at baseline (M1) and after 8 weeks (M2) of the preparatory period of periodization training to measure total and differential leukocyte counts, serum interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α).

Results: There was a significant decrease in monocytes at M2 compared to M1 ($P = 0.004$). There were no significant alterations in total leukocytes ($P = 0.07$), neutrophils ($P = 0.07$), or lymphocytes ($P = 0.09$). No significant changes in plasma concentrations of TNF-α ($P = 0.30$) or IL-6 ($P = 0.90$) were found. The weekly load from week 6 was higher when compared with weeks 1, 2, 4, and 8 ($P < 0.05$), and week 8 was the lowest when compared with week 5 ($P < 0.05$). Self-reported URI incidences were highest at weeks 1 and 2.

Conclusion: Variations in weekly training load during the preparatory period were not correlated with changes in self-reported occurrence of URI incidences, suggesting that young athletes may have an attenuated response to exercise-induced perturbations to the immune system.

Keywords: immune system, upper respiratory illness, young athletes, cytokines

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Introduction

The theory of training highlights the importance of the development of strong basic skills for the purpose of increasing work capacity, skill effectiveness, and the psychological qualities required to improve an athlete's performance and achieve a specific goal in competitions.[1,2] Throughout the training process, human physiological and psychological functions are modeled to respond to the demands of the tasks involved.[3]

When physical demands outweigh the body's ability to fully recover between training sessions and competitions, athletes can be more susceptible to upper respiratory illness (URI), the occurrence of injury, or both.[4] In this context, it is fundamental that training programs for young athletes in any field of physical activity should take into account the physiological effects brought on by different training interventions. In particular, the physical development process can influence the immunological system

and the good health of young athletes, as well as reduce their performance levels.[5]

It has been suggested that the relationship between exercise and URI follows a "J-curve hypothesis,"[6] with moderate and intermittent levels of exercise improving the immune function and reducing susceptibility to URI.[7–9]

On the other hand, intense exercise has been shown to temporarily alter and/or suppress some immune parameters, including the number of circulating leukocytes, plasma cytokine concentrations, neutrophils, and macrophage phagocytic activity,[7–10] and it is also associated with an increased susceptibility to URI.[7,8,11] In addition, studies show a positive correlation between exercise workload and URI.[9,12]

While these observations hold true for adults, similar research on interactions between exercise workload, the occurrence of URI, and the behavior of the immune system in young individuals is deficient.[5] Thus, the aim of this study was to investigate immune responses, the occurrence of URI symptoms, and training load changes during the preparatory period of the training periodization in young basketball athletes.

Materials and methods
Subjects
Twelve young male basketball athletes (12.7 ± 0.6 years; 57.6 ± 12.6 kg; 18.7 ± 5.9% fat-free mass; 170 ± 10 cm) playing in the São Paulo State Regional Basketball League, Brazil, were recruited for the present study. The inclusion criterion was a minimum of two years' experience of team basketball training. Exclusion criteria included: use of medication that could affect the immune parameters, diseases or joint/muscle problems, and less than 80% frequency and/or interruption during the training period. Participants with

heart, pulmonary, or orthopedic complications, as well as diabetes and severe muscle injuries, were excluded from the study. All participants were classified as stage III or IV as regards secondary sexual characteristics, in accordance with the descriptions proposed by Tanner.[13]

Training experience and habitual physical activity were determined by the use of a questionnaire and interview. All subjects had experience in the exercises used during the preparatory period of the training periodization. Individuals responsible for each participant were informed of the potential risks associated with the study and were asked to sign informed consent before study participation. The experimental methods and procedures were approved by the Research Ethics Committee of the University of Campinas, Brazil.

Procedures
Upon return from off-season training (three months after the last sports season), the baseline levels of all subjects were measured. These included: a 12-hour fasting blood draw; the Tanner stages of secondary sexual characteristics; and anthropometric measures, such as body composition, height, and weight.

The preparatory period of the training periodization[3] comprised three weekly sessions of 94.3 ± 8.1 minutes duration, each one performed for 8 weeks. Training sessions were divided into both technical and functional skills according to the objectives of training[3] (Table 1). The duration (in minutes) and the rating of perceived exertion (RPE)[4] were recorded 30 minutes after the end of each session to evaluate the training load. The occurrence of URI symptoms was recorded weekly over the entire period of the study. Blood samples (10 mL) were collected before M1 and after

Table 1 Schematic evaluation of the load, monotony, strain, and self-reported occurrence of URI symptoms throughout the preparatory period of the training periodization in young basketball players (n = 12)

Weeks	Objectives of the training (%)		Weekly training duration (min)	Sessional RPE	Weekly load	Strain	Monotony	Self-reported URI symptoms (%)
	Technical	Functional						
1	25.5	74.5	98.0 ± 2.8	4.1 ± 1.4	867*	1712	1.97	50
2	15.5	84.5	92.5 ± 4.9	3.9 ± 2.3	739*	1561	2.11	50
3	18.3	81.7	94.7 ± 6.1	3.5 ± 2.4	1046	1728	1.65	33
4	27.0	73.0	91.0 ± 8.5	3.5 ± 2.3	1032*	1747	1.69	42
5	19.0	81.0	99.3 ± 0.6	4.1 ± 3.3	1233#	2132	1.73	25
6	33.0	67.0	98.0 ± 8.5	4.4 ± 4.2	1753	3431	1.96	42
7	17.5	82.5	88.5 ± 19.1	4.4 ± 1.9	820	1841	2.25	33
8	17.5	82.5	87.5 ± 10.6	3.9 ± 1.8	707*	1801	2.55	33

Notes: *Significant difference as compared to week 6 ($P < 0.05$); #significant difference as compared to week 8 ($P < 0.05$).
Abbreviations: URI, upper respiratory illness; RPE, rating of perceived exertion.

8 weeks, at the end of the preparatory period (M2), and all blood analyses were performed successively.

Anthropometry

Height was measured using a wall-mounted stadiometer, and weight was taken using a calibrated manual scale. Body composition was determined using skinfold thickness with a Lange skinfold caliper. The equation of Slaughter et al[14] for children and youths was used to estimate body density using the triceps and subscapular skinfolds. Body fat percentage was estimated by Siri's[15] equation and used to estimate fat mass (kilograms) and fat-free mass (kilograms). The same investigators performed all tests.

Training load indicators

After each training session, the training volume, defined as the duration in minutes was recorded. In addition to this procedure, the RPE proposed by Foster[4] was presented to the athletes to calculate the load of each session. The following question was posed to each participant: "What was the intensity of the training in regard to the scale?" The daily training load is defined as the relationship between effort quality (intensity) and volume of training. Thus, training load indicators:[4] daily load (DL = the RPE × daily session training duration in minutes); weekly mean load (WML = the sum of the DLs); and total weekly load (WL = the sum of DLs/number of training days) were determined in arbitrary units.[4] Monotony was calculated by dividing the daily mean load over each week by the standard deviation of load, and strain was calculated by the weekly load × monotony.[4]

Symptoms of upper respiratory illness

At the beginning of each training week, athletes were asked about any URI symptoms experienced in the previous week, and the number of symptoms was recorded. A URI was defined by symptoms of a runny nose, cold, otitis, headache, sore throat, fever, and other symptoms for at least 2 consecutive days as described by Tsai et al.[16] To minimize over reporting and exclude trivial symptoms, only reports of two or more symptoms that lasted for more than 1 day were regarded as indicative of the existence of a URI. Results are presented as a percentage (%) of individuals presenting with URI symptoms for each week.

Blood collection and analysis

Fasting blood samples were obtained from the antecubital vein and collected into Vacutainer® tubes (Becton Dickinson Ltd,

Oxford, UK) containing anticoagulant (EDTA) between 8 and 9 hours in the morning at baseline (M1) and after 8 weeks (M2), with the individuals at rest (72 hours after the last training session). All blood was collected, processed, and centrifuged (20 minutes at 1400 rpm at 18°C). Plasma aliquots were stored at 70°C for subsequent cytokine analysis.

Total leukocyte counts and leukocyte subsets were measured using an automated cell counter (BX Micros 60 – CT; ABX Diagnostics, Irvine, CA), and the results were presented in terms of cell number × mm^3.

Tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were determined in duplicate by enzyme-linked immunosorbent assay (ELISA), following the specifications of the manufacturer (Quantikine High Sensitivity Kit; R&D Systems, Minneapolis, MN). The results for TNF-α and IL-6 are presented in terms of picograms per milliliter (pg/mL). The sensitivity, intra-assay, and inter-assay were as follows: 0.106 pg/mL, 4.3% and 7.3% for TNF-α; 0.039 pg/mL, 7.8% and 7.2% for IL-6.

Statistical analyses

Initially, the Shapiro–Wilk test of normality and the homoscedasticity test (Bartlett criterion) were evaluated. Since all variables presented a normal distribution and homoscedasticity ($P < 0.05$), a one-way repeated-measures analysis of variance (ANOVA) was used, and when the difference was significant, the Tukey post hoc test for multiple comparisons was applied. The Spearman correlation test was used to determine possible correlations between occurrence of URI symptoms and weekly load, occurrence of URI symptoms and monotony, occurrence of URI symptoms and strain, and strain and monotony. All data were evaluated using the SPSS® software (version 16.0; SPSS Lead Technologies Inc, Chicago, IL). All results are presented as mean ± standard deviation (SD). The threshold for significance was set at $P < 0.05$.

Results

Post hoc tests revealed significant decreases in monocytes in M2 when compared with M1 ($P = 0.004$). There were no significant alterations in total leukocytes, neutrophils, or lymphocytes after the preparatory period of the training periodization (Figure 1).

There were no significant differences in plasma concentrations of TNF-α or IL-6 at M2 when compared with M1 (Table 2).

There was a significant difference in weekly load at week 6 compared with weeks 1, 2, 4, and 8 ($P < 0.05$).
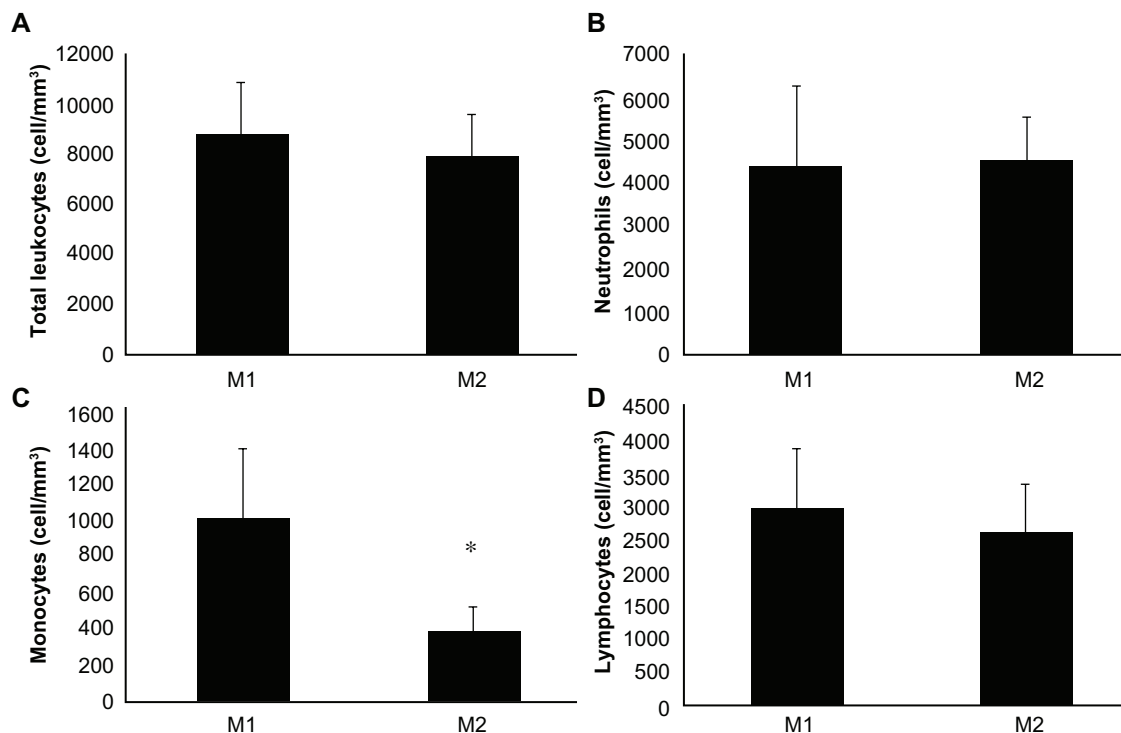
Figure 1 Total leukocytes (**A**), neutrophils (**B**), monocytes (**C**), and lymphocytes (**D**) at baseline (M1) and after 8 weeks (M2).
Notes: Values are presented as mean ± SD. *Significantly different from M1 ($P < 0.05$).

It was also found that weekly load for week 8 was lowest as compared with week 5 ($P < 0.05$) (Table 1).

Self-reported occurrence of URI symptoms was highest at weeks 1 and 2, as compared to others (Table 1). No significant correlations were found between self-reported occurrence of URI symptoms and weekly load, self-reported occurrence of URI symptoms and strain, and self-reported occurrence of URI symptoms and monotony during the experimental protocol (Table 3). Significant correlation was found between strain and monotony in all experimental weeks ($P < 0.05$) (Figure 2).

Discussion

Athletes commonly experience a variety of injuries and illnesses throughout a competitive season, impacting the performance of the team and the success of the coach.[9] Although adequate data for adults is available, similar understanding of

Table 2 Serum TNF-α and IL-6 at baseline (M1) and after 8 weeks (M2)

Variable	M1	M2
TNF-α	1.13 ± 0.27	1.21 ± 0.32
IL-6	1.16 ± 0.13	1.10 ± 0.16

Notes: Values are presented in pictograms per milliliter (pg/mL). Mean ± SD ($P < 0.05$).

the interactions between exercise workload and the immune system in young individuals is deficient.[5] The objective of the present study was to investigate the immune responses, occurrence of URI symptoms, and weekly training load throughout the preparatory period of the training periodization in young males who were involved in competitive basketball. The results of our study revealed a decrease in monocytes in M2 as compared to M1, but no alterations in total leukocytes, monocytes, lymphocytes, IL-6, and TNF-α at the time when measures were taken. Moreover, there were no correlations between training load, strain, and monotony with URI-recorded incidences during the experimental period.

Previous studies reported immune modulations in leukocyte counts throughout a season of training and competition.[17–21] Benoni et al[21] found an increase in total leukocytes, monocytes, neutrophils, eosinophils, and lymphocytes in basketball players after a sports season, with the values returning to the pre-start levels 3 weeks after the end of the championship. In addition, an increase in total leukocytes, monocytes, and neutrophils, without modification in eosinophils and lymphocytes, was reported in soccer players,[19] and elevation in neutrophils accompanied by a reduction in lymphocytes was found in football players throughout a sports season.[20] Meanwhile, the present study revealed a decrease in monocytes and no significant alterations in total

Table 3 Spearman correlation between self-reported upper respiratory illness (URI) symptoms and weekly load, strain, and monotony during the preparatory period of the training periodization in 12 young male basketball athletes

Week	URI × weekly load		URI × monotony		URI × strain		Strain × monotony	
	r	P	r	P	r	P	r	P
1	0.30	0.336	0.10	0.751	0.00	0.990	0.97*	0.00
2	0.22	0.484	0.05	0.885	0.07	0.812	0.99*	0.00
3	0.18	0.568	0.04	0.912	0.04	0.891	0.96*	0.00
4	0.41	0.180	0.45	0.146	0.39	0.204	0.83*	0.00
5	0.41	1.180	0.44	0.153	0.49	0.099	0.98*	0.00
6	0.02	0.939	0.27	0.397	−0.17	0.594	0.90*	0.00
7	0.07	0.812	0.13	0.686	0.18	0.577	0.63*	0.03
8	0.02	0.936	0.18	0.570	0.18	0.576	0.95*	0.00

Note: *Significant correlation ($P < 0.05$).

leukocytes, neutrophils, or lymphocytes after the end of the preparatory stage of periodization (8 weeks), suggesting that young athletes tend to be more resistant to exercise-induced perturbations to the immune system, since the changes in IL-6 and TNF-α in response to exercise tended to be smaller than those recorded in adults.

Concomitantly, a season's preparatory period is frequently the most difficult and physically demanding series of practices in the season.[3] This regimen is often believed to be necessary so that the athletes reach their "optimal" level of training as the season proceeds.[22] Modified muscle use during this stage can produce a stereotypic inflammatory response in the muscle tissue, in which neutrophils rapidly invade, followed by monocytes/macrophages, which are closely involved in tissue damage repair, and remodeling.[23,24] A likely role of neutrophils in muscle repair or remodeling is the oxidative or proteolytic modification of damaged tissue to allow phagocytosis of debris by neutrophils or macrophages.[23] In humans,

monocytes represent immune effector cells, equipped with chemokine receptors and adhesion receptors that mediate migration from blood to tissues in response to inflammation.[25] Monocytes are derived and differentiated from precursor cells in the bone marrow in response to cytokines such as IL-3, granulocyte–macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), IL-1, IL-6, and TNF-α among others.[25,26] The blood concentrations of these cytokines increase during inflammation, thus promoting monocyte efflux from the marrow to the blood with concurrent maturation.[26] Related to a season of training and/or competition, we have recently observed that professional athletes (19.47 ± 2.49 years) did not show changes in IL-2, IL-6, or TNF-α during and after a volleyball season, even with the highest training load and number of URI incidences.[27] Concomitantly, Suzui et al[28] found no alterations in monocyte counts, IL-6, INF-γ, or TNF-α during and after one month of intensive volleyball training in 15 university studies.
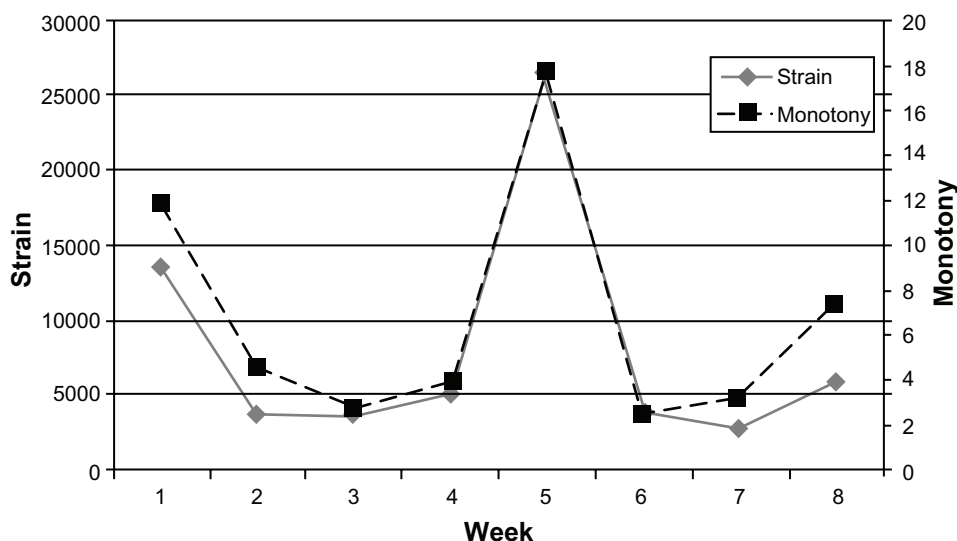

Figure 2 Correlations between strain and monotony throughout the preparatory period of the periodization ($P < 0.05$).

On the other hand, Nemet and colleagues[29] demonstrated a catabolic-type hormonal environment in 13 healthy adolescent boys during a wrestling season, marked by a decrease in IGF-1 with increased IL-6 and IL-1ra. However, the authors observed a significant increase in fitness level during this seemingly catabolic hormonal environment, suggesting that proinflammatory cytokines at lower levels may actually promote growth of muscle blood vessels and serve as a beneficial response to exercise.[29]

Although we noted a significant decrease for monocytes in M2 as compared to M1, no significant alterations were found for systemic IL-6 or TNF-α at the same time, despite the changes in weekly training load during the preparatory period. These results suggest an associated muscle tissue remodelation due to possible damage caused by the training load applied during the preparatory period and monocyte recruitment from local muscle inflammation to repair the damaged tissue, once the systemic proinflammatory cytokines were not altered.

In conclusion, considering that training load can be associated with environmental and psychological aspects, as well as the modulation induced by stress and the neuroendocrine system,[30,31] our results suggest that variations in weekly training load during the preparatory period were not correlated with changes in self-reported occurrence of URI symptoms and did not promote chronic alterations that negatively impair the immune system, since the systemic proinflammatory cytokines were not altered and the number of individuals with URI incidences were higher during the first two weeks. This suggests that coaches and physiologists should monitor the immunological markers of athletes during all stages of periodization training for a better understanding of individual changes that can occur throughout a sports season. Other aspects not controlled in this investigation, such as salivary-IgA, could also play an important role in the complex relationship between training, immunity, and the occurrence of URI symptoms. However, for comparison purposes, there are few investigations relating to the immune system, training load, and seasonal training in young athletes during the sports season. Thus, the accumulated effects of sport-specific training loads on chronic immune modulations in young athletes warrants further investigation.

Acknowledgments

Disclosure

References

1. Issurin VB. New horizons for the methodology and physiology of training periodization. *Sports Med*. 2010;40(3):189–206.
2. Bompa T. *Total Training for Young Champions*. Champaign, IL: Human Kinetics; 2000.
3. Bompa T, Half GG. *Periodization 5th edition: Theory and Methodology of Training*, 5th ed. Champaign, IL: Human Kinetics; 2009.
4. Foster C. Monitoring training in athletes with reference to overtraining syndrome. *Med Sci Sports Exerc*. 1998;30(7):1164–1168.
5. Timmons BW. Exercise and Immune Function in Children. *Am J Lifestyle Med*. 2010;1(1):59–66.
6. Heat GW, Macera CA, Nieman DC. Exercise and upper respiratory tract infections. Is there a relationship? *Sports Med*. 1992;14:353–365.
7. Moreira A, Delgado L, Moreira P, et al. Does exercise increase the risk of upper respiratory tract infections? *Br Med Bull*. 2009;90(1):111–131.
8. Gleeson M. Immune function in sport and exercise. *J Appl Physiol*. 2007;103(2):693–699.
9. Plutur P, Foster C, Miskowski JA, et al. Alteration of immune function in women collegiate soccer players and college students. *J Sports Sci Med*. 2004;3:234–243.
10. Mackinnon LT. Immunity in athletes. *Int J Sports Med*. 1997;18 Suppl 1: S62–S68.
11. Nieman DC. Is infection risk linked to exercise workload? *Med Sci Sports Exerc*. 2000;32(7):S406–S411.
12. Gleeson M, Pyne DB. Special feature for the Olympics: effects of exercise on the immune system: exercise effects on mucosal immunity. *Immunol Cell Biol*. 2000;78(5):536–544.
13. Tanner JM. *Growth at Adolescence*, 2nd ed. Oxford: Blackwell Scientific Publications; 1962.
14. Slaughter MH, Lohman TG, Boileau RA, et al. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol*. 1988;60(5):709–723.
15. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. *Nutrition*. 1993;9(5):480–491; discussion 480, 492.
16. Tsai ML, Chou KM, Chang CK, et al. Changes of mucosal immunity and antioxidation activity in elite male Taiwanese taekwondo athletes associated with intensive training and rapid weight loss. *Br J Sports Med*. 2011;45(9):729–734.
17. Cordova A, Sureda A, Tur JA, et al. Immune response to exercise in elite sportsmen during the competitive season. *J Physiol Biochem*. 2010;66(1):1–6.
18. Novas AM, Rowbottom DG, Jenkins DG. Tennis, incidence of URTI and salivary IgA. *Int J Sports Med*. 2003;24(3):223–229.
19. Rebelo AN, Candeias JR, Fraga MM, et al. The impact of soccer training on the immune system. *J Sports Med Phys Fitness*. 1998;38(3): 258–261.
20. Bury T, Marechal R, Mahieu P, et al. Immunological status of competitive football players during the training season. *Int J Sports Med*. 1998;19(5):364–368.
21. Benoni G, Bellavite P, Adami A, et al. Changes in several neutrophil functions in basketball players before, during and after the sports season. *Int J Sports Med*. 1995;16(1):34–37.
22. Anderson L, Triplett-McBride T, Foster C, et al. Impact of training patterns on incidence of illness and injury during a women's collegiate basketball season. *J Strength Cond Res*. 2003;17(4):734–738.
23. Tidball JG. Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol*. 2005;288(2):R345–R353.
24. Walsh NP, Gleeson M, Shephard RJ, et al. Position statement. Part one: Immune function and exercise. *Exerc Immunol Rev*. 2011;17:6–63.
25. Geissmann F, Manz MG, Jung S, et al. Development of monocytes, macrophages, and dendritic cells. *Science*. 2010;327(5966):656–661.

26. Woods J, Lu Q, Ceddia MA, et al. Special feature for the Olympics: effects of exercise on the immune system: exercise-induced modulation of macrophage function. *Immunol Cell Biol*. 2000;78(5):545–553.

27. Dias R, Frollini AB, Brunelli DT, et al. Immune parameters, symptoms of upper respiratory tract infections, and training-load indicators in volleyball athletes. *Int J Gen Med*. 2011;4:837–844.

28. Suzui M, Kawai T, Kimura H, et al. Natural killer cell lytic activity and CD56(dim) and CD56(bright) cell distributions during and after intensive training. *J Appl Physiol*. 2004;96(6):2167–2173.

29. Nemet D, Pontello AM, Rose-Gottron C, et al. Cytokines and growth factors during and after a wrestling season in adolescent boys. *Med Sci Sports Exerc*. 2004;36(5):794–800.

30. Pedersen BK, Toft AD. Effects of exercise on lymphocytes and cytokines. *Br J Sports Med*. 2000;34(4):246–251.

31. Pedersen BK, Bruunsgaard H. How physical exercise influences the establishment of infections. *Sports Med*. 1995;19(6):393–400.