TRAINING LOAD, IMMUNE SYSTEM, UPPER RESPIRATORY SYMPTOMS AND PERFORMANCE IN WELL-TRAINED CYCLISTS THROUGHOUT A COMPETITIVE SEASON

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ABSTRACT: This study aimed to evaluate the leukocyte subset counts, serum immunoglobulin A, performance and upper respiratory symptoms (URS), as well as their interrelationships, of well-trained cyclists for a 29-week training season using monitored loads. The season was divided into three phases: preparatory (nine weeks), first competitive phase (nine weeks) and second competitive phase (11 weeks). The sample consisted of eight well-trained cyclists, aged 18 ± 2 years. Immunological parameters and performance were evaluated during weeks 1 (baseline), 10 (early first competitive phase), 19 (early second competitive phase) and 29 (end of the second competitive phase). The training loads (volume x rating of perceived exertion) were monitored daily while the monitoring of URS was performed every 15 days using the WURSS-44 questionnaire. The data were analyzed using a one-way ANOVA and a Pearson correlation test with the significance level set at $p \leq 0.05$. No significant differences were found for training load, leukocyte subset counts or serum immunoglobulin A among the three phases. However, serum immunoglobulin A was 50.9% below the control group values. URS were significantly higher during the preparatory period, and there were significant correlations between URS and training load (strain) in the preparatory period ($r = 0.72$, $p = 0.032$) and second competitive phase ($r = 0.73$, $p = 0.036$). In conclusion, indicators of training load without a significant change throughout the season did not significantly affect immune parameters measured; however, the increase of strain can cause an increase of upper respiratory symptoms throughout the season, but without loss of performance.

KEY WORDS: cycling, training load, immune system, upper respiratory symptoms

INTRODUCTION

The main objective of training in elite sports is to increase performance during competitions. Achieving increased performance requires successful interaction between the training load and recovery time, ideally achieving the phenomenon of supercompensation [12]. In contrast, failures in this process may promote overreaching and overtraining, which are associated with negative changes in physiological, psychological, immunological and biochemical variables, resulting in decreased performance and increased risk of injury [10,11].

One way to prevent overreaching and overtraining and to increase athletic performance is to organize the training and competitions during the season, known as training periodization [12].

Of the many negative effects correlated with overtraining in athletes, immune system disorders have been the focus of several studies [3,5,6]. Exercise can modulate immune function either positively or negatively, depending on the type, duration and intensity of the exercise [21,8]. Regular exercise of moderate intensity has been associated with improved immune function while high-intensity exercise or prolonged and strenuous training periods can cause decreased immune function and increased susceptibility to opportunist infections [8,21]. Studies have shown increased frequency of upper respiratory tract infections (URTI) after increased training loads [4] or loads of prolonged exercise such as marathons and ultramarathons [5,23].

Many immune system changes can affect the body's ability to fight infections, such as changes in leukocyte counts and in the function and production of immunoglobulins [8]. The depression of immune system function is a result of multifactorial stress including physiological, psychological, environmental and behavioural stresses [19]. Thus, these changes may increase susceptibility to URTI in athletes [21].

Several studies have reported that the increased incidence of URTI in athletes is related to decreased concentrations and secretion of secretory immunoglobulin A (IgA) [5,19,23].

Immunoglobulins (Ig) or antibodies are a group of proteins that are part of the humoral response of the immune system and are divided into five classes (isotypes): IgA, IgM, IgD, IgG and IgE [28]. Secretory IgA is the most abundant immunoglobulin class of body fluids such as saliva, tears, colostrum and mucus, and is considered

the first line of host defence against pathogens that invade mucosal surfaces by contributing to local immunity, preventing microbial adherence and neutralizing enzymes, toxins and viruses [10]. IgA is also present in the blood (serum IgA), but its function is still not very well understood [28]. However, some individuals with serum IgA deficiency have recurrent respiratory and gastrointestinal infections [15].

Relatively few studies have assessed the response of serum IgA to exercise [20], and its relation to training loads, performance and upper respiratory symptoms (URS) in athletes submitted to long periods of training has not been investigated.

Therefore, this study aimed to evaluate the leukocyte subset counts, serum immunoglobulin A, performance and upper respiratory symptoms, as well as their interrelationships, in well-trained cyclists during a 29-week training season using monitored loads.

MATERIALS AND METHODS

Subjects. Eight well-trained road cyclists (age 18 ± 2 years; weight 64.9 ± 8.6 kg; height 174.7 ± 10.1 cm and 10.7 ± 1.5 % fat) with a mean of seven years of systematic training participated in the experiment. All of the athletes were involved in regular training (7 to 10 weekly sessions) and were participating in official competitions at national level. The cyclists were considered "well-trained" according to the criteria suggested by Jeukendrup, Craig and Hawley [13] related to training and race status as training frequency, training duration, training background, race days per year and International Cycling Union ranking. The study was performed in accordance with the ethical standards of the Helsinki Declaration and was approval by the local Ethics Committee (protocol no. 10-05/108) and all subjects provided written informed consent before participation.

Experimental design of the study

The cycling season was composed of 29 weeks and was divided into three phases: preparatory (1st to 9th week, with five days competition), first competitive phase (10th to 18th week, with 13 days competition) and second competitive phase (19th to 29th week, with 23 days competition). Collection of blood samples (to measure IgA and leukocyte counts) and field performance tests were performed during weeks 1 (baseline), 10 (early first competitive phase), 19

(early second competitive phase) and 29 (end of the second competitive phase). The upper respiratory symptoms (URS) were checked every 15 days during the season (Figure 1). The training load was calculated daily 20-30 minutes after the training session.

Characteristics of training phases

This study was conducted throughout one season of competitions and training, and at no time did the study influence the training of the athletes. The training was divided into three phases by the coach of the athletes based on the competitive calendar of the season. General guidelines for training were provided to all athletes on the team. In the preparatory phase, the athletes were encouraged to train at high volumes but not at any particular intensity of effort (average speed in km/h). During the first and second competitive phases, the athletes were encouraged to maintain their training schedule and to increase the intensity of effort.

Blood sample collection

All blood collections occurred in the morning between 8:00 and 9:00 am at least 36 hours after the last training session or competition. The blood sample was collected from a superficial forearm vein with subjects in the seated position (after 10–15 minutes of rest) using standard venipuncture techniques. Eight millilitres of blood was collected into K3EDTA and clot-activator serum-separation tubes (BD Vacutainer, New Jersey, USA) for a serum IgA analysis. The serum was separated by centrifugation at 4000 rpm for 5 minutes and stored frozen at –80°C until the analysis. Four millilitres of blood was collected in K2EDTA tubes (BD Vacutainer, New Jersey, USA) for leukocyte subset counts. For the group of athletes four blood collections were performed throughout the competitive season (weeks 1, 10, 19 and 29) while for the control group there was only one (week 1, baseline).

Determination of serum IgA and leukocytes

Serum IgA levels were quantified by incubation with appropriate antisera (anti-human IgA, Dade Behering, Germany). The amount of complex (immunoglobulin-anti-immunoglobulin) formed was measured by light scatter, using laser nephelometry (Behring Nephelometer II, Dade Behering, Germany), and the amount of antibody present quantified by comparison with standards of known concentration.
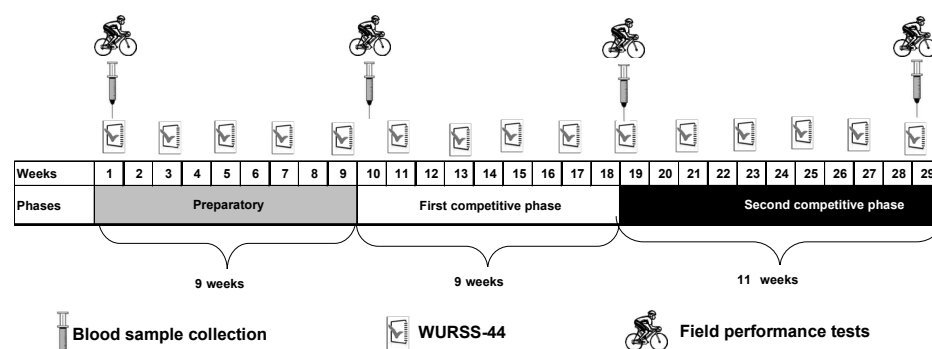

| Weeks | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
| Phases | | | | Preparatory | | | | | | | | First competitive phase | | | | | | | | | | | Second competitive phase | | | | | | |

9 weeks   9 weeks   11 weeks

⊓ Blood sample collection   ☑ WURSS-44   🚴 Field performance tests

FIG. 1. EXPERIMENTAL DESIGN OF THE STUDY

The leukocyte subset counts (LSC) for the enumeration of neutrophil, lymphocyte, monocyte, basophil, and eosinophil populations were performed on whole blood samples within 2 hours of sample collection using an automatic counter and an ADVIA 120 Hematology System (Bayer, Pittsburgh, USA).

Control group for reference values of IgA
To compare values of IgA between athletes and reference values we used a control group comprising 18 male college athletes (6 handball players, 4 cyclists and 8 soccer players) 18-21 years old.

Upper respiratory symptoms
The upper respiratory symptoms were determined by the Wisconsin Upper Respiratory Symptom Survey-44 (WURSS-44) [1]. The WURSS-44 includes one global severity question, 32 symptom-based questions, 10 functional impairment or quality-of-life questions, and one global change question. The severity of each reported symptom was rated on a seven-point scale: 1 (very mild), 3 (mild), 5 (moderate), and 7 (severe). Symptoms not experienced were recorded as 0. An overall symptom score was calculated by adding the severity scores from the first 43 items. The questionnaire was performed every 15 days during the training season and was administered at the same period of the day in a quiet place. All athletes had prior knowledge about the completion of the questionnaire. For comparison among the phases of training the average of all answered questionnaires at each stage was used.

Training load
The training load was calculated using a method proposed by Foster [7] using the rating of perceived exertion (RPE). For this approximately 30 min following the conclusion of each training session, each subject was instructed to rate the global intensity of that training session using Borg's Category Ratio-10 scale (CR-10) [2] modified by Foster et al. [7]. The athletes had previous experience using the RPE scale. The "load" was calculated as the product of the RPE and session duration in minutes. Additionally, we calculated the "monotony" (mean weekly load/standard deviation) and "strain" (total weekly load * monotony). All variables are expressed in arbitrary units (AU).

Field performance tests
For monitoring the performance a time trial of 15 km (TT-15), held on a flat highway, was performed. The test was divided into two routes of 7.5 km in opposite directions, in order to minimize wind interference, and performed at the maximum possible intensity. Before the start of the test a warm-up of 10 to 15 min, at light intensity, was allowed, and after the warm-up the athletes took a rest for five minutes. To maintain the specificity, the TT-15 test was performed with the athletes' own equipment of training and competitions, such as bike, clothing and protective equipment. Before and during the test, the participants were instructed to consume only water.

Statistical analysis
The data are presented as means ± standard error of the mean (SE). A one-way ANOVA and Scheffé post-hoc test were used to compare the load, monotony, strain, IgA, performance and URS among the training phases. Correlations among the variables were determined using a Pearson's product–moment correlation. Statistical significance was set at $p<0.05$, and the Statistica statistical software package version 7.0 (Statistica, Tulsa, USA) was used for all statistical calculations.

RESULTS

During the preparatory phase, first competitive phase and second competitive phase, the variables measured had the following average values, respectively: load (4670 ± 584, 4251 ± 268 and 4231 ± 129 UA); monotony (1.9 ± 0.3, 1.6 ± 0.1 and 2.0 ± 0.1 UA); strain (9633 ± 2267, 6898 ± 656 and 9501 ± 563 UA) and volume (364 ± 40, 352 ± 21 and 342 ± 6.7 km). No significant difference was found among the phases for all variables related to training load; on the other hand, higher values ($p=0.012$) were found
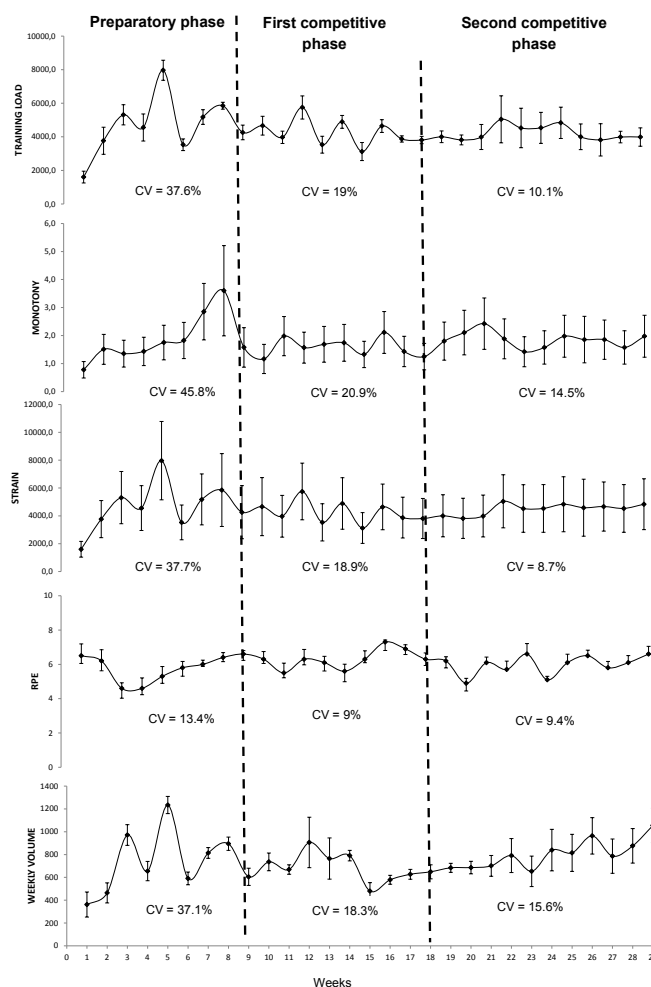
FIG. 2. INDICATORS OF TRAINING LOAD AND RATING OF PERCEIVED EXERTION (RPE) DURING THE 29-WEEK TRAINING SEASON.
Note: CV = coefficient of variation within each period

for RPE in the first competitive phase (5.9 ± 0.2) regarding the preparatory phase (5.1 ± 0.2) and second competitive phase (5.0 ± 0.3). Figure 2 shows the distribution of indicators of training load and intensity (RPE) throughout the season, showing the highest coefficients of variation (CV) during the preparatory period.

The performance remained stable in tests performed at baseline, the 10th week and the 29th week. However, there was an increase in performance at the 19th week, as shown by the significant decrease in test time (Table 1).

TABLE I. FIELD PERFORMANCE TESTS EVALUATED AT DIFFERENT TIMES OF THE SEASON

Performance TT-15	Baseline	10th week	19th week	29th week
Time (s)	1383 ± 47	1365 ± 62	1317 ± 43[a]	1367 ± 40

Note: [a] Significantly different (p<0.05) from baseline, 10th week and 29th week.
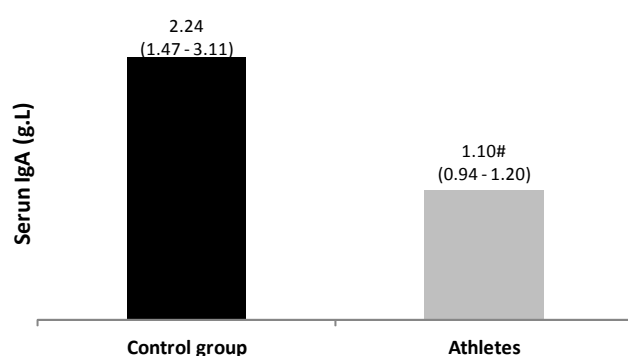
FIG. 3. COMPARISON OF SERUM IGA BETWEEN ATHLETES AND CONTROL GROUP.
Note: # The values of IgA of athletes are the mean values obtained in the four samples collected.

No significant difference in immunological parameters was observed in the leukocyte subset counts or serum IgA concentration during the season (Table 2). However, the average concentration of serum IgA of the athletes was significantly lower (50.9%) than the control group values (Figure 3).

No significant correlation was found among all variables (immunological parameters, performance and the indicators of training load).

The WURSS-44 total score and total number of symptoms reported were significantly higher during the preparatory phase than baseline or during the first and second competitive phase. The number of URS for a week was not different among the three phases (Table 3).

Significant correlations were obtained between the WURSS-44 total score and strain during both the preparatory phase (r = 0.72, p = 0.032) and the second competitive phase (r = 0.70, p = 0.045), as well as between the total symptoms of URS and strain during the preparatory phase (r = 0.73, p = 0.036).

DISCUSSION

Despite the small sample used, which we consider a limitation of the study, it is important to highlight that monitoring athletic training loads is quite difficult, especially during a real competitive season in elite athletes. Monitoring the training loads is important because it is helpful to understand the behaviour of performance over long periods of training and to prevent overtraining [7].

No significant differences were found in the indicators of training load (load, monotony, strain and volume) between the training phases evaluated. On the other hand, higher values were found for RPE in the first competitive phase, indicating a higher training intensity in this phase, which may partly explain the increase in performance at the end of this phase.

TABLE 2. IMMUNOLOGICAL VARIABLES EVALUATED AT DIFFERENT TIMES OF THE SEASON

Immunological variables	Baseline	10th week	19th week	29th week
Total Leukocytes ($\times 10^9 \cdot L^{-1}$)	5.47 ± 0.36	6.01 ± 0.46	6.27 ± 0.65	6.29 ± 0.72
Lymphocytes ($\times 10^9 \cdot L^{-1}$)	1.97 ± 0.13	2.18 ± 0.13	2.21 ± 0.17	2.35 ± 0.18
Monocytes ($\times 10^9 \cdot L^{-1}$)	0.57 ± 0.06	0.50 ± 0.05	0.50 ± 0.07	0.43 ± 0.06
Neutrophils ($\times 10^9 \cdot L^{-1}$)	2.73 ± 0.26	3.07 ± 0.35	3.25 ± 0.55	3.23 ± 0.53
Eosinophils ($\times 10^9 \cdot L^{-1}$)	0.21 ± 0.06	0.26 ± 0.05	0.30 ± 0.05	0.27 ± 0.07
Basophils ($\times 10^9 \cdot L^{-1}$)	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Serum IgA ($g \cdot L^{-1}$)	1.16 ± 0.10	1.01 ± 0.10	1.12 ± 0.07	1.10 ± 0.09

TABLE 3. VARIABLES OBTAINED FROM WURSS-44 QUESTIONNAIRE

WURSS-44 scores	Baseline	Preparatory phase	First Competitive phase	Second Competitive phase
Total score	7.8 ± 3.2	32 ± 8.5[a]	12.8 ± 4.0	14.1 ± 4.0
Total symptoms	3.5 ± 1.2	13.1 ± 2.7[b]	6.0 ± 1.4	7.6 ± 2.7
Symptoms per week	=====	1.5 ± 0.3	0.7 ± 0.2	0.9 ± 0.3

Note: [a] Significantly different (p<0.01) from baseline, first competitive phase and second competitive phase.
[b] Significantly different (p<0.01) from baseline and first competitive phase

In contrast, analysing the distribution of these indicators throughout the season indicates that there was a larger CV for all indicators during the preparatory phase. In part, this behaviour can be explained by the large variation in training volume during this phase, whereas the smaller CV variability during competitive phases can be attributed to the extensive competitive period (69% of the entire season), a characteristic that is true of several sports, including road cycling. Road cycling competitions increased from 35-45 days in the 1980s [12] to 88-112 competition days per year currently [13]. Thus, coaches, trainers and athletes should seek to make reasonable adjustments and adaptations to the organization of training that will effectively prepare the athletes for the competition calendar while preserving their physical and mental integrity.

The immune system is directly influenced by excessive training loads and, in this study, was represented by the leukocyte subset counts and serum IgA. As noted in Table 3, the leukocyte values are within the references for healthy individuals [16], and the training loads imposed were not reflected in differences in the total count of white blood cells during the training season. In addition, no significant correlations were obtained between immunological parameters, performance and indicators of training load.

Exercise sessions of high intensity or long duration may cause temporary suppression of the immune system, caused by changes in either the count of white blood cells or their function, which tends to return to normal after 3-24 hours [8]. Many of these acute effects on immune function are attributed to neuroendocrine changes, particularly the release of stress hormones such as catecholamines and corticosteroids, which are known to modulate the immune system [29]. On the other hand, chronic changes in the count of white blood cells have not been observed in athletes of different sports subjected to different periods of training, such as 20 days in rugby players [17], six weeks in triathletes [4] and seven months in swimmers [9]. In addition, no differences in the total count of white blood cells between athletes and non athletes have been observed [22].

Total serum IgA levels were not significantly different between the training phases evaluated. However, the mean values were 50.9% lower than those of the control group. Corroborating these findings, other studies have also identified serum IgA values below the reference values in athletes involved in intense training routines for long periods [9,18,25].

Few studies have evaluated the acute or chronic responses of serum IgA to exercise, and the available information is conflicting [20]. Acute changes in serum IgA in athletes have not been observed after maximal exercise of short duration (30 seconds) [14]. However, after prolonged exercise, such as ultramarathons, serum IgA production is suppressed and may remain below baseline levels for two [20] or three days [18]. In contrast, changes in serum IgA after different periods of training have not been observed in athletes in different sports, such as after 20 days of training in rugby players [17], four months in volleyball players [3] and 12 months in rowers [25].

In this study, the low concentration of serum IgA observed during the season may indicate chronic immunosuppression in these athletes. Possible explanations for the chronic suppression of immunoglobulins, particularly IgA, may be related to the accumulation of training loads caused by several days, months and years of intense and continuous training performed by elite athletes, leading to a gradual decrease of immunoglobulins [9].

Evidence indicates that T and B cell functions appear to be sensitive to increases in training load in well-trained athletes, with decreases in circulating numbers of type 1 T cells, reduced T cell proliferative responses and falls in stimulated B cell Ig synthesis [29].

The clinical implications of serum IgA levels below normal levels in athletes are not well understood. However, it is unlikely that below normal levels are related to increased frequency of URTI in athletes, unlike salivary IgA [20]. Epidemiological studies indicate that 85-90% of individuals with serum IgA deficiency are asymptomatic and that only a few develop respiratory infections or gastrointestinal problems [15], which may explain the lack of significant correlations between URS and serum IgA in this study.

One of the limitations of studies involving exercise and URTI in athletes is the use of number and severity of URS collected through self-reported questionnaires as an indicator of URTI [5,23,24], because this relationship cannot be directly related to the disease confirmed clinically [27]. Self-reported symptoms may produce false positive results because some symptoms, such as a sore throat, runny nose, congestion and fever, may also be related to allergies, the inhalation of air pollutants or airway inflammation [29]. However, self-reported questionnaires during training serve as an additional tool for coaches and trainers by signalling possible respiratory disease. Moreover, recently, Pyne et al. [26] found that in elite swimmers, regardless of whether the URS were directly related to a respiratory disease, the increase of the symptoms can impair performance.

During the preparatory phase, the athletes answering the WURSS-44 questionnaire had a greater total score and reported an increased amount of URS; however, this did not impair the performance of athletes.

Other studies have also shown positive correlations between intensified training loads and increases in URTI [6,24]. However, Spence et al. [27] found no such relationship when confirming the disease clinically in elite athletes training for five months. In this study, the authors found that only 29% of athletes exhibited URS that were caused by pathogens identified by biochemical tests.

In the present study, the URS reported by the athletes during the season did not allow us to affirm their relationship to respiratory illness, especially the URTI, although such a relationship is possible. The URS reported during the study also may have been linked to other factors such as allergies, inhalation of air pollutants and airway inflammation.

Interesting results were observed regarding the relationship between URS and strain (parameter of training load). Significant correlations were found between the total score with strain during the

preparatory phase (r = 0.72, p = 0.032) and the second competitive phase (r = 0.70, p = 0.045) and between the total number of symptoms and strain (r = 0.73, p = 0.036) during the preparatory phase. These results corroborate the findings of Foster [7], who found that 89% of illnesses reported in elite athletes were explained by an increase in strain of 59% over the individual's threshold.

In summary, the results of this study suggest that training and the competitive season did not cause significant changes in the immunological parameters measured (leukocyte subset counts and serum IgA). However, URS increased significantly during the periods of intensified training that correlated significantly with the strain. In a similar way, the low levels of serum IgA observed in the athletes may indicate chronic depression of the immune system without significant apparent clinical implications or negative effects on performance. In addition, the low concentrations of IgA were not significantly related to URS, performance or training loads. On the other hand, the significant relationship found between strain and URS indicates the importance of monitoring these indicators in athletes because URS may be related to respiratory diseases.

CONCLUSIONS

Indicators of training load without a significant change throughout the season did not significantly affect leukocyte subset counts or serum immunoglobulin A; however, a long period of training seems to suppress the production of serum immunoglobulin A in the athletes assessed. The results suggest that there is no relationship among serum immunoglobulin A, upper respiratory symptoms and performance, but they also suggest that the increase of strain can cause an increase of upper respiratory symptoms throughout the season.

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