

Research article

## Evidence of a Non-Linear Dose-Response Relationship between Training Load and Stress Markers in Elite Female Futsal Players

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### Abstract

The aim of this study was: to describe typical training load (TL) carried out by a professional female futsal team for a period of 5 weeks; and to verify the relationship between TL, stress symptoms, salivary secretory immunoglobulin A (SIgA) levels, and symptoms of upper respiratory infections (URI). Over 45 sessions, the TL of the athletes was monitored daily by means of session-RPE method during the in-season period prior to the main national competition. Stress symptoms were measured weekly by means of the “Daily Analysis of Life Demands in Athletes Questionnaire” (DALDA), SIgA levels, and by symptoms of URI by the “Wisconsin Upper Respiratory Symptom Survey-21” (WURSS). There was a significant increase in TL, monotony, and training strain in week 3, with a concomitant and significant reduction in percentage variation ( $\Delta\%$ ) of SIgA concentration and secretion rate ( $p < 0.05$ ). Additionally, a second order regression model showed a high goodness of fit ( $R^2 = 0.64 - 0.89$ ) between TL and strain with SIgA concentration, secretion rate, and “worse than normal” responses of stress symptoms from the questionnaire. In conclusion, a link between TL and SIgA levels, and stress symptoms in female futsal players was evident in a non linear fashion. There appears to be an optimal range of values of daily TL between ~343 and ~419 AU and strain between ~2639 and 3060 AU, because at levels below and above these values there was an increase in stress symptoms and above ~435 and ~3160 AU to TL and strain there were a decrease in SIgA levels. In contrast, symptoms of URI failed to demonstrate relationship with the variables studied.

**Key words:** Team sports, mucosal immunity, psychometric measures, overtraining.

### Introduction

Monitoring training loads (TL) in combination with psychophysiological responses has been suggested as necessary to prevent overreaching and overtraining. The session-rating of perceived exertion (i.e. session-RPE) has been demonstrated to be a simple and practical method for quantifying internal TL in team sports (Alexiou and Coutts, 2008; Foster et al., 2001; Impellizzeri et al., 2004). A way to evaluate the impact of physiological stress on immunity is to analyze the salivary secretory immunoglobulin A (SIgA) level, which is considered to be a marker inversely related to the risk of developing upper respiratory infection (URI) symptoms in athletes (Fahlman and Engels, 2005; Walsh et al., 2011). Some

studies have shown that low SIgA levels may reduce resistance to infections and also increase the risk of impaired performance in competitions, which is often regarded as a higher than normal psychophysiological stress level (Gleeson et al., 1999; Tsai et al., 2011).

Additionally, “Daily Analysis of Life Demands in Athletes Questionnaire” (DALDA) (Coutts et al., 2007b; Neville et al., 2008) and the “Wisconsin Upper Respiratory Symptom Survey – 21” (WURSS-21) questionnaire (Moreira et al., 2011a; Rushall, 1990) have previously been used as simple tools to monitor the immune response and prevent excessive psychophysiological stress that may negatively alter athletes’ health and performance. In this regard, Coutts et al. (2007b) and Moreira et al. (2011a) have shown that the DALDA questionnaire is a sensitive tool for monitoring individual athletes’ responses to internal TL. Furthermore, significant relationships between increased TL (Foster, 1998) and volume of exercise (Gleeson et al., 2013) with symptoms of URI have been also documented. However, some authors presented inconsistent results between the relationship of TL, stress symptoms, SIgA levels, and URI (Cox et al., 2007; Fahlman and Engels, 2005; Gleeson et al., 2000; Leicht et al., 2012; Neville et al., 2008).

To date, the relationship between session-RPE and corresponding psychophysiological responses has not been sufficiently addressed, especially in female athletes. The clarification of this relationship is important because it is not known if typical TL carried out by professional female team sports players can induce alterations in stress symptoms, SIgA levels, and URI incidence in the same way as their male counterparts. For instance, female athletes may experience more stress in similar TL than male athletes (di Fronso et al., 2013; Kellmann et al., 2001), and consequently they may require special attention from coaches and physical trainers in order to manage TL appropriately on a daily basis.

Thus, the objectives of this study were twofold: 1) to describe typical TL experienced by a professional female futsal team during the 5 weeks before the main national competition by means of the session-RPE method and; 2) to verify the relationship between TL, stress symptoms, SIgA levels and symptoms of URI. We hypothesized that periods of intensified TL would increase stress symptoms, decrease SIgA levels with a corresponding increase in the susceptibility to URI.

## Methods

### Participants

Thirteen top-level professional female futsal players (mean and SD; age:  $22.1 \pm 4.2$  years; body mass:  $60.7 \pm 5.9$  kg; height:  $1.65 \pm 0.5$  m and BMI  $22.3 \pm 1.4$  kg·m<sup>-2</sup>) who were second place in the Brazilian National League in 2009 were enrolled in the study. The players had a training experienced of 4-5 years. They signed a written informed consent form. The study was approved by the Institutional Ethics Committee.

### Design

Firstly, the players performed an incremental running test to determine the ventilatory threshold (VT), respiratory compensation point (RCP), and maximal oxygen consumption (VO<sub>2</sub>max). The players were also submitted to an oral examination for detection of clinical signs of periodontal disease, active caries or mucosal lesions. The follow-up study initiated after the State championship played in the first half of the year and was comprised of 5 weeks during the preparation period for a national main championship in Brazil, in the second half of the year. The TL of the players was monitored on a daily basis by means of the session-RPE method for 45 training sessions. Stress symptoms as assessed by DALDA, symptoms of URI as assessed by WURSS-21 and salivary SIgA levels were measured in the afternoon, prior to the last training session of each week. Subsequently, the relationships between TL and training strain (independent variable) with stress symptoms, SIgA, and symptoms of URI responses (dependent variables) were determined to ascertain possible associations.

### Procedures

#### Incremental test

The incremental test on the treadmill (Super ATL - Inbrasport®, Brazil) started at 6 km·h<sup>-1</sup>. The inclination was kept constant at 1%, and the speed was increased by 1 km·h<sup>-1</sup> every minute until voluntary exhaustion. Heart rate (HR) was recorded with a short-range telemetry system (RS800, Polar Electro Oy, Finland). Pulmonary gas exchange was averaged every 20-s using a metabolic cart (Metalyzer 3B, CPX System, Germany). The O<sub>2</sub> and CO<sub>2</sub> analyzers were calibrated using gases of known concentration and the volume signal was calibrated with a 3 L syringe. The VT and RCP were determined as suggested by Lucia et al. (2003). All the players met at least two of the following criteria at exhaustion to validate the VO<sub>2</sub>max attainment: a plateau in oxygen consumption, RER values above 1.10, and HR within 5 bpm of age-predicted maximum (Billat et al., 1996).

### Training program

The designated training sessions consisted of resistance training (RT), technical-tactical training (TT), and physical training (PT) over a 5 week period. In the typical training sessions (1-3 weeks), RT consisted of 3 sets of 15 reps at 70% of repetition maximum (RM) with rest intervals of 45 s, carried out 4 times a week with a mean duration of ~30-40 minutes per day. The PT was developed

together with TT on court, 5 times a week with a mean duration of ~80-120 min per day. In the week 4, RT was decreased to 2 times a week while no RT sessions were performed in the week 5. During the last two weeks, volume and intensity of both TT and PT were maintained relatively constant.

### Quantification of internal training load

The internal TL was computed by using the session-RPE method. Approximately 30 minutes following the completion of every training session, the players were asked to rate the intensity of the whole session by means of a modified 10-point RPE scale (Foster et al., 2001). This value of RPE was multiplied by the total duration of the training session. All the players were previously familiarized with the use of the RPE scale. The session-RPE loads were recorded as total weekly and daily average units. Concurrently with the session-RPE, the "strain" and "monotony" were calculated weekly in accordance with Foster (1998). The monotony was calculated weekly by dividing the weekly mean TL by the standard deviation, while training strain was calculated as the overall weekly TL multiplied by monotony.

### Stress symptoms

The DALDA (Rushall, 1990) was administered to measure weekly stress sources/symptoms. The DALDA questionnaire is divided into two parts, namely Part A and Part B, which represent the sources of life stress and symptoms of stress, respectively. Each subject was required to complete the DALDA prior the last training session of each week at the same time of the day. The players marked every question as being either "worse than the normal", "normal" or "better than the normal". This questionnaire was filled out at the end of every week of training and number of responses labeled as "worse than normal" was retained for analysis (Moreira et al., 2011b). We considered for analysis only part B of the questionnaire, in accordance with Coutts et al. (2007c).

### Salivary secretory immunoglobulin A (SIgA)

Enzyme-linked immunosorbent assay (ELISA) was used for analyses of SIgA levels. The samples were collected at rest. The baseline values were determined from saliva samples collected at rest one week before the start of the training period. Saliva samples were collected prior the last training session of each week at the same time of the day. Unstimulated whole saliva samples were collected after individuals had rinsed their mouth twice with water. Participants were asked to spit saliva into sterile tubes for a period of 5 min. Saliva samples were centrifuged at 12,000 rpm for 10 min, and the supernatants were stored at -20 °C until use. Saliva flow rate was determined by the volume of secreted saliva per minute (ml·min<sup>-1</sup>).

Total levels of salivary SIgA were determined using microtiter plates (Costar 3590, Corning, NY, USA) and a commercial kit (Human IgA ELISA Quantification set, E80-102, Bethyl laboratories, Montgomery, USA) according to the manufacturer's instructions. After being coated with primary antibody and blocking plates, saliva samples were diluted at 1:1000 and incubated for 1 h at

room temperature. After washing, plates were incubated with anti IgA peroxidase conjugated antibody. For the determination of SIgA concentration ( $\mu\text{g}\cdot\text{ml}^{-1}$ ), absorbance values at 450 nm were plotted against the standard curve obtained for the serial dilutions of a known concentration of purified human IgA. The SIgA secretion rate was expressed by the amount of IgA secreted per minute ( $\mu\text{g}\cdot\text{ml}^{-1}$ ).

### Upper respiratory infection (URI)

The WURSS-21 (Barrett et al., 2005) was used to compute symptoms of URI prior to the last training session of each week at the same time of the day. It was assumed that the responses to WURSS-21 would be a reasonable marker of URI and functional impairment, in agreement with previous study of Spence et al. (2007). This questionnaire includes 10 items assessing symptoms, with nine items assessing functional impairments and one item assessing global severity and global change ('How sick do you feel today?' and 'compared to yesterday, I feel that my cold is . . .'). All the items are responded to using a Likert scale of severity, ranging from 0–7. The total number of occurrences regardless of severity level was retained for analysis.

### Statistical analyses

The distribution of data was analyzed by the Shapiro-Wilk test. The sphericity of data was analyzed by Mauchly's test with Greenhouse-Geisser correction. All variables are presented as mean  $\pm$  standard deviation (SD). In addition, saliva flow rate and SIgA levels were also presented as percentage variation ( $\Delta\%$ ) taking into account the relative change of each week investigated compared to the baseline period [i.e (week 1 value – baseline value)/baseline value\*100]. All the variables over the five weeks were compared by means of repeated measures analysis of variance (ANOVA). *Post-hoc* analyses were carried out using Fisher's least squares difference

(LSD) test. The relationship between the TL and strain with SIgA levels, symptoms of URI and stress symptoms were estimated from a second-order regression as used by Manzi et al. (2009) based on mean values of 8 subjects to each week. Differences were considered significant if  $p < 0.05$ . SPSS (version 17.0 for Windows; Chicago, IL) was used for all statistical calculations.

### Results

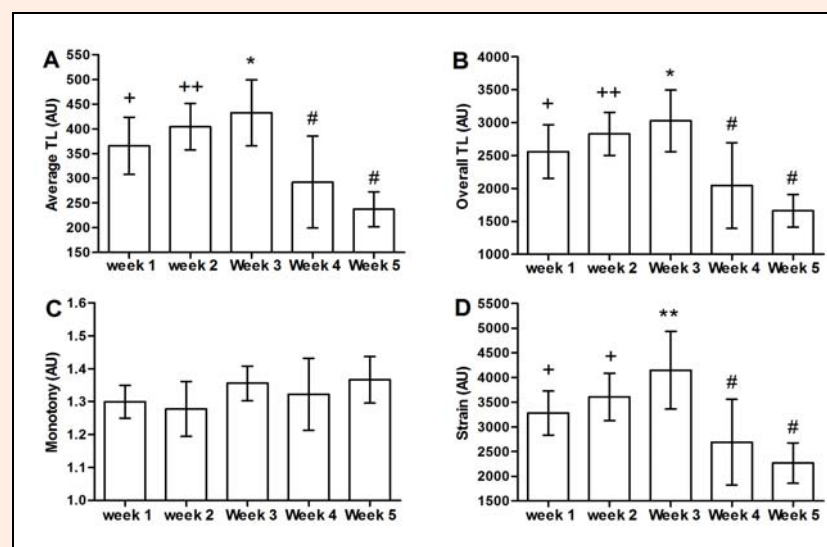
The physiological characteristics of the 13 players are shown in Table 1. Five did not complete the study. One contracted an oral infection, 2 suffered injuries during the observation period and 2 players were called to the Brazilian national team. Hence, the results for TL, stress symptoms, SIgA levels and symptoms of URI were obtained from 8 players.

**Table 1.** Mean and standard deviation of physiological variables (n = 13).

Variables	Mean (SD)
VO <sub>2</sub> max ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	53.1 (7.0)
VO <sub>2</sub> at VT ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	40.9 (8.1)
VO <sub>2</sub> at RCP ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	47.6 (6.3)
HR at VT ( $\text{beats}\cdot\text{min}^{-1}$ )	162 (19)
HR at RCP ( $\text{beats}\cdot\text{min}^{-1}$ )	181 (9)
HRmax ( $\text{beats}\cdot\text{min}^{-1}$ )	190 (6)

Maximum oxygen uptake (VO<sub>2</sub>max), ventilatory threshold (VT), respiratory compensation point (RCP), heart rate at ventilatory threshold (HR at VT), heart rate at respiratory compensation point (HR at RCP), maximum heart rate (HRmax).

The main effects on average TL, overall TL and training strain across the 5 weeks of observation were significant ( $F = 29.064$ ,  $df = 4$ ,  $p < 0.001$ ;  $F = 29.963$ ,  $df = 4$ ,  $p < 0.001$ ;  $F = 23.298$ ,  $df = 4$ ,  $p < 0.001$ ). The average (Figure 1A) and overall (Figure 1B) TL were significantly higher in week 3 than in weeks 1, 4 and 5. During the two weeks (4 and 5) preceding the competition, the



**Figure 1.** (A) Average weekly training load (TL), (B) overall TL, (C) monotony, and (D) strain training over 5 weeks of monitoring (n = 8). + different from weeks 3, 4 and 5 ( $p < 0.05$ ). ++ different from weeks 4 and 5 ( $p < 0.05$ ). \* different from weeks 1, 4 and 5 ( $p < 0.05$ ). # different from week 1, 2 and 3 ( $p < 0.05$ ). \*\* different from week 3 to weeks 1, 2, 4 and 5 ( $p < 0.05$ ).

**Table 2.** Mean and standard deviation of saliva flow rate and SIgA levels and percentage variation ( $\Delta\%$ ), upper respiratory illness symptoms and stress symptoms ( $n = 8$ ). Data are means ( $\pm$ SD).

	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5
Saliva flow rate ( $\text{ml}\cdot\text{min}^{-1}$ )	.84 (.26)	.91 (.18)	.81 (.17)	.81 (.27)	.78 (.32)	.83 (.46)
$\Delta\%$ saliva flow rate	-	12.8 (32.3)	.99 (28.50)	3.49 (41.90)	-2.60 (44.7)	-.75 (51.3)
SIgA concentration ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	52.2 (32.1)	55.5 (32.3)	78.2 (30.3)	38.8 (16.6)	69.3 (34.9)	59.8 (31.4)
$\Delta\%$ SIgA concentration	-	14.3 (34.1)	67.0 (37.2)	-15.0 (27.8) $\dagger\dagger$	97.1 (90.7)	35.8 (73.5)
SIgA secretion rate ( $\mu\text{g}\cdot\text{ml}^{-1}$ )	52.2 (59.4)	49.0 (27.1)	61.0 (21.9)	30.3 (13.7)	56.3 (35.3)	49.0 (27.3)
$\Delta\%$ SIgA secretion rate	-	31.7 (68.4)	69.1 (74.7)	-10.7 (54.3)	60.0 (127.6)	54.6 (135.7)
URI – total reports	-	-	-	17	3	-
URI No. of affected individuals	-	-	-	2	2	-
URI episodes (max and min)	-	-	-	0–10	0–2	-
Stress symptoms	-	2.4 (2.2)	2.6 (3.4)	3.1 (4.5)	2.3 (3.9)	2.6 (3.7)
Stress symptoms (max and min)	-	(0–6)	(0–7)	(0–11)	(0–7)	(0–10)

$\dagger\dagger$   $p < 0.05$ , in relation to weeks 2 and 4.

TL was significantly reduced compared with weeks 1, 2 and 3. The training strain (Figure 1D) was also higher in week 3 compared with weeks 1, 2, 4 and 5 ( $p < 0.05$ ). During the weeks 4 and 5 the values were significantly reduced compared with weeks 1, 2 and 3. The monotony of training (Figure 1C) was not significantly altered across the weeks.

The SIgA levels were highly variable between subjects across the weeks. The greatest variation was in the baseline, with values of 66% and 111% regarding the concentration and secretion rate of SIgA, respectively. The SIgA concentration showed tendency of change across the weeks ( $F = 2.05$ ,  $p = 0.070$ ). However, neither SIgA concentration, SIgA secretion rate nor saliva flow rates were significantly altered during the training period (Table 2). However, when values were normalized for the individual's mean baseline values, the  $\Delta\%$  of SIgA concentration significantly changed across the weeks ( $F = 5.81$ ,  $p = 0.002$ ). The  $\Delta\%$  of SIgA concentration was lower in week 3 in relation to weeks 2 and 4 (Table 2)

Two players reported 17 URI symptom items in week 3 while other 2 reported 3 symptom items in week

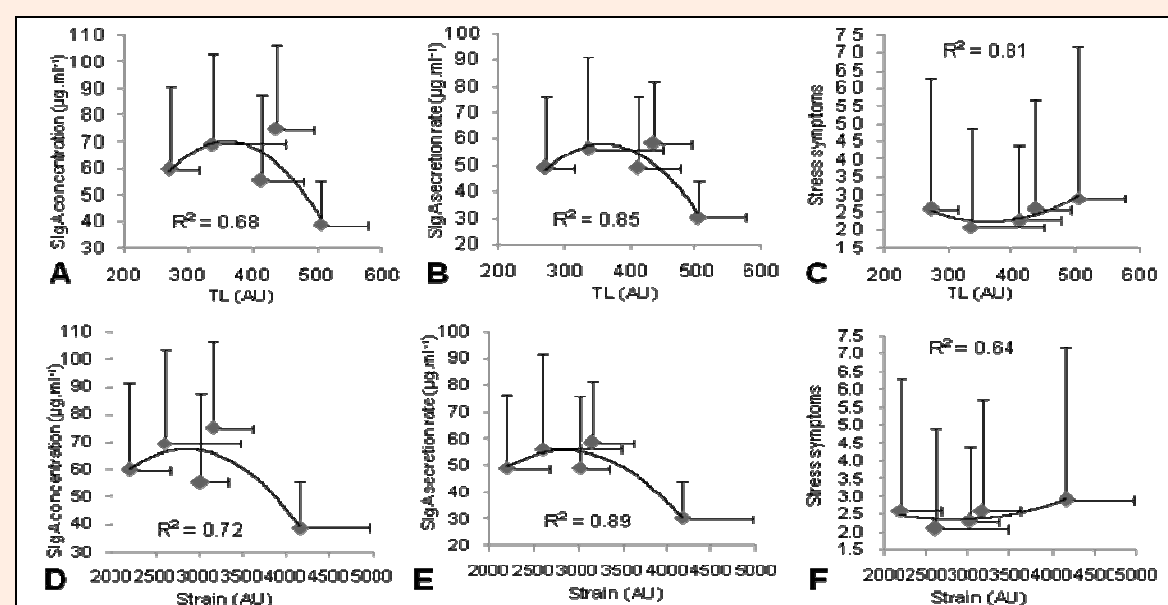
4. There were no significant differences in weekly stress symptoms, assessed by DALDA scores across the training period ( $p > 0.05$ ) (Table 2).

A second order regression model showed a high goodness of fit ( $R^2 = 0.64 - 0.89$ ) between TL and strain with SIgA concentration and secretion rate, and with the “worse than normal” responses for stress symptoms in the DALDA questionnaire (Figures 2 A, B and C).

## Discussion

To the best of our knowledge, this is the first study describing the typical TL and the psychophysiological stress experienced by a female professional futsal team during a 5-week training mesocycle. This study demonstrates that increased TL, monotony and training strain may be associated with alterations in SIgA levels and stress symptoms in a non-linear fashion, therefore suggesting a non-linear dose-response relationship. Nevertheless, our results failed to demonstrate any correlation among these variables.

The team coaches programmed the taper strategy

**Figure 2.** Weekly dose-response relationships based on mean values from 8 players (weeks 1-5) of training load (TL) and strain, with SIgA concentration (A and D), SIgA secretion rate (B and E), and “worse than normal” responses of stress symptoms (C and F) ( $n = 8$ ).



to reduce training loads in the last 2 weeks (weeks 4 and 5) of training period before the competition, decreasing volume and maintaining training intensity of the sessions. In the week 4, RT frequency was decreased to 2 times per week, while no RT was performed in the week 5. Technical tactical training volume and intensity were maintained constant during this period. Internal TL varied in accordance to the planned external training loads (Figure 1). The strategy of reducing TL prior to the competition is a common practice among strength and conditioning coaches as a recovery period because low TL may result in a transient improvement in performance due to super-compensation (Coutts et al., 2007b; 2007c).

In the present study there was a reduction of about 45% of TL during the taper period. Coutts et al. (2007a) observed that large reduction (~45%) of internal TL during taper period in male rugby players induced an increase in the testosterone/cortisol (T/C) ratio and glutamine/glutamate (Gln/Glu) ratio and decreased plasma glutamate and creatine kinase (CK) activity concomitant with positive endurance and power performance changes. The tapering period also allows athletes to recover from psychophysiological distress or illness. In agreement with the findings of Papacosta et al. (2013) that reported changes in SIgA levels during the tapering period in judo athletes, the present study also presented a significant positive change in  $\Delta\%$  of SIgA concentration in the same period of training, suggesting that this tapering strategy is a suitable approach to allow some degree of immune function recovery.

The effectiveness of the training programs depends on the successful manipulation of the total training volume and intensity. High scores of monotony and training strain are a result of low TL variability, which in turn has been suggested to be related to the onset of overtraining, when combined with high TL (Foster, 1998). In the present study, the third week presented the highest overall weekly TL (3057 AU), monotony (1.6 AU) and training strain (4186 AU), as noted in Figure 1A, B, C and D, respectively. Foster et al. (1998) found that for top-level speed skaters the incidence of banal infections, which is thought to be a marker of the early stages of overtraining, was higher in the weeks at which accumulated TL, monotony and strain exceeded approximately 4400, 2.2, and 6000 AU, respectively. However, to determine the relationships between TL and infection risk or to determine a secure TL threshold for individual training is still a matter of debate, instigating further investigations in sports sciences.

The perceived TL can be influenced by innate characteristics, quantity and the nature of external TL and fitness level (Impellizzeri et al., 2004; Milanez et al., 2011). In male futsal, for example, players with a higher aerobic fitness reported lower TL values compared with their less fit counterparts, despite undergoing similar external TL (Milanez et al., 2011). Furthermore, gender differences would be also influencing psychophysiological response to TL in individual and team sports (Kellmann et al., 2001; di Fronso et al., 2013). For instance, Kellmann et al. (2001), suggested that female rowers would experience higher levels of stress and lower levels of recovery

than males when exposed to similar TL. Further, Di Fronso et al. (2013) found lower scores of physical recovery, sleep quality, and self efficacy in female basketball players when compared to males. Consequently, female athletes may require more attention from coaches and physical trainers during the training monitoring process. However, further evidence is necessary in this area for a better understanding of the role of aerobic fitness, competitive experience and immunological responses in stress tolerance to training and competitive loads in female athletes.

Previous studies evaluated the salivary SIgA levels in order to monitor psychophysiological stress in response to TL in similar training periods (Fahlman and Engels, 2005; Leicht et al., 2012) but few of them quantified the TL by the session-RPE method (Moreira et al., 2009; 2011a). In the present study, the significant reduction in  $\Delta\%$  SIgA concentration was found in week 3 in response to increased TL, monotony and strain. Our results are in agreement with previous studies in the literature (Leicht et al., 2012; Moreira et al., 2011b). For instance, Moreira et al. (2011b) found a significant decrease in the SIgA secretion rate after a period of 4 weeks of training in basketball players. Subsequently, Leicht et al. (2012) described a negative relationship between TL and SIgA levels in tetraplegic wheelchair rugby players. In this respect, the goodness of fit ( $R^2$  ranged from 0.68 to 0.89) found in the present study would suggest a non-linear dose-response relationship between SIgA with TL and strain. That is, for this group of players, values of TL and strain ~435 and ~3160 AU respectively would be desirable because higher values would decrease SIgA levels (Figure 2A, B, D and E). These results provide important information for coaches and sport scientists regarding the utilization of SIgA as useful markers of physiological stress and the “optimal” TL to potentially minimize the risk of URI.

Impairment of salivary SIgA secretion in response to TL and psychophysiological stress before or during the URI symptom items has been suggested by other authors as a symptom of overreaching/overtraining (Gleeson et al., 2011; Neville et al., 2008; Tsai et al., 2011). It is assumed that increases in the symptom items of intense and rigorous training periods may lead to the formation of the “open-window” of immunosuppression and increase the risk of URI (Koch et al., 2007; Nieman, 1997). Fahlman and Engels (2005) observed, over a 12 month training period, that college football players had a greater risk of contracting infections when SIgA secretion was below  $40 \mu\text{g}\cdot\text{min}^{-1}$ . Gleeson et al. (1999) observed, over a 7 month training period, that SIgA concentration values  $\leq 40 \mu\text{g}\cdot\text{min}^{-1}$  were associated with an increased number of URI symptom items over a training season in elite swimmers. In the present study, mean SIgA concentration and SIgA secretion rate of the team reached risk levels (in week 3) as suggested by Gleeson et al. (1999) and Fahlman and Engels (2005) respectively, but the large increase of URI symptom items in the week 3 was not significant.

Our results are in agreement with previous studies that reported no significant relationships between SIgA and symptoms of URI in different sports like tennis, fe-

male soccer, elite tetraplegic rugby and basketball (Leicht et al., 2012; Novas et al., 2002; Novas et al., 2003). Although Novas et al. (2002) found a relationship between the increase in energy expenditure and URI symptoms, these authors did not find a relationship between URI symptoms and SIgA levels in female tennis players (Novas et al., 2003). Thus, the relationship between SIgA levels and URI is still not clear as there are contradictory results in the literature (Fahlman and Engels, 2005; Gleeson et al., 1999; Leicht et al., 2012; Novas et al., 2002; Novas et al., 2003; Vardiman et al., 2011). Hence, such a relationship must be considered with caution because factors other than salivary antibody levels may contribute to infection development (Diamond et al., 2008). Some of those studies found increased symptoms of URI concomitant with a decrease in SIgA levels, although a non-linear relationship among these variables is expected (Fahlman and Engels, 2005; Gleeson et al., 1999).

DALDA questionnaire also has been suggested to be useful for monitoring psychophysiological stress in response to TL (Coutts and Reaburn, 2008; Moreira et al., 2011b). This tool has been shown to be sensitive to TL (Coutts and Reaburn, 2008; Moreira et al., 2011b) as well as to bodily reactions to training stress (Coutts and Reaburn, 2008). For instance, Nicholls et al. (2009) found that DALDA was able to discriminate between different periods such as rest, training and pre- and post-match days. In this previous study, professional rugby players reported greater stress on training days, when compared to rest period and match days. In the present study we found a goodness of fit ( $R^2$  ranged from 0.64 to 0.81) between an increased number of “worse than normal” scores with TL and strain. To the best of our knowledge, this is the first study reporting such as relationships. Therefore, it could be suggested that the DALDA questionnaire is sensitive for monitoring psychophysiological stress in response to the variations of TL. For this group of players an optimal range of values between 343 and 419 UA to TL and 2639 and 3060 AU to strain training would be suggested, since below and above these values increased responses of stress symptoms were observed (see Figure 2 C and F). Higher levels of stress symptoms at low TL values coincided with the period immediately preceding the competition. Hence, they may have been mainly caused by anxiety and psychological stress rather than by TL.

The main limitation of the present investigation is the small sample size, but some players were injured while others were called to the National Brazilian team and could not complete the study. Additionally, the goalkeepers were excluded from the study because their training routine and the TL experienced are quite different from the outfield players. Furthermore, the training program period investigated was relatively shorter than those used in previous studies that found some relationship between SIgA and URI (Fahlman and Engels, 2005; Gleeson et al., 1999). Additionally, URI symptoms could have been more reliable if diagnosed by a medical doctor rather than using the questionnaire.

In summary, the present study demonstrated an in-

teresting link between TL, monotony and training strain with SIgA levels and stress symptoms. However, although two players reported altogether 17 URI symptoms in week 3 concurrent with an increase in TL, monotony, strain training, stress symptoms and a decrease in SIgA concentration, this increase was not statistically significant. However, a non-linear dose-response relationship between TL and strain with SIgA and stress symptoms was detected in the present study.

## Conclusion

The present study confirms the need for coaches and physical trainers to monitor TL, monotony and strain in combination with psychophysiological responses during periods of training before an important competition. The study demonstrated that increased TL, monotony and training strain may be associated with SIgA levels and stress symptoms. In the present study a significant increase in SIgA levels was observed during the tapering period, suggesting that this is a suitable approach to allow immune function recovery. Furthermore, for this group of players, there appears to be an optimal range of values of daily TL between ~343 and ~419 AU and strain between ~2639 and 3060, because at levels below and above these values there was an increase in stress symptoms and above of ~435 and ~3160 AU to TL and strain there were a decrease in SIgA levels. These results provide important information regarding the utilization of SIgA and stress symptoms derived from the DALDA questionnaire as useful markers of training stress. On the other hand, URI symptom items were not directly related to the variation in SIgA and responses to the DALDA questionnaire.

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### Key points

- There is a dose-response relationship between SIgA levels and stress symptoms with TL.
- For the athletes of the present study, values of ~436 AU and ~3161 AU to TL and strain training would be desirable because higher values would decrease responses of SIgA levels.
- An optimal range of values of TL between ~336 and ~412 AU to TL and ~2610 and ~3016 AU to strain training would be suggested for this group of athletes, since below and above these values increased responses of stress symptoms were observed.

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