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ORIGINAL ARTICLE

Relationships among salivary immunoglobulin A, lactoferrin and cortisol in basketball players during a basketball season

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Abstract The aim of this study was to examine the changes and relationships of immune and stress parameters of basketball players during a basketball season. Eight members of National Taichung University basketball team volunteered to participate. Saliva samples were collected at rest and before the start of practice or competition at seven time points during the intense training, competition and recovery period. Salivary immunoglobulin A (sIgA), cortisol, and lactoferrin were measured during training and competition period and compared with those measured at the fourth recovery week. Relationships among immune and stress parameters were evaluated. Compared with those detected at the fourth recovery week, significant decreases in secretion rates and absolute concentrations of sIgA and lactoferrin were observed at times of intense training and competition. In addition, significant increases in secretion rates and absolute concentrations of salivary cortisol were observed during intense training and competition period and the first week of recovery. Moreover, a significant inverse correlation ($r = -0.28$; $P < 0.05$) that existed between secretion rates of sIgA and cortisol as well as a positive correlation ($r = 0.32$; $P < 0.05$) that existed between secretion rates of sIgA and lactoferrin was measured. Our results demonstrated that the secreted cortisol was induced and the mucosal immunity of the participants was suppressed during the basketball season. The inverse correlation existed between secretion rates of sIgA and cortisol may indicate a possible role of cortisol in the strenuous exercise-induced immunosuppression. Our results also suggest that a delicate balance may exist between mucosal innate and adaptive immune responses.

Keywords Basketball · Immune function · Stress response to exercise

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Introduction

Basketball is a demanding sport that involves repeated bouts of intense physical exercise. The pre-competition training sessions are aimed to enhance the speed, agility, aerobic endurance, anaerobic power as well as sport-specific skills of basketball players (Castagna et al. 2008; Simenz et al. 2005). Therefore, the pre-competition training sessions during the basketball season include intensified physical and technical trainings. Strenuous bouts of intense training and competitions are known to affect immunological/stress functions in elite athletes (Gleeson 2007). However, only a limited number of studies have been conducted to examine the influence of training and competition on physiological status of basketball players or the relationships among important immune and stress parameters (Moreira et al. 2008).

The mucosal immune system, especially sIgA, functions as the first line of defense against pathogen invasion by

preventing the attachments of infectious agents to mucosal surfaces (Bishop and Gleeson 2009; Gleeson and Pyne 2000; Marcotte and Lavoie 1998). Studies revealed that prolonged strenuous physical stress suppressed the sIgA concentration and secretion rate (Gleeson and Pyne 2000; Novas et al. 2003). Decreased secretion of sIgA may result in impaired immune responsiveness, and consequently, increased pathogen colonization and infection. For example, our previous work demonstrated that the upper respiratory tract infection of elite taekwondo athletes who had rapid weight changes during intense training and competition period were significantly increased due to suppressed mucosal immunity (Tsai et al. 2009). Lactoferrin is one of the most abundant antimicrobial proteins and plays a key role in mucosal immunity against pathogen infection (Legrand et al. 2004). Previous studies reported that salivary lactoferrin concentrations are modulated immediately after strenuous exercise (Inoue et al. 2004; Legrand et al. 2004; West et al. 2006). Cortisol is known to be a physiological indicator of stress (de Kloet et al. 2005). Physical stress, such as exercise or training, increases secretion of cortisol from the adrenal cortex (Duclos et al. 2003). Therefore, it has been suggested that cortisol concentration can act as an indicator for assessing physical stress during training and competitions.

It has been reported that an increased secretion of cortisol may contribute to the exercise-induced immunosuppression (Elenkov and Chrousos 1999; Gleeson 2007; Ronsen et al. 2001). Although the relationships among cortisol and mucosal immune parameters have been investigated previously, contradictory results were reported. Hucklebridge et al. (1998) showed an inverse correlation between salivary cortisol and sIgA levels (Hucklebridge et al. 1998). Other studies reported that no significant correlation was found between sIgA and cortisol levels (Cieslak et al. 2003; Kugler et al. 1992; McDowell et al. 1992). Nevertheless, the relationships among cortisol and mucosal immune variables were not sufficiently investigated in athletes undergoing intense training and competition. The aim of this study was to examine the changes and relationships of mucosal immune variables and stress hormone response of basketball players during training, competition and recovery period.

Methods

Participants

Eight members of National Taichung University (NTCU) basketball team in Taiwan volunteered to participate in this study. All participants signed a written inform consent form, which was approved by the Human Ethics Committee of the National Taiwan Sport University, prior to the onset of the study. The University Basketball Association (UBA) in Taiwan is divided into three different grade levels. The NTCU basketball team is grouped into the second level. Participants' mean (±SEM) age, height, body mass, muscle mass and body fat were 20.5 ± 0.3 years, 176.6 ± 2.0 cm, 75.1 ± 3.9 kg, 59.0 ± 3.1 kg and 12.7 ± 1.5%, respectively. The participants were not taking any medication and had no history of endocrine/immunological disorders before or during this study.

Study design

Regular training was initiated 2 months before the start of the intense training program. During this off-season period, basketball players performed muscle strength and endurance training with the weekly total training volume similar to that in T4 (Fig. 1). These basketball players participated in a 4-week intensive training program in preparation for a national tournament. Figure 1 shows the weekly total training volume (shown as minutes for each session) during the saliva collection period. This variable was utilized to represent the training load. Black columns represent the strength sessions that include muscle strength and power development, increased muscle endurance, improved flexibility, explosive power, basketball speed, quickness and agility (ability to quickly change direction). Gray columns represent the technical sessions that include a series of shot training, team work and tactical training. No training sessions were scheduled during the recovery period (from R1 to R4). In order to assess the impact of intense training or competition to the athletes, physiological parameters measured during the training and competition period were compared to these measured at R4 when athletes were rested for 4 weeks.

Saliva collection

A schematic representation of the experimental design is shown in Fig. 2. Saliva samples were collected on days 3 (T4) and 24 (T1) during the intense training period. The competition was held with one game per day for each team during the competition weeks. A total of seven competitions were held on days 29, 30, 31, 43, 44, 45 and 46 for the NTCU basketball team. During the first competition week (C1), basketball team played for three consecutive games on days 29, 30 and 31. Saliva was collected on day 31 (C1) prior to the start of the competition. This was followed by a 1-week rest period (M1). During the C1, M1 and C2 weeks, except for 300 min/week of technical training courses, no strength training courses were arranged (Fig. 1). Saliva was collected on day 38 (M1) when athletes were rested for 7 days after the last competition of the

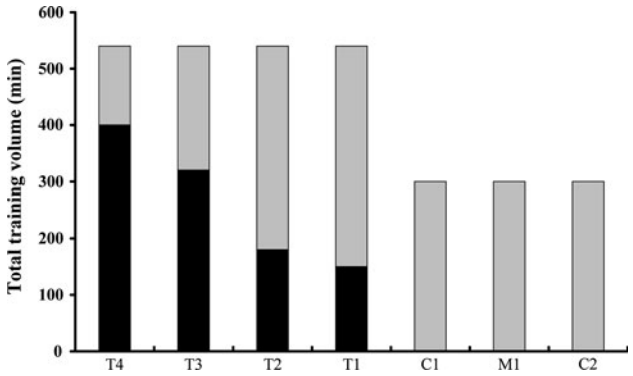

Fig. 1 Weekly total training volume during the basketball season. Training volume was shown as minutes for each session. *Black columns* represent the strength training courses. *Gray columns* represent the technical training courses. The training was stopped completely after the second competition week during recovering period

C1 week. Then, during the C2 week, basketball team played four consecutive games on days 43, 44, 45 and 46. Saliva was collected on day 45 before the onset of the competition. This was followed by a recovery period during which saliva samples were collected on days 52 (R1) and 73 (R4). Circadian variations have been shown to cause alterations in levels of salivary cortisol and immunoglobin (Dimitriou et al. 2002). To minimize the circadian variations on these physiological variables, all samples were collected before the start of practice in the afternoon (1,730–1,830 h) (Fig. 2). Prior to sample collection, each participant was asked to thoroughly rinse his mouth with sterile distilled water without swallowing to minimize possible contamination that could interfere with the analyses. However, this procedure can potentially affect the saliva flow rate and/or rinse the saliva protein of interest from the mouth if saliva samples were collected immediately after rinsing the mouth. Therefore, 10 min after the rinsing procedure, unstimulated whole-saliva specimens were collected for 2 min. Volume of saliva was estimated by weighting the tube immediately after collection and saliva density was assumed to be 1.00 g/ml (Cole and Eastao 1988). Salivary flow rate was calculated as volume (ml)/collection time (min). Saliva specimens were stored in sterile plastic containers at −80°C until use.

Assays

All saliva samples were centrifuged before analysis. Experiment procedures for measuring total protein, sIgA, lactoferrin and cortisol concentrations were essentially the same as described in our previous work (Tsai et al. 2009). Briefly, total protein concentration was measured using the Bio-RAD protein assay kit (Bio-RAD, Hercules, CA, USA). SIgA concentration was measured by enzyme-linked immunosorbent assay (ELISA). Primary (anti-human IgA; I-9889) and secondary antibodies (peroxidase-conjugated anti-human IgA; A3062) were purchased from Sigma (Poole, UK). Assays were calibrated using serial dilutions of human colostrum IgA (I-2636, Sigma, Poole, UK). The concentration of salivary lactoferrin was measured using a commercial ELISA assay kits (Calbiochem, Darmstadt, Germany) according to the manufacturer's instructions. The DRG salivary cortisol ELISA kit (DRG Diagnostics, Marburg, Germany) was used for the measurement of cortisol in saliva samples. All salivary variables are normalized by salivary flow rate. The average intra-assay coefficient of variation (CV) for sIgA, cortisol and lactoferrin was 3, 4 and 4%, respectively.

Statistical analysis

Data are reported as mean values and standard error of mean values (SEM). The Shapiro–Wilk test showed that all of the salivary variables were normally distributed ($P > 0.05$). The data were analyzed using one-way repeated measures ANOVA and LSD post hoc comparisons. The relationships that existed between salivary variables were assessed by Pearson's correlation coefficient analysis. Statistical significance was set at $P < 0.05$.

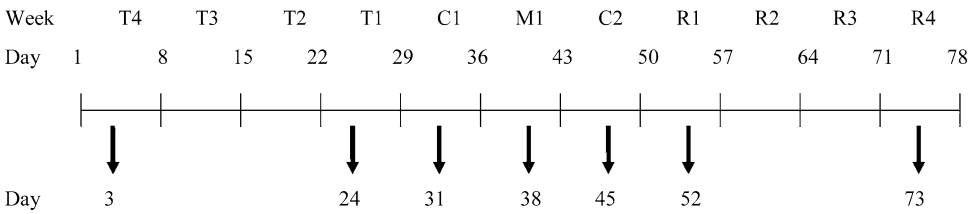

Fig. 2 Schematic representation of the experimental design. Saliva was collected at seven time points during the basketball season. *T1*, *T2*, *T3* and *T4* represent 1, 2, 3 and 4 weeks before the first competition week, respectively. *C1* and *C2* represent the first and second competition week, respectively. *M1* is the break week between two competition sessions. *R1*, *R2*, *R3* and *R4* represent 1, 2, 3 and 4 weeks after the second competition week, respectively. *Black arrows* indicate the seven time points when saliva samples were collected. *Numbers at top of each bar* represent days after the starting date of the study. *Numbers at the end of black arrowheads* represent days at which saliva samples were collected

Results

Immunological and stress functions of athletes were differentially modulated

Compared with R4, secretion levels and absolute concentrations of salivary total protein measured at different time points were not significantly varied (Fig. 3; Table 1). Significant decreases in sIgA secretion rates and absolute concentrations were observed during the training and competition period. As shown in Fig. 4, when compared with R4, there were significant decreases in sIgA secretion rate at T4 ($P < 0.01$), T1 ($P < 0.05$), C1 ($P < 0.01$) and C2 ($P < 0.05$). However, sIgA secretion rate measured at M1 and R1 were not significantly different from those measured at R4 ($P > 0.05$). On the other hand, compared with R4, significant decreases in salivary lactoferrin secretion rate and absolute concentrations were observed at T4, T1, C1 and M1 (Fig. 5; Table 1); while the levels at C2 and R1 were not significantly altered. As shown in Fig. 6 and Table 1, significant increases in salivary cortisol secretion rates and absolute concentrations were detected at T4, T1, C1, C2 and R1; while the secretion rates and absolute concentrations of cortisol measured at M1 were not significantly different from those measured at R4.

Correlations between immunological and stress parameters

To examine the relationships among the secretion rates of sIgA, lactoferrin, cortisol and total protein of basketball players during the basketball season, Pearson's correlation coefficient was calculated (Table 2). An inverse correlation that existed between levels of sIgA and cortisol was measured ($r = -0.28$; $P < 0.05$). A positive correlation was observed between secretion rates of sIgA and lactoferrin ($r = 0.32$; $P < 0.05$). In addition, Pearson's correlation coefficients revealed a significant positive correlation that existed between secretion rates of total protein and sIgA ($r = 0.39$; $P < 0.01$) as well as between total protein and lactoferrin ($r = 0.52$; $P < 0.01$). However, no significant correlation was detected between secretion rates of lactoferrin and cortisol ($r = -0.12$; $P = 0.363$).

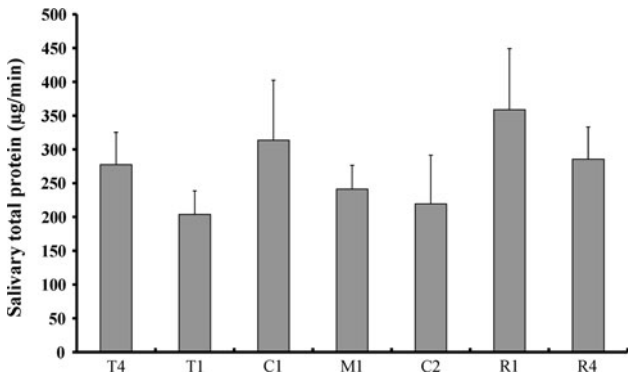

Fig. 3 The salivary total protein measured during the basketball season. Values are expressed as total protein concentration/flow rate (μg/min). The data are expressed as the mean ± SEM, $n = 8$

Discussion

The main findings of the present study were as follows: (1) secretion rates and absolute concentrations of salivary total protein were not significantly altered when compared with R4 and a positive correlation was observed between secretion rates of sIgA and total protein; (2) secretion rates and absolute concentrations of sIgA were significantly decreased during the training and competition periods and 1 week after competition (M1), the secretion rates and absolute concentrations of sIgA were quickly recovered; (3) secretion rates and absolute concentrations of lactoferrin were significantly decreased during the training and competition periods and a positive correlation was observed between secretion rates of sIgA and lactoferrin; (4) secretion rates and absolute concentrations of cortisol were significantly increased during the training and competition periods and there exists an inverse correlation between sIgA and cortisol; and (5) the sIgA, lactoferrin and cortisol levels measured at the beginning of the study (T4) were significantly different from those measured at R4.

Levels of salivary total protein have been used to estimate the hydration status of athletes during training and competition (Walsh et al. 2004). Our results suggest that the secretion rates of salivary total protein were not significantly changed during the study period. These findings suggest that the basketball players consumed sufficient amount of fluid to avoid acute dehydration during the intense training and competition period. Therefore, the hydration status of these athletes was not significantly affected. Human saliva contains various kinds of proteins and its composition is selectively altered in response to exercise (Chicharro et al. 1998). Although the secretion rates of total protein measured during training and competition were not significantly different from that measured at R4, a positive correlation was observed between secretion rates of total protein and sIgA as well as between total protein and lactoferrin. These data suggest that, although the difference was not statistically significant, the secretion rates of salivary total proteins were influenced by intense training and competition with a similar pattern found for sIgA and lactoferrin.

Previous studies showed that intense training and training of a longer duration have a suppressive effect on the mucosal sIgA response. Tharp and Barnes (1990)

Table 1 Absolute concentrations of salivary total protein, sIgA, lactoferrin and cortisol

Week	Total protein (μg/ml)	sIgA (μg/ml)	Lactoferrin (ng/ml)	Cortisol (ng/ml)
T4	1109.5 ± 192.0	146.7 ± 18.0**	3247.1 ± 635.7*	71.0 ± 2.2**
T1	815.7 ± 139.4	144.9 ± 22.7*	3440.8 ± 739.1*	48.0 ± 4.9*
C1	1254.5 ± 355.6	142.9 ± 11.9**	2634.4 ± 546.9*	63.6 ± 4.1*
M1	964.6 ± 141.3	204.9 ± 9.5	2728.6 ± 441.6*	46.6 ± 4.5
C2	877.6 ± 288.8	153.2 ± 18.0*	3684.1 ± 602.7	84.4 ± 4.1**
R1	1434.9 ± 362.6	204.3 ± 20.5	4619.8 ± 819.7	47.2 ± 4.0*
R4	1141.6 ± 191.7	210.7 ± 15.0	4300.8 ± 905.3	40.6 ± 3.9

Values are mean ± SD

*$P < 0.05$; **$P < 0.01$, significantly different from R4

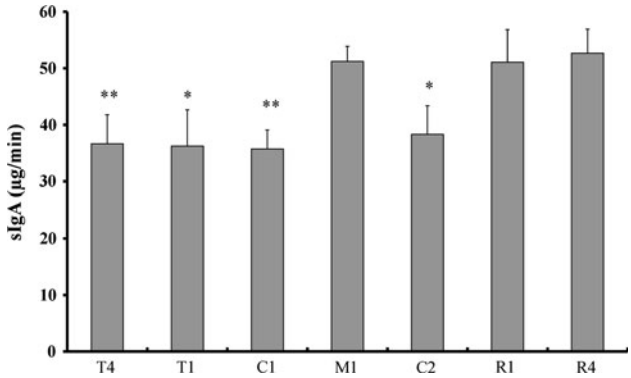

Fig. 4 The secretion rates of sIgA measured during the basketball season. Values are expressed as sIgA concentration/flow rate (μg/min). The data are expressed as the mean ± SEM, $n = 8$. Significant difference between values obtained at each sampling time and those measured at the fourth recovery week (R4) was set at *$P < 0.05$; **$P < 0.01$

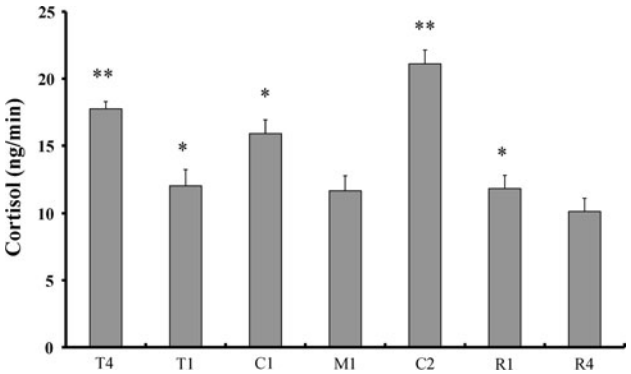

Fig. 6 The secretion rates of salivary cortisol measured during the basketball season. Values are expressed as cortisol concentration/flow rate (ng/min). The data are expressed as the mean ± SEM, $n = 8$. Significant difference between values obtained at each sampling time and those measured at the fourth recovery week (R4) was set at *$P < 0.05$; **$P < 0.01$

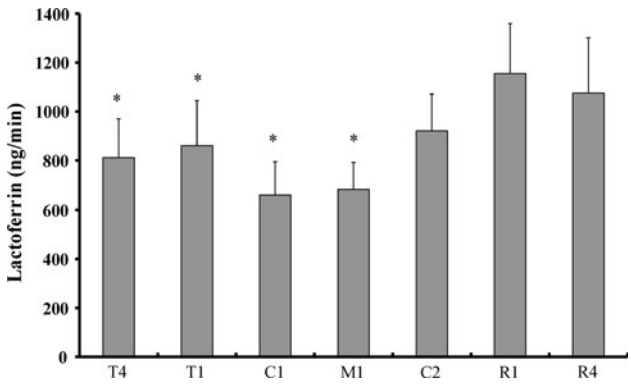

Fig. 5 The secretion rates of salivary lactoferrin measured during the basketball season. Values are expressed as lactoferrin concentration/flow rate (ng/min). The data are expressed as the mean ± SEM, $n = 8$. Significant difference between values obtained at each sampling time and those measured at the fourth recovery week (*R4*) was set at *$P < 0.05$

reported a decrease in sIgA secretion across a 3-month training period in swimmers (Tharp and Barnes 1990). Gleeson et al. (1999) also reported a downward trend in sIgA levels across a 7-month training period in elite swimmers (Gleeson et al. 1999). The findings of the current study are in line with these observations. We observed that the sIgA levels measured at T4, T1, C2 and C1 were significantly lower than those measured at R4. It indicates that the resting sIgA secretion of basketball players was significantly decreased during the intense training and competition period than during the recovery period. These results demonstrate that the effects of prolonged intensive training and competition might have caused immune suppression in basketball players. In addition, Tiollier et al. (2005) examined the impact of a multi-stressor situation on sIgA levels during the training of French commandos and found that the suppressed sIgA secretion was restored to the basal level after a 1-week recovery period (Tiollier et al. 2005). Our data are in good agreement with those of Tiollier et al. (2005) that the secretion rates and absolute concentrations of sIgA measured after 1 week of rest at M1 were significantly higher than those measured at T4, T1, C2 and C1. It suggests that the suppressed secretion of sIgA is quickly restored after 1 week of rest.

Lactoferrin was selected as a marker of innate mucosal immunity because of its documented ability to sequester iron, bind to bacteria, and antimicrobial activities in

Table 2 Pearson's correlation coefficients among secretion rates of sIgA, lactoferrin, cortisol and total protein

	Correlation coefficient *r* (*P*)		
	Lactoferrin (ng/min)	Cortisol (ng/min)	Total protein (μg/min)
sIgA (μg/min)	0.32* (0.015)	−0.28* (0.037)	0.39** (0.003)
Lactoferrin (ng/min)		−0.12 (0.363)	0.52** (0.001)
Cortisol (ng/min)			−0.06 (0.661)

The numbers in brackets represent the *P* values from the correlation analysis

Two-tailed *P* values: $*P < 0.05$; $**P < 0.01$

synergy with sIgA and lysozyme (Ellison et al. 1988). Our results show that the secretion rates and absolute concentrations of salivary lactoferrin were suppressed during the intense training and competition period in basketball players. These data indicate that prolonged intense basketball training lowers the secretion rate of a key salivary protein of innate mucosal immunity, which might leave individuals at greater risk of contracting infection and negatively impacting training and competitive performance. Moreover, there exists a positive correlation between the secretion rates of sIgA and lactoferrin. Some previous observations are in line with this finding, suggesting a weak positive correlation existed between levels of sIgA and lactoferrin in stimulated saliva (Rudney 1989; Rudney and Smith 1985). Although the underlying mechanisms remain elusive, our results suggest that a delicate balance existed between salivary innate and adaptive immune responses may be affected by different physiological conditions.

The cortisol levels measured during the training period and competition weeks were significantly higher than that measured at the fourth week of recovery. Studies revealed that the cortisol levels are increased in response to physical stress (Duclos et al. 2003). Moreover, Kindermann et al. (1982) have highlighted the fact that the hormonal response to exercise was different depending on the type and extent of the exercise (Kindermann et al. 1982). Therefore, the elevated cortisol secretion rates and absolute concentrations during the training and competition period may be caused by the physical stress of exercise. Although the physiological mechanisms underlying the decline in sIgA are still unclear, it is possible that the immune response was influenced by increased secretion of cortisol (Hucklebridge et al. 1998; Fleshner 2000; Li 2007; Wira and Rossoll 1991). An inverse correlation that existed between the secretion rates of sIgA and cortisol was measured in this study. Our results are in good agreement with those of Hucklebridge et al. (1998) and Fleshner (2000), who reported an inverse correlation existed between salivary cortisol and sIgA levels (Hucklebridge et al. 1998; Fleshner 2000). Moreover, Wira and Rossoll (1991) demonstrated that sIgA levels were significantly decreased due to a redistribution of polymeric IgA from mucosa to the circulation controlled by glucocorticoids (Wira and Rossoll 1991). However, this inverse correlation is not always observed. Previous studies reported that no significant correlation was found between sIgA and cortisol levels (Cieslak et al. 2003; Kugler et al. 1992; McDowell et al. 1992; Tiollier et al. 2005). Therefore, the relationship that existed between the secretion of sIgA and cortisol may be affected by multiple factors, such as intensity of stress, duration of training, types of exercise, individual differences between athletes and others. Additional studies and sophisticated experimental designs are needed to explicate the relationship between the secretion of sIgA and cortisol.

Our data show that the secretion rates and absolute concentrations of sIgA and lactoferrin measured at the beginning of the study (T4) were significantly lower than those measured at R4. In addition, the secretion rates and absolute concentrations of cortisol measured at T4 were significantly higher than those measured at R4. The saliva samples were collected 2 days after the start of the intense regular training. In addition, regular training was initiated 2 months before T4. Therefore, it indicates that the accumulative effects of long-term, regular training and/or short-term intense training lead to the stimulation of stress response and the suppression of mucosal immunity.

Conclusions

In summary, this study provides evidence that the secretion rates and absolute concentrations of sIgA and lactoferrin of basketball players were significantly reduced during the training and competition period in the season. A positive correlation that existed between secretion rates of sIgA and lactoferrin was revealed. In addition, secretion rates and absolute concentrations of cortisol were significantly increased during the intensive training and competition period. Taken together, these data indicated that intensive training and competition have adverse effects on the mucosal immunity in basketball players and further demonstrated an inverse correlation that existed between secretion rates of cortisol and sIgA during intense training

and competition period. The results of the current study may provide valuable insights and information for future study on basketball players during the season.

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Conflict of interest None.

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