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Research article

ALTERATION OF IMMUNE FUNCTION IN WOMEN COLLEGIATE SOCCER PLAYERS AND COLLEGE STUDENTS

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ABSTRACT
The purpose of this study was to monitor the stress-induced alteration in concentrations of salivary immunoglobulin (S-IgA) and cortisol and the incidence of upper respiratory tract infections (URTI) over the course of a 9-week competitive season in college student-athletes and college students. The subjects consisted of 14 NCAA Division III collegiate female soccer athletes (19.8 ± 1.0 years, mean ± SD) and 14 female college students (22.5 ± 2.6 years). Salivary samples were collected for 9 weeks during a competitive soccer season. S-IgA and cortisol concentrations were determined by enzyme linked immunosorbent assay (ELISA). A training and performance questionnaire was given to the subjects every week, to record the subjects' session rating of perceived exertion (RPE) for all the training, load, monotony and strain, as well as any injuries or illnesses experienced. The between groups ANOVA procedure for repeated measures showed no changes in salivary concentrations of IgA and cortisol. Chi-square analysis showed that during the 9-week training season injury and illness occurred at a higher rate among the soccer players. There was a significant difference at baseline between soccer and control S-IgA levels ($p \leq 0.05$). Decreased levels of S-IgA and increases in the indices of training (load, strain and monotony) were associated with an increase in the incidence of illness during the 9-week competitive soccer season.

KEY WORDS: Training, football, endocrine, illness.

INTRODUCTION

Moderate, regular physical training is generally considered to be associated with improved health, including decreased blood pressure and body weight, improved glucose tolerance, and possibly a decreased sensitivity to upper respiratory tract infection (URTI) (Mackinnon, 1997). Among elite athletes and their coaches, a common perception is that heavy exertion lowers immune resistance and is the predisposing factor to URTIs. Recent epidemiological evidence is consistent with this perception (Mackinnon, 1997). Recent studies have focused on the effects of exercise on immune parameters in order to better understand mechanisms by which exercise training may influence resistance to infection (Tomasi et al., 1982; Mackinnon 1997; Gleeson and Pyne, 2000). Intense exercise has been shown to transiently alter and/or suppress a number of immune parameters including the number of circulating leukocytes, plasma cytokine concentrations, salivary immunoglobin A (S-IgA) secretion rate, and neutrophil and macrophage phagocytic activity (Mackinnon, 1997). Studies show that there is a positive correlation between exercise workload and URTIs (Tomasi et al., 1982; Gleeson and Pyne, 2000).

Moderate exercise may stimulate the immune system but hard training actually suppresses the immune system, thus increasing the risk of infection

(Shephard and Shek, 1993). Moderate exercise may decrease the risk of acquiring an URTI, but too much exercise may increase the risk. Moderate exercise in sedentary subjects with naturally acquired URTI does not appear to alter the overall sensitivity and duration of the illness (Weidner and Schurr, 2003). Illness and/or injury occur when the physical demands outweigh the ability of the body to recover between training sessions and competitions (Anderson et al., 2003). Observations of the training patterns and incidence of illness and injury during a women's collegiate basketball season have suggested that there was no correlation between total weekly training loads and illness rates (Anderson et al., 2003). However, no measures of immune or hormonal markers were made in that study (Anderson et al., 2003).

The risk of URTI appears to be especially high during the 1- or 2-week period following marathon-type race events and 1-9 hour following prolonged endurance exercise, when the host defense mechanism is decreased and the risk of URTI is elevated (Neiman et al., 1990). Training at an intense level over many years can result in a chronic suppression of salivary immunoglobulin levels (Gleeson and Pyne, 2000). The S-IgA levels show an inverse correlation with the number of URTIs, which indicates that the measurement of S-IgA levels over a training season, may be predictive for athletes at risk for infection (Gleeson et al., 1999). However, very few studies have investigated S- IgA changes over the course of a competitive season.

Current knowledge suggests that heavy acute and/or chronic exercise is associated with an increased risk of URTIs. Some studies have suggested that the incidence of infections, which are often thought to be a marker of early stages of overtraining syndrome, were related to excursions above individually identifiable thresholds of training strain (Foster, 1998). The purpose of this study was to investigate the stress-induced alterations in immune function and stress hormones in college students and college student-athletes.

METHODS

Experimental design

This investigation utilized NCAA Division III collegiate female soccer athletes. The control subjects were college students not involved in competitive athletics. Using a longitudinal study design, the soccer and control groups were monitored throughout 9 weeks of a semester, which corresponded to the competitive season for the soccer players. At the beginning of every week, for 9 continuous weeks, the subjects provided salivary samples for measurement of S-IgA and cortisol concentrations. A training and performance questionnaire was given to the subjects at the beginning of every week's practice session, which was utilized to record the subjects' session rating of perceived exertion (RPE), as well as any injuries or illness suffered.

Subjects

Fourteen female soccer players volunteered for this study (age 19.8 ± 1.0 years, mean ± SD, weight 65.9 ± 7.3 kg). All participants had previous clearance from a physician to participate in intercollegiate athletics and all went through an orthopedic screening by a certified athletic trainer to determine their health status and musculoskeletal injury history. The control group consisted of 14 healthy, physically active female college students (age 22.5 ± 2.6 years, body weight 62.2 ± 7.3 kg). The control subjects were not involved in competitive athletics but several of them were involved in regular exercise programs. Subjects did not adjust their diets or lifestyles significantly during the course of the season. Informed consent was provided by all participants, in accordance with the guidelines of the University of Wisconsin-La Crosse Institutional Review Board for the Protection of Human Subjects.

Saliva Collection

At the beginning of each week, unstimulated saliva samples were collected over a 1-minute period into pre-weighed plastic tubes. The subjects were asked to not eat immediately prior to providing their saliva sample and to maintain their normal dietary habits over the course of the study period. The samples were collected at the same time of the day to control for diurnal variations. They were measured for volume, immediately frozen and stored at –80 °C until analysis.

S-IgA and cortisol measures

S-IgA concentrations were determined in duplicate by an enzyme linked immunosorbent assay (ELISA) (Salimetrics, State College, PA). S-IgA secretion rate was calculated by multiplying absolute S-IgA concentration ($\mu g \cdot mL^{-1}$) by saliva flow rate ($mL \cdot min^{-1}$). Saliva flow rate was calculated by dividing the total volume of saliva obtained in each sample (mL) by the time taken to produce the saliva sample (minutes). Salivary cortisol concentrations were determined in duplicate by ELISA (Diagnostic System Laboratories, Webster, TX). Assay plates were read using an *Opsys* MR™ Microplate Reader (Dynex Technologies, Chantilly, USA). For S-IgA, intra-assay variance was 8.2 % and the sensitivity of the assay was 2.5 $\mu g \cdot mL^{-1}$. For cortisol intra-assay variance was 7.6 % and the sensitivity of the assay was 0.011 $\mu g \cdot dL^{-1}$.

Training questionnaire

The subjects in both the soccer and control groups were given a training and performance questionnaire at the beginning of each week. The questionnaire was given at the beginning of the week for recording the RPEs for that week's practices/exercise sessions and for that week's illnesses and injuries. The questionnaire was comprised of questions about the subjects training and exercise patterns as well as any injuries or illness suffered. Questions included lists of problems that make athletes stop or reduce their training because of illness (colds, flu, viruses, asthma, upsets, infections, etc.), training injuries (sprains, fractures, pain in muscles, cramps, etc.), and other problems (accidents, family, travel or work problems, bad weather, exams, worry, apathy, loss of interest, etc). The soccer and control subjects were asked to provide information about the exercise or training that they had done that week. For the soccer group this included matches, practice sessions, and individual conditioning sessions. For the control subjects this included any exercise sessions they did. The subjects recorded the duration of each session and outlined what type of exercise they did. They also provided a rating of how hard each session was using the session RPE scale (Figure 1). Briefly, the subject used the scale approximately 30 minutes after completion of any workout, match, or training session, and rated " how hard was the workout?" from the scale, in which, zero is resting and ten is maximal effort (Foster et al., 2001).

Rating = Descriptor
0 = Rest
1 = Very, Very Easy
2 = Easy
3 = Moderate
4 = Somewhat Hard
5 = Hard
6 = *
7 = Very Hard
8 = *
9 = *
10 = Maximal

Figure 1. Rating for Perceived Exertion Scale.
Note: Briefly, the athlete was shown the scale approximately 30 minutes after completion of the workout, match or training session, and asked, "How hard was the workout?"

The goal of the session RPE is to encourage the athlete to view the training session globally and to simplify the myriad of exercise intensity cues during the exercise bout. This allowed for evaluation of trends in training, injury and illness in relation to the session RPE and the global intensity of the exercise session (Foster, 1998). It has been demonstrated to be a valid measure of both aerobic and anaerobic exercise (Foster et al., 2001; Gabbett, 2003). From the session RPE method, the accessory indices of training, monotony and strain were calculated, potentially providing an index of the training outcomes (Foster, 1998). Monotony was defined as the variability of practices for the entire season, while strain was defined as the overall stress demanded on the athlete for a period of a week (Foster, 1998). Monotony was calculated by dividing the mean daily load over each week by the standard deviation of load. Strain was calculated by multiplying monotony by the weekly load (Foster, 1998).

Injury

An injury was defined as a circumstance in which the athlete received an evaluation from the team's student athletic trainer and required limiting their practice for at least 1 day. Each injury was counted as single injury, whether seen for 2 days or 2 weeks; consequently, if the athlete had two separate injuries, they were counted as two. Injury for the college students was defined as a circumstance during which the subject felt that she could not or was unable to perform any exercise.

Illness

An illness was defined as a circumstance in which the subject felt that they were limited or unable to perform the training or the exercise session (eg. flu, cold, virus, etc.). Research has shown that athletes, as well as the general population (control groups), are quite able to correctly diagnose symptoms of URTI such as running nose, sore throat, and cough in combination with fatigue, headache and fever (Cohen et al., 1991). Each individual's illness was recorded on the questionnaire in the same manner as injury.

The correspondence between spikes in the indices of training (load, monotony and strain), decreases in S-IgA and subsequent (within 7 days) illness was noted, and individual thresholds that allowed for optimal explanation of illnesses were computed. From this we attempted to define the percentage of illnesses that could be explained by each index of training.

Statistical analysis

S-IgA concentration, cortisol concentration, saliva flow rate, and S-IgA secretion rate were analyzed separately by between groups repeated measures ANOVA. Pearson's product-moment correlation was performed independently for both the groups to determine the strength of the relationship between S-

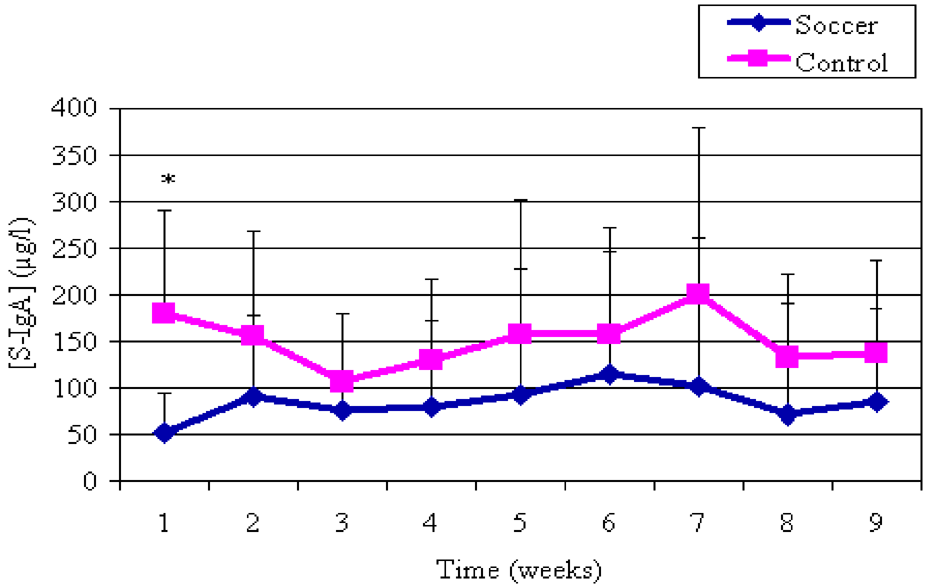

Figure 2. Changes in S-IgA concentration during the course of a 9-week competitive soccer season.

IgA and cortisol concentrations with mean load, standard deviation of load, weekly load, monotony, strain, and the number of reported days with injury or illness. A chi square analysis was performed to determine the frequency of injury and illness in the soccer players and college students during the course of study. Effect size was calculated as the difference between the means divided by the SD of the two samples. A $p \leq 0.05$ was accepted as statistically significant.

RESULTS

There were no significant differences in age or bodyweight between the soccer group and the control group at baseline

Salivary IgA

The weekly variations of S-IgA levels over the 9-week competitive season for the soccer group in contrast to that of the control group are shown in Figure 2. The repeated measures ANOVA showed no changes in salivary concentrations of IgA or secretion rate over the course of the training season. There was a significant difference at baseline between the soccer and control groups S-IgA levels ($p \leq 0.05$), with the soccer group having lower levels than the control group (Effect size = 1.1).

Cortisol

ANOVA for repeated measures showed no changes in salivary concentrations of cortisol between the soccer and control groups over the course of the training season. The pattern of variation in cortisol concentration of soccer and control groups over the 9- weeks is shown in Figure 3. There were no significant differences over the course of 9-week competitive season.

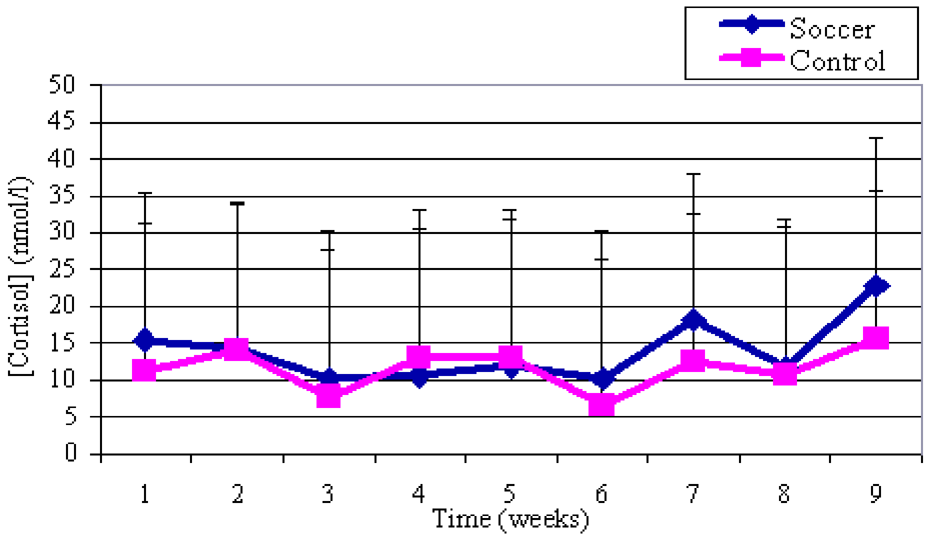

Figure 3. Changes in cortisol concentration during the course of a 9-week competitive soccer season.

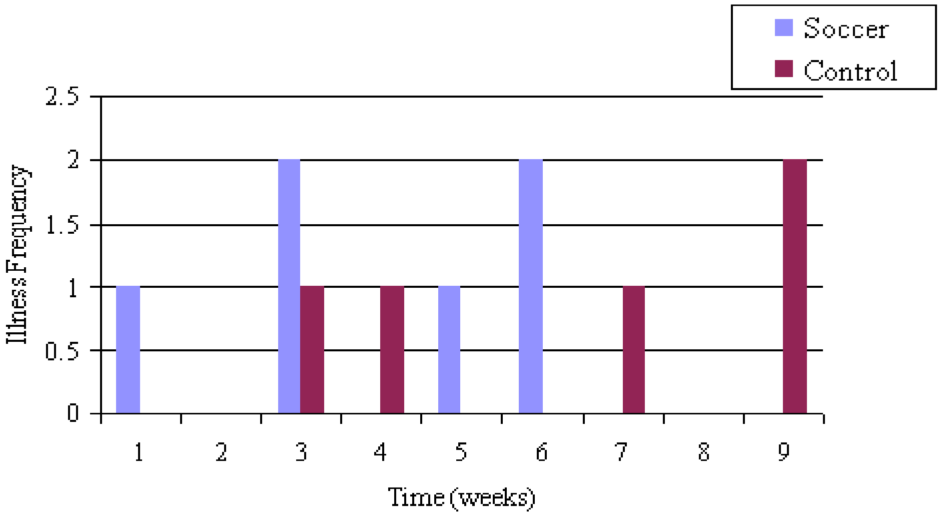

Figure 4. Frequency of injuries during the course of a 9-week competitive soccer season.

Injury

The total number of days of injury and the difference in the injury pattern among soccer and control groups is shown in Figure 4. During the first 3 weeks of the season the number of injuries among the soccer group was high in comparison with control group. There was a crossover during week 4, after which the number of injuries experienced by the control group was more than the soccer group. But the occurrence of injuries among the soccer group was at a significantly higher rate during the 9-week competitive season.

Illness

The total number of days of illness and the difference in the pattern of illness among the soccer and control groups is shown in Figure 5. During the course of the study, illness occurred at a significantly higher rate among the soccer group. No illness was recorded during the weeks 7, 8 and 9 of the season, during which the training load was reduced.

Monotony and Strain

Monotony and strain for the soccer and control groups are shown in Figures 6 and 7. In both the soccer and control groups, strain followed a pattern very similar to monotony, with slight changes due to different training loads. The training load for the soccer group was higher when compared to the control group (Figure 8).

With our approach of correlating the incidence of illnesses with indices of training, 55% of illnesses could be explained by a preceding spike in training load. In addition, 64% of illnesses could be explained by a preceding spike in strain and monotony. After correlating the incidence of illnesses with a decrease in S-IgA, 82% of illnesses could be explained by a preceding decrease in S-IgA. Using an increase in cortisol levels, 55% of the

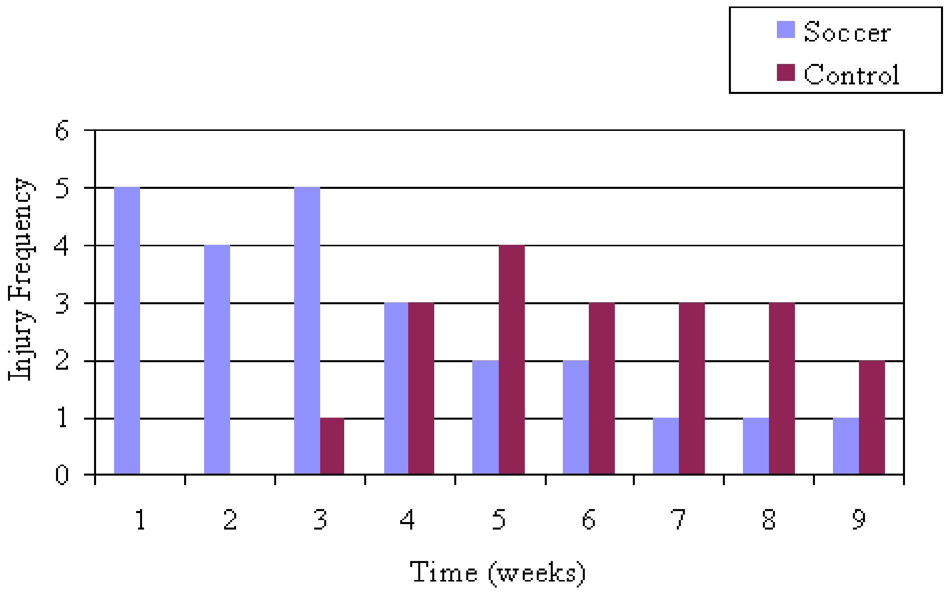

Figure 5. Frequency of illness during the course of a 9-week competitive soccer season.

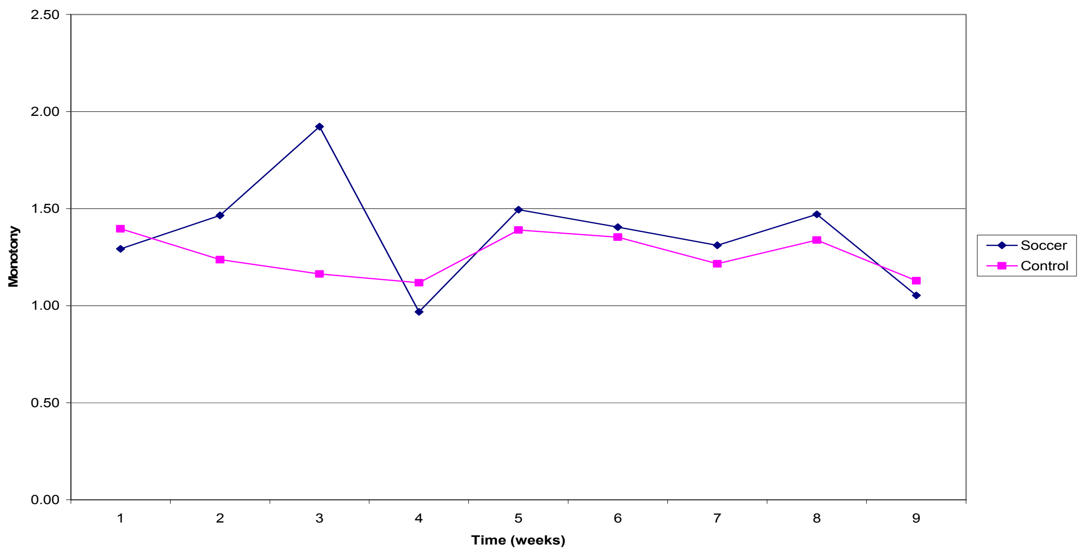

Figure 6. Changes in monotony during the course of a 9-week competitive soccer season.

illnesses could be explained by a preceding decrease in S-IgA and increase in salivary cortisol.

Correlations

Significant correlations were found in the soccer group between S-IgA and monotony ($r = 0.75$) during week 4 ($p \leq 0.05$). Correlations were also found between cortisol concentration and injury ($r = 0.73$) during week 5 ($p \leq 0.05$). Among the control group, significant correlations were found between cortisol and strain ($r = 0.72$) during week 1 ($p \leq 0.05$). Week 4 showed a correlation between cortisol and stress ($r = 0.72$). Week five showed correlation between cortisol and injury ($r = 0.79$) ($p \leq 0.05$). During week 9 a significant correlation was found between cortisol and mean load ($r = 0.73$), cortisol and weekly load ($r = 0.73$), cortisol and strain ($r = 0.72$) ($p \leq 0.05$).

DISCUSION

Athletes experience a variety of injuries and illnesses throughout a competitive season, impacting the performance of a team and the success of a coach. Research has been conducted on the S-IgA levels in elite athletes (McDowell et al., 1992; Tomasi et al., 1982; Pyne and Gleeson, 1998; Gleeson et al., 1999; Kraemer et al., 2004), but there is very limited research on occurrence of injury and illness in college student-athletes and non-athletic college students. The purpose of this study was to compare the stress-induced alterations in S-IgA and cortisol concentrations in female college students who were athletes (soccer group) and college students who were not involved in competitive athletics (control group). The major finding of this study was that there was no significant difference in

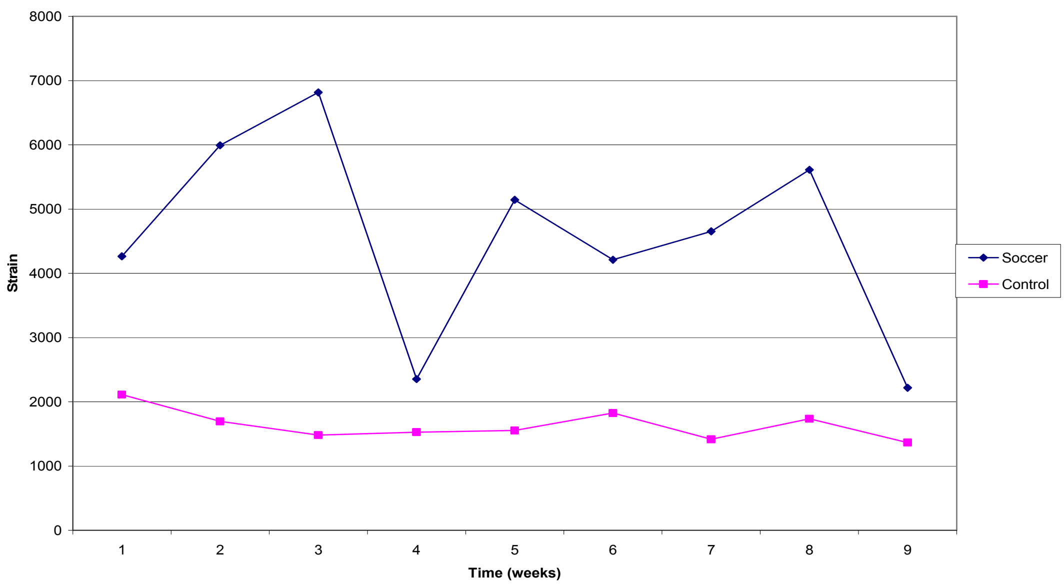

Figure 7. Changes in strain during the course of a 9-week competitive soccer season.

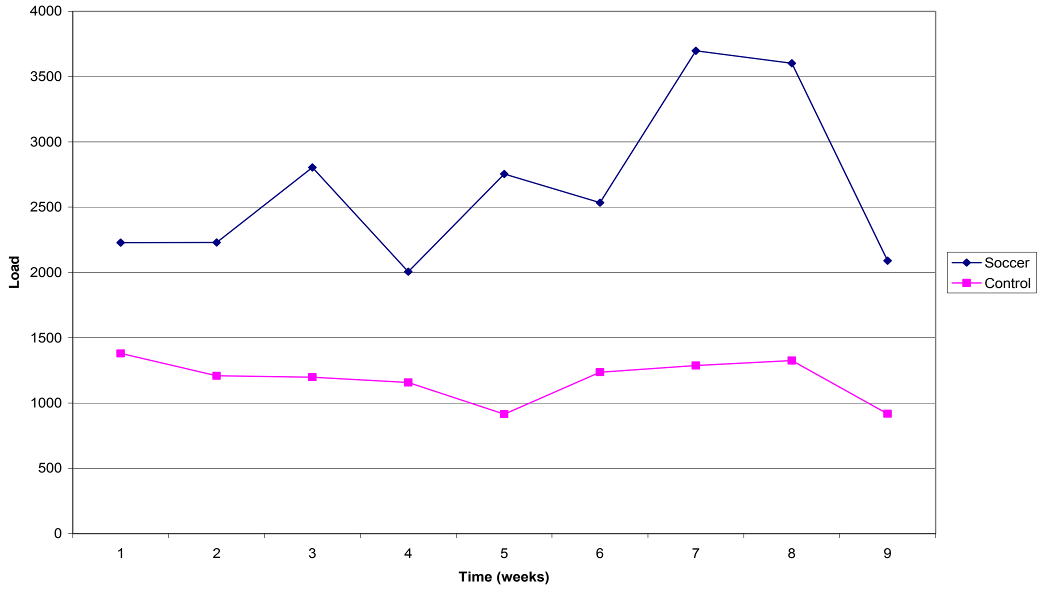

Figure 8. Changes in load during the course of a 9-week competitive soccer season.

S-IgA and cortisol concentrations in the soccer and control groups in relation to URTI during a 9-week competitive season. The frequency of injury and illness in the soccer group was significantly greater than the control group.

This study demonstrated that there was no significant relationship between the S-IgA concentrations and the incidence of URTIs among the soccer and control groups during the course of the study. At the beginning of the study there was a significant difference in the S-IgA levels between the soccer and control groups, with the soccer group having lower levels. This could be a result of the soccer players being in a state of overreaching or overtraining following their pre-season training. However, we do not have any quantitative training data from the pre-season to confirm this. During week 3 a decrease in the S-IgA levels among the soccer and control groups was seen (Figure 2). This drop in S-IgA levels in soccer group could be because the first 2 weeks of a season are frequently the most difficult and physically demanding practices of the season. The athletes are working towards reaching their "optimal" level of training. However, it did appear that the soccer group had lower resting S-IgA levels over the course of the season when compared to the control group. There is a well known association between either chronic or acute heavy exertion, and an increased incidence of infection (Foster, 1998). This may relate to a modest immunosupression during periods of heavy training (or high levels of other stressors) that renders the individual more susceptible to infection by pathogens in the environment. We did observe that 82% of illnesses could be explained by a preceding decrease in S-IgA. Previous research has shown a quantitative relationship between various indices of training and the presence of negative adaptations to training (Foster, 1998). This is supported by our data which demonstrated that increased amounts of load, strain and monotony were associated with the incidence of illness.

The results showed that there was no significant change between the soccer and control groups for cortisol concentrations by the end of the 9-week training season. Kraemer and colleagues (2004) showed that intense training prior to the start of the season, combined with continued high intensity training during both practice and competition, contribute to chronically elevated cortisol concentrations. This study showed that there was large intersubject variability in cortisol concentration over the course of the season. Elevated levels of cortisol could be because of the extreme stressors experienced during the competitive season and academic semester. Elevated cortisol concentrations lead to increased binding at the glucocorticoid receptor, which results in reduced protein synthesis and concomitant losses in muscular force and functional performance (Kraemer et al., 2004). Thus the measurement of cortisol concentration has been suggested to be a possible endocrinological marker of physiological stress associated with exercise. Elevation of cortisol concentration could be the result of stress, diet, inflammation, or high intensity exercise (Hoffman et al., 2002).

Gottschall (1999) noted that an increase in illness paralleled an increase in the training patterns during a men's basketball season. It was concluded that higher training patterns increase the potential for illness. This study revealed a significant correlation between S-IgA and monotony (r = 0.75) in the soccer group during week 4. The majority of significant correlations were for cortisol and the various indices of training. This further highlights the important role of cortisol as a possible marker of physiological stress with exercise. However, there was no consistent pattern throughout the study. IgA is the predominant immunoglobulin in mucosal secretions providing the body's main defense against pathogens. One such pathogen is the rhinovirus, which if allowed to replicate, can cause URTIs. However, there are numerous other factors that contribute to actual disease pathology (Fahlman et al., 2001). Whether one gets sick with cold after a sufficient amount of virus has entered the body depends on many factors that affect the immune system other than just physical activity and nutrition. Smoking, alcohol consumption, mental stress, and lack of sleep have all been associated with the impaired immune function, and an increased risk of infection (Neiman, 2000). Gleeson et al. (1999) determined the mean pre-training S-IgA concentration in elite swimmers. It was shown that the lower the initial S-IgA, the higher the incidence of infection. It was observed in this study that, though the frequency of injury and illness in the soccer group was more than the control group there was no significant relationship between S-IgA concentrations with illness or injury. When an individual takes part in physical activity, there is always an inherent risk of injury. Coaches, athletic trainers, and strength and conditioning professionals work not only to decrease the number of injuries that occur, but also to try to prevent injury occurrence. They need to be aware of the trends that follow when injuries are most likely to occur. Knowledge of injury trends could be useful for helping to implement the proper intensity of practices during the season (Anderson et al., 2003).

Foster (1998) demonstrated that there was link between the load of practices and the strain and monotony of practices. Monotony was lower when practices varied considerably in volume and intensity (6). During week 4 of the 9-week training season the training load of the soccer group was low. Therefore, the lower monotony values indicate variability in practice for the soccer group. Practices need to be adjusted according to the number of games played during the training season. In both the soccer and control groups, strain and monotony followed the same pattern. High levels of monotony did not exist during this particular soccer season. Gabbett (2003) showed that the incidence of training injuries in rugby league players was highly correlated with intensity, duration, and load of training. Although we did not demonstrate high correlations between training indices and illness, we did observe that 54-63% of the illnesses were associated with a preceding spike in training load, monotony or strain.

Some limitations of this study need to be addressed. Experience and daily interaction with athletes have clearly shown that they are quite aware of the history of past infections including training days lost due to URTI. In addition most of the investigated athletes had training logs helping them to answer the questions correctly. Another aspect that could be criticized is that the diagnosis of URTI was self-reported and not confirmed by a physician. The athletes as well as the college students appeared to be able to correctly diagnose symptoms of URTI such as running nose, sore throat, and cough in combination with fatigue, headache, and fever.

Previous research has suggested that the menstrual cycle may have an impact of the cortisol profile (Kirschbaum, et al., 1999). It has been observed that women in the follicular phase or taking oral contraceptives had blunted free cortisol responses (Kirschbaum et al., 1999). A limitation of the present study was that we did not control for the phase of the menstrual cycle that the subjects were in. However there is evidence that suggests that morning cortisol response is influenced by the awakening time but not by menstrual cycle phase (Kudielka and Kirschbaum, 2003) and not all studies have shown cortisol responses to acute exercise to vary across menstrual cycle phase (Galliven et al., 1997).

CONCLUSIONS

During the course of a competitive season collegiate soccer players are exposed to a number of physical and psychological stressors from practice, conditioning, and competition. The ability of the players to recover following such activities can ultimately affect the quality of the performance for ensuing physical activity. This study showed that there were no significant stress-induced alterations in the S-IgA and cortisol concentrations in female college students who are athletes (soccer group) and college students who are not athletes (control group). There was no significant difference in S-IgA and cortisol concentrations in soccer and control groups in relation to URTI during the course of a 9-week competitive season. It did appear that the levels of S-IgA were suppressed in the soccer group throughout the course of the season. The frequency of injury and illness in the soccer group was significantly greater than the control group.

Injury and illness were not significantly correlated to S-IgA in this particular study. However, we did observe a relationship with a decrease in S-IgA and the occurrence of illness. In addition, there appears to be a relationship between indices of training such as strain and monotony with incidence of illness. This is in agreement with previous research with athletes (Foster, 1998). It is important to monitor players prior to preseason conditioning to gain a more complete understanding of the changes that occur during the course of a competitive soccer season. As most sport seasons are now extending to year-round training, a year long study is needed to fully document changes in collegiate soccer players compared to those of the college students.

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KEY POINTS

- There was a significant difference at baseline between soccer and control S-IgA levels
- Eighty-two percent of illnesses could be explained by a preceding decrease in S-IgA.
- Increases in the indices of training (load, strain and monotony) were associated with an increase in the incidence of illness

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