IMMUNE RESPONSES ACROSS A WOMEN'S SOCCER SEASON: AN EXPLORATORY STUDY

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ABSTRACT

BRADY J. WRIGHT: Immune Responses Across a Women's Soccer Season: An Exploratory Study
(Under the direction of Dr. Robert McMurray)

It is important for coaches and strength training professionals to know at what times during the season, athletes are most susceptible to infection. As a result, the immune system was tracked every two weeks throughout a women's soccer season to see if a relationship existed between C-reactive protein (CRP), the incidence of infection and the level of soccer activity. CRP levels were paired with a questionnaire regarding soccer, physical activity, total exercise levels and self-reported upper respiratory tract infections. The results of the study indicate that soccer levels were highest at the beginning of the season when physical conditioning took place. The two weeks following the high levels of soccer activity demonstrated the largest increase in the number of infections reported. However, CRP exhibited no trend with either the incidence of infection or soccer/exercise levels. The early stages of the season may leave athletes most susceptible to infection.

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CHAPTER I

INTRODUCTION

The literature as summarized by Mackinnon (1999) has shown that a relationship exists between exercise and the immune system. In general, the immune system is divided into two systems; innate and adaptive immunity. Innate immunity is the first line of defense in the immune system and is important in reducing the susceptibility to infections. This is activated within the first 10-12 hours of bacterial recognition, while adaptive immunity may take up to several days to respond to pathogens or infection (Mackinnon, 1999). Innate immunity consists of nonspecific defense mechanisms including monocytes, macrophages, natural killer (NK) cells, acute phase proteins (such as C-reactive protein), and several pro-inflammatory cytokines. Adaptive immunity consists of specialized lymphocytes such as B and T cells. Innate immunity plays a vital role in reducing the susceptibility to infections until adaptive immunity can be fully activated.

In response to trauma, infection or exercise, there is an inflammatory response which occurs at the site of injury or infection. Increased movement of plasma and leukocytes to the location is a key step in this process. Additionally, an increase in macrophages release pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α). TNF-α combines with interleukin-6 (IL-6) to induce the acute phase response (Mackinnon, 1999). The acute phase response can last for several days and may be responsible for promoting clearance of damaged tissue, prompting the muscle for growth and repair and recognizing foreign

pathogens (Nieman, 1993). These pro-inflammatory cytokines stimulate the liver to release C-reactive protein (CRP). CRP is a key acute phase protein and is released during bacterial infection, tissue injury or prolonged intense exercise (Taylor et al. 1987). CRP has anti-body qualities and can bind to bacteria surfaces. CRP also stimulates phagocytosis and activates the complement system, further eliminating bacteria (Liesen et al. 1977). CRP is an important innate immune marker and is critical to the inflammatory response of the body.

Exercise & Immune Responses

Several studies have followed this immune parameter during exercise and the results have been varied. CRP levels have shown to decline with chronic exercise training in runners (Mattusch et al. 2000; Kasapis & Thompson, 2005) suggesting a reduced acute phase response. In contrast, Taylor et al. (1987) showed that an acute bout of prolonged aerobic exercise may increase CRP levels by up to 300%. This may be attributed to the increased inflammation and muscle micro-trauma elicited by the exercise. In general, research on exercise and CRP has found that an acute bout causes an increased inflammatory response, elevating CRP levels, while chronic exercise training tends to have an anti-inflammatory effect, lowering CRP levels. Resting values of CRP have been documented in a number of athletes previously (Dufaux et al. 1984). Although not significantly different, CRP levels of soccer players were higher than any other athletes or their controls.

There is a growing body of literature which suggests that a paradoxical relationship exists between exercise and the immune system. Athletes, especially at the collegiate/elite level are more susceptible to a variety of minor illnesses, including upper respiratory tract infections (URTI) such as the common cold or flu (Gleeson et al. 1995; Mackinnon, 2000; Nieman, 2000). It is also generally accepted that moderate levels of exercise may act to

improve immune function and reduce susceptibility to infections (Nieman, 1993; Stewart et al. 2007). In response to the increased incidence of URTI in the athletic population, a growing number of studies have examined immune function with chronic exercise (Gleeson et al. 1995; Verde et al. 1992; Mattusch et al. 2000; Stewart et al. 2007; Kasapis & Thompson, 2005). The majority of studies on this topic have focused on prolonged aerobic activities such as swimming, cycling or running. Although some literature exists, there has been considerably less research conducted on team sports.

Immunity & Soccer

Soccer is a game utilizing significant aerobic and anaerobic contributions characterized by multiple, intermittent sprints of high intensity over a 90 minute period (Kraemer et al. 2004). Only a few studies have examined the immune system in response to either a single soccer game or short-term soccer training camps (Ispirlidis et al. 2008; Malm et al. 2003; Malm et al. 2004). Malm et al. (2003, 2004) tracked the immune system after several days of consecutive soccer games (Malm et al. 2004) and after five days of an intense soccer training camp (Malm et al. 2003). The investigators examined lymphocyte changes and URTI incidence over the course of the course of the soccer camp. It was found that lymphocytes were suppressed post-camp, while the number of URTI significantly increased post-camp. It has also been shown that a single game of soccer can induce short-term muscle damage and an inflammatory response consisting of an increase in cytokines, CRP and leukocytes which is similar to endurance activities (Ispirlidis et al. 2008). The large amount of eccentric muscle activity combined with a high degree of physical contact from other players could explain why this occurs. These inflammatory responses have the potential to alter the immune system and increase susceptibility to infection (Nieman, 2000). Our present

state of knowledge does not provide adequate information across an entire soccer season to see the effects of games and training on the immune system over a prolonged period of time.

Few studies have examined the immune system across an entire soccer season (Bury et al. 1998; Rebelo et al. 1998; Fallon et al. 2001), with Fallon et al being the only study examining females. Blood samples were taken pre-season, mid-season and post-season with up to six months between samples. None of these studies have profiled the subjects' immune systems on multiple occasions per month to see when immune function was altered. While these studies found that certain immune cells were altered across the season, the results were contrasting. Bury et al. (1998) found a decreased neutrophil count at the end of the season, suggesting suppressed immunity. Alternatively, Rebelo et al. (1998) found increased leukocyte and lymphocyte numbers at the end of the season, which they suggested might be a possible improved immune function. The most recent study (Fallon et al. 2001), found that the acute phase response was reduced following a soccer season suggesting an anti-inflammatory effect of chronic exercise. The major limitation of these studies is the length of time between each blood sample. There is a need to track immune functions in shorter time increments to better understand the impact that a sports season may have on the immune system. Additionally, the incidence of URTI was documented in only one of the three studies (Bury et al. 1998). No investigation into the relationship between URTI and immune parameters was reported. Further research into the immune system and the incidence of URTI across a sports season is needed.

Summary

Knowledge of when and how immune system function alters during training can lead to improved training programs to keep athletes healthier. No study has closely profiled

collegiate athlete's immune system across a sports season or closely followed CRP levels in females. Resting CRP levels were analyzed with information regarding training volume, intensity and number of URTIs reported. Additionally, little research has focused on the relationship between CRP levels and URTIs in response to a season of exercise training. The purpose of the study was to monitor CRP levels across the entire sports season to determine if training volume and intensity influence these inflammatory markers and any incidence of URTI in females. Both immune parameters were analyzed along with information obtained from a questionnaire documenting training volume, intensity and number of URTIs. One outcome of the study was to explore if there are periods during the season athletes are more susceptible to infection. This study was design to help aid coaches in development of training schedules to reduce potential illness.

Research Questions

The specific research questions of this study were based around the soccer season which began in early September and ended in the middle of November.

1. Do resting CRP levels change throughout the soccer season?

2. Is there a relationship between number of infections and resting CRP levels throughout the soccer season?

3. Is there a relationship between the amount of physical activity and URTI on CRP levels throughout the soccer season?

Hypotheses

Since this is an exploratory study, specific hypotheses were not tested. For the purposes of this study, there was only one hypothesis:

There will be no relationship between CRP, soccer exposure and number of URTIs.

Operational Definitions

Training volume: The amount of time (in minutes) spent at soccer training

Training intensity: A 1-10 scale similar to a rating of perceived exertion scale (RPE). 1 is the easiest, while 10 is the most difficult

Soccer exposure: A calculated number used to determine the soccer load (training volume, training intensity and actual games)

Soccer exposure = (training intensity x training time) + (game intensity x game time)

Physical activity exposure = Any physical activity undertaken by the subject that is *not* scheduled soccer training or games

Physical activity exposure = (exercise intensity x exercise time)

Total exercise exposure = Soccer exposure + physical activity exposure

Upper Respiratory Tract Infection (URTI) = A common infection such as the cold or flu which is self diagnosed by the subject

Assumptions

There was an assumption that menstrual stage did not influence the immune system. It was also assumed that the subjects answered the questionnaire as truthfully as possible. Lastly, it was assumed that any medication used by subjects, remained constant throughout the soccer season.

Limitations

The study was a 12 week longitudinal protocol. The longevity of the study allowed for confounding variables outside the control of the principal investigator to potentially impact results. Such factors included: additional exercise activities undertaken by the subjects outside of soccer practice, fluctuating levels of stress due to the rigors of academics and irregular sleep patterns. The longevity of the study also meant that a change in weather would occur. As the study progressed, the temperature decreased which may have an impact on the incidences of URTI observed.

Additionally, the questionnaire administered throughout the study had not been proven to be valid. Lastly, the incidences of URTI were self-documented with no proper diagnosis by a medical physician required.

Delimitations

Only full-time members from the UNC women's club soccer team were used in the study. Women were between the ages of 18-30 years old. All women were in generally good health with no chronic inflammatory diseases. Pregnant women were excluded from the study. Pregnancy would hinder their ability to train and play soccer with the team and would therefore impact the results of the study. Subjects using chronic anti-inflammatory medication were excluded from participating in the study.

All data collection was conducted in the morning at approximately the same time each testing session. Each testing window occurred between 8:30-10:30 am. All blood draws were conducted on the same day of the week for each subject. Subjects rested in a supine position for 15 minutes prior to any of the blood draws to account for the effect of posture and to ensure measurement of resting values.

Significance

No study has profiled the immune system of athletes for an entire sports season or tracked CRP levels across a season. In addition to this, only one previous study on soccer and the immune system has used female subjects. The results of this study will add to the limited literature available on immune function across a sports season and in regards to the female population. Results from the study are relevant to athletes and coaches for the optimization of training regimes. This study attempted to help determine specifically when collegiate athletes are more susceptible to infection. Knowing when athletes' immune system is most vulnerable

will allow coaches to take the necessary precautions to limit URTIs. Increased knowledge in the area of exercise immunology will allow the athletes to stay healthier and thus perform better throughout the sports season.

CHAPTER II

LITERATURE REVIEW

The main focus of this chapter is innate immunity parameters, in particular; C-reactive protein (CRP) and its responses to chronic exercise. A brief summary of the entire immune system is also provided. Prolonged exercise and soccer are the predominant exercise related topics which are discussed throughout the chapter. The chapter also highlights areas in which a lack of research has hindered our understanding of the immune system and chronic exercise.

The Immune System

The immune system is a very complex system involving the integration of many different aspects of immunity. Generally, however, it is divided in two distinct systems: innate and adaptive immunity. Innate immunity is the first line of defense in the immune system and is important in the reducing the susceptibility to infections. This is activated within the first 10-12 hours of bacterial recognition. Adaptive immunity on the other hand, takes several days to become activated and primarily consists of specialized lymphocytes such as B and T cells. The adaptive responses can generate memory of previous exposure to certain pathogens (Mackinnon, 1999). This provides a more effective barrier to specific infection. Memory B and T cells are two lymphocytes which are predominant in the adaptive immune response and are critical in the elimination of foreign bacteria. It is important to

understand the role of adaptive immunity within the entire immune system, but the primary focus of this study is on innate immunity. Innate immunity is vital to reducing the susceptibility of infection due to the delayed nature of the adaptive immune system. The primary purpose of this system is to reduce the susceptibility to infection until adaptive immunity can be fully activated. Innate immunity consists of physical barriers, chemical barriers, immune cells and pro-inflammatory cytokines. Physical barriers include skin, mucus and the epithelial cell barrier (Mackinnon, 1999). Chemical barriers include the acute phase proteins, complement system and the pH of body fluids. Lastly, immune cells consist of monocytes, macrophages and natural killer (NK) cells.

The Role of CRP in Innate Immunity

CRP is a protein that is synthesized by the liver and found in the blood. It is one the major proteins released during the acute phase response (Du Clos, 2000). CRP is also one of the first responders to infection and is an important marker of both innate immunity and the inflammatory process. CRP is associated with a host of chronic diseases factors although this is beyond the scope of the present study.

In response to trauma, infection or exercise, there is an inflammatory response which occurs at the site of injury or infection. One of the first responses of inflammation is an increase in plasma and leukocytes to the location of trauma which helps to remove infection and repair damaged tissue (Burger & Dayer, 2002). At this point, several other immune cells become active, including macrophages and monocytes. An increase in macrophages can lead to the release of pro-inflammatory cytokines such as TNF-α. TNF-α has a myriad of functions, one of which is to combine with interleukin-6 (IL-6) to induce the acute phase response (Mackinnon, 1999) via the liver. The acute phase response can last for several days

and may be responsible for promoting clearance of damaged tissue, prompting the muscle for growth and repair, mobilization of neutrophils (Taylor et al.1987) and recognizing foreign pathogens (Nieman, 1993). The acute phase response is characterized by a release of glycoproteins. The most notable of these glycoproteins is CRP. CRP, like other acute phase reactants, is released during bacterial infection, tissue injury or prolonged intense exercise (Taylor et al. 1987). CRP performs several important functions in the body. CRP acts as a protective molecule which can identify certain pathogens (Du Clos, 2000). This occurs through the opsonization of bacteria and binding to bacteria surfaces. CRP also stimulates phagocytosis which is a complex process of eliminating foreign particles. In addition to this, CRP activates the complement system which is a group of 20 unrelated proteins involved in the destruction of bacteria (Liesen et al. 1977).

As highlighted, CRP is an important innate immune marker and is critical to the inflammatory response of the body. Throughout the remainder of this chapter this inflammatory marker will be discussed with relation to chronic exercise training and if applicable, across a sports season. Literature on soccer and the immune system will also be discussed in detail.

CRP and Chronic Exercise

Research on the inflammatory response to chronic physical activity levels has generally found an anti-inflammatory effect of exercise. This reduced inflammatory response is characterized by a suppressed acute phase response and lower CRP levels during resting conditions. Lower CRP level and reduced inflammation means that there is less activation of the immune system. As a result of this, lower levels of stress are placed on the body. Although not specifically pertinent to this study, lower CRP levels are associated with

reduced risk of several chronic diseases such as cardiovascular disease. Cross-sectional studies have highlighted the relationship between high levels of either physical activity or aerobic fitness and reduced CRP values (Church et al. 2002; Ford, 2002). Church et al. (2002) collected data on 722 men and found that an inverse relationship exists between CRP and cardiorespiratory fitness. The individuals with the lower levels of aerobic fitness had the highest CRP values. Individuals with the highest aerobic fitness levels were determined to have the lowest CRP level. A study by Ford (2002) looking at the Third National Health and Nutrition Examination Survey (NHANES III) data found that subjects who engaged in regular physical activity had decreased CRP levels compared with their sedentary counterparts. The study included 13,748 subjects and highlights the relationship between physical activity levels and CRP. The majority of studies that have focused on CRP levels with chronic exercise training (Lakka et al. 2005; Liesen et al. 1977; Mattusch et al. 2000; Stewart et al. 2007) agree that a reduction in CRP levels is evident following chronic training. This suggests an anti-inflammatory effect of chronic exercise as previously mentioned.

Liesen et al. (1977) tracked CRP levels across nine months of training. A two hour run was conducted before and after the training program and blood was taken immediately after the run, three and seven days later. The exercise training program consisted of the three subjects running around 40 km per week. The main finding of the study was that a reduced CRP response followed the bout of exercise after the nine months of training. The results indicate an anti-inflammatory response to chronic exercise training. The major limitation of this study is the small sample size which makes it difficult to draw meaningful conclusions. It

is also important to highlight that most other research on CRP and chronic exercise reported resting values of the acute phase protein rather than after a prolonged aerobic exercise session. As a result, it is hard to compare this study to the other CRP and exercise related literature. This study does however, demonstrate that exercise training has the potential to attenuate exercise-induced CRP levels and is therefore included in this discussion.

Resting CRP levels have been reported by Mattusch et al. (2000) before and after nine months of exercise training. There were 14 male runners, aged between 25-40 years, participating in the study. The subjects were moderately trained marathon runners who ran between 31 and 50 km per week throughout the study. The results indicated that resting CRP levels significantly declined from 1.19 mg/L before training to 0.82 mg/L after the nine months of training. At the same time, CRP levels were increased in the control group. CRP levels of a non-training control group were also documented. In the control group CRP levels were increased. The results of the study highlight the CRP reduction following chronic exercise training. The study conducted by Mattusch et al. (2000) differs from Liesen et al. (1977) as only resting values of CRP were reported.

Stewart et al. (2007) examined resting CRP levels before and after a 12 month exercise training program. There were 29 subjects aged between 18-35 years old (younger group) and 31 subjects aged between 65-85 years old (older group) that participated in the study. Each age group was divided into either a physically active or physically inactive group. The physically active group did nothing different to their regular exercise program, while the physically inactive group used a combination of aerobic and resistance training performed three times per week. Each exercise session included 20 minutes of aerobic training and then resistance training consisting of two sets of eight exercises. The results

showed that the physically inactive groups experienced a decrease in CRP following the training program. Collectively, CRP levels were reduced by 58% in the physically inactive group. The physically active group did not have significantly different CRP levels at the end of the 12 months compared the original baseline values. There was no mention of an increase in training volume or load for the physically active group which may help to explain why CRP levels did not significantly change. This study is the only to use a combination of aerobic and resistance training into the exercise regime. The decreased CRP reported in the physically inactive group is in agreement with both Liesen et al. and Mattusch et al., although the results from the physically active group are not. As discussed, no alteration of the training protocol in the healthy subjects may be a potential reason for the CRP values staying constant.

Lakka et al. (2005) reported resting CRP before and after a 20 week standardized exercise training protocol. There were a total of 652 subjects who participated in the study. The study was divided into three groups based on initial resting CRP level (high, medium, low). The exercise program consisted of three training sessions per week on the cycle ergometer. Throughout the duration of the study, progressive overload was applied. Across the entire study population, there was no difference in resting CRP levels before and after the 20 weeks. In subjects with both low and medium initial resting CRP levels, no changes in CRP levels were reported. The subjects with high initial CRP levels did show a significantly lower CRP level following the twenty week exercise protocol. The results suggest sedentary healthy individuals with high initial CRP levels may show an anti-inflammatory effect of chronic exercise.

The training volume and intensity differences between the studies could explain the contrasting findings reported in healthy active subjects. Both Liesen et al. (1977) and Mattusch et al. (2000) used exercise training programs consisting of prolonged aerobic exercise. The high volume of the training protocol may be needed to elicit anti-inflammatory effects in already active individuals. Stewart et al. (2007) used an exercise protocol for physically active individuals that involved no alteration to the exercise regime. Lastly, the exercise training program of Lakka et al. (2005) consisted of three sessions per week on the cycle ergometer. This is the only study to have used cycling rather than a combination of running or resistance training and may not have provided a sufficient stimulus to the healthy individuals. The varying modes and training volumes between the studies low may lead to the difference in results between the studies.

It can be concluded that prolonged aerobic exercise training of sufficient volume leads to a reduction in resting CRP levels of physically active individuals. In addition to this, moderate levels of physical activity may reduce CRP levels in sedentary, physically inactive individuals. The results of the studies highlight the anti-inflammatory nature of chronic exercise. This is seen in the reduction in CRP levels throughout an exercise training program.

The exact mechanism behind the reduction in CRP levels is currently unknown. Kasapis & Thompson (2005) suggested that an increase in physical activity could decrease levels of both IL-6 and TNF-α which would inhibit CRP production, reducing baseline levels. This potential mechanism has also been proposed by Stewart et al. (2007) although the study failed to reveal evidence suggesting this. Kasapis & Thompson (2005) also suggested that a possible increase in antioxidant defenses through an upregulation of antioxidant enzymes occurs with long term physical activity. The antioxidant effect could

reduce injury to cell tissues therefore reducing inflammation and thus CRP levels. This potential mechanism has also been mentioned by Mattusch et al. (2000) although no concrete relationships were discovered. Overall further research into the specific mechanisms related to this CRP reduction is needed to fully understand the anti-inflammatory effect of chronic exercise training.

Immune System Responses and Soccer

There has been very limited research conducted on CRP levels across a sports season. Fallon et al. (2001) reported acute phase reactants before, during and after a netball and soccer season in competitive female athletes. The results suggest that there is a similar anti-inflammatory response to soccer as in prolonged aerobic exercise training. A further discussion of this study will appear later in the chapter (The immune system across a soccer season). Soccer is a high intensity, intermittent activity with considerable eccentric muscle action. Physical contact with other players may also induce muscle micro-trauma which has the capacity to alter pro-inflammatory immune system markers. Research conducted on the immune system following either a single soccer match (Ispirlidis et al. 2008), two consecutive matches (Malm et al. 2004) or short-term training camps (Malm et al. 2003) have shown immune system alteration.

Ispirlidis et al. (2008) tracked inflammatory responses for six days after the completion of a competitive soccer match. Twenty four elite male soccer players were used in the study. The purpose of the study was to determine the effects of a single game of soccer on performance, muscle damage and inflammation during a six day recovery period. The results of the study indicated that an acute phase response occurred following the completion of the match. The acute phase response was highlighted by a peak elevation of CRP 24 hours

post game along with elevation of leukocytes and cytokines immediately after the game. Creatine kinase (CK) activity peaked 48 hours after the soccer game and levels indicated similar muscle damage compared to running a marathon. The large amount of eccentric muscle action combined with the physical contact of the game is the main contributor to this muscle damage. The results of the study indicate that a competitive soccer game can stimulate the innate immunity of the body in a similar fashion to prolonged aerobic activities such as running or cycling. This causes a marked but transient acute phase response. It is important to note that the study highlights immune system alteration does occur following a single competitive soccer game.

Malm et al. (2004) conducted a study that tracked immune system alteration in response to two consecutive soccer games. Both soccer games were 90 minutes in length and were only separated by 20 hours. The subjects consisted of ten elite male soccer players and blood was drawn before the first game, between games, and 6, 24, 48 and 72 hours after the second game. The purpose of the study was to describe the immunological alterations to consecutive soccer games. Markers of both innate immunity and adaptive immunity were tracked during the study. NK cells (innate immunity) showed a decrease following the two soccer games which may highlight a potential innate immune suppression post-game. Both B and T cells (adaptive immunity) were also tracked following the soccer game but the results were contrasting. B cell levels increased in response to the soccer games, suggesting a possible enhancement of the adaptive immune system. On the other hand, T cells significantly decreased after the two games, suggesting a reduction in the body's ability to fight infection. The contrasting results of the study indicate that further research is needed to

understand why these changes occur. In addition to this, it may take up to 72 hours to express normalized immune system variables, which is consistent with Ispirlidis et al. (2008).

Another study by Malm et al. (2003) looked at the immune system in response to a five day soccer training camp. As in the previous study, ten elite male soccer players were subjects. The purpose of the study was to highlight immune system alterations following a five day soccer training camp. The five day soccer training camp involved an increase in physical training as well as several practice games. Blood was drawn before and after the training camp. The major findings of the study showed that both B and T cells were suppressed following the training camp indicating that adaptive immunity following heavy soccer training may be compromised, potentially leading to increased susceptibility to infections. The study recorded the number of URTI before and after the soccer training camp. The results indicated that a significantly higher amount of URTI occurred post-camp. In the three weeks prior to the training camp only two reported URTI occurred. In the three weeks post-camp, there were 12 reported incidences of URTI. This is a six-fold increase in the number of infections reported. The increased rate of URTI may help to explain the decreased B and T cells. The results indicate that a prolonged period of high intensity soccer training has the capacity to suppress the immune system.

The three studies that tracked the immune system with short-term soccer training or games demonstrated immune system alteration does occur. Further research is needed to understand why some of the changes occur but a possible immune suppression is evident when consecutive games/training camps are conducted without adequate rest.

The Immune System Across a Soccer Season

To date, only three studies have examined the effects of a soccer season on the immune system (Bury et al. 1998; Rebelo et al. 1998; Fallon et al. 2001). Although the findings are largely inconclusive, these studies have all demonstrated that the immune system is altered across an entire soccer season.

Bury et al. (1998) studied the immunological status of competitive soccer players during the entire training season. The subject pool consisted of 15 elite soccer players from Belgium. Blood sampling occurred four times throughout the year; preseason, middle of the season, end of the season and before the start of the following season. The entire study length was 12 months. The study tracked the total number of leukocytes, lymphocytes, NK cells and neutrophils. Activity of these immune parameters was recorded, as well as the incidence of URTI. The study reported an increase in neutrophil number both halfway through the season and at the end of season. Although the number of neutrophils increased, the activity of these cells decreased (chemotaxis and phagocytosis). It is unclear as to the impact and mechanism behind these alterations. It has been proposed in this study that a decreased neutrophil function may help to explain the increased URTI reported. There was also a decrease in total lymphocyte numbers at mid season and at the end of the season. This has the potential to suppress adaptive immunity. There was no change in NK cell number or activity throughout the season. Although the incidence of URTI was documented, it was not reported to be associated with any measured immune cell responses. The majority of URTI occurred in the winter months (77%) which occurred in the middle of the season.

Another study examining the immune system across a soccer season was conducted by Rebelo et al. (1998). The study consisted of 13 professional soccer players from Portugal.

The study lasted for 11 months and blood samples were drawn before the season, six weeks into the season, six months into the season and 11 months into the season. Circulating leukocyte, lymphocyte and NK cell subpopulations were investigated. The study reported an increased in total leukocytes after six weeks, six months and 11 months. The lymphocyte population initially increased after six weeks and then decreased after six months. Eleven months later, the lymphocyte population had elevated to its highest level. Although it appears that this would lead to an immune function enhancement, the study iterates the point that more investigation is needed before meaningful conclusions can be drawn. NK cell activity was recorded and no alteration in function was reported, which is in agreement with Bury et al. (1998). The study did not report the incidence of URTI.

Fallon et al. (2001) is the only study to track acute phase reactants across a soccer season or use female subjects. Eighteen members of the Australian women's soccer team participated in the study. The purpose of the study was to determine if an acute phase response occurred after training for field sports. There were a total of three blood samples taken throughout the study: after a two week rest period, five months later which occurred after a week of moderate training and nine months from the beginning of the study which was taken after a week of heavy training. All data collection occurred within the training and competitive season. Although 12 different acute phase reactants were recorded, CRP was the primary focus of the study. It was anticipated that the heavier training load would result in increased CRP levels. Contrastingly, CRP levels decreased throughout the season, regardless of training intensity. The results suggest a chronic anti-inflammatory effect of soccer similar to prolonged aerobic type activities. There were no specific mechanisms proposed as to why this anti-inflammatory reaction occurred.

Summary

In general the research conducted on the immune system across a soccer season is limited and the time points used are irregular. More research needs to be conducted to understand why certain immune alterations occur. In only one study has CRP and the acute phase response been documented across a season with the majority of soccer studies focusing on a) men and b) adaptive immunity rather than innate immunity. One of the major limitations to the current base of literature is the length in between data collection points. There is a need to profile the athlete's immune system multiple times each month to properly understand when, how, or if the immune system is altered. A collection period of 5-6 months between samples is the only available data at the moment. Finally, the incidence of URTI needs to be recorded in a similar timeframe to understand when athletes are more susceptible to infection.

CHAPTER III

METHODS

Subjects

Eleven members of the UNC women's club soccer team participated in the study. Initial inclusion criteria were that subjects must be full members of the club soccer team, be between the ages of 18-30 and were in generally good health with no chronic inflammatory diseases. Initial exclusion criteria was any subject who was uncomfortable with blood draws, had any major medical concerns including inflammatory diseases, routinely used anti-inflammatory medications or became pregnant throughout the duration of the study. The head coach gave permission for subjects to participate in the study. Recruitment, protocol and procedures were approved by the Institutional Review Board (IRB) of the University of North Carolina at Chapel Hill. All subjects gave informed consent through written documentation and a completed medical history form that included a question on inflammatory diseases and medications.

Instrumentation

An enzyme-linked immunosorbent assay (ELISA) system was used for the study (MTX Lab Systems, Vienna, VA). CRP was determined using a commercially available ELISA kit (R & D Systems, Minneapolis, MN) which included standard CRP solutions. The sensitivity of the assay was 0.005-0.022 ng/mL. A soccer exposure questionnaire was administered (Appendix I). The questionnaire contained information regarding training

volume, training intensity, number of games, other physical activity, injuries, incidence of infection, sleep patterns and use of anti-inflammatory medicine over the previous two week period. The information obtained from the soccer exposure questionnaire was used to determine the amount of soccer exposure, physical activity exposure and total exercise exposure at each data collection and was calculated in metabolic equivalent (MET) minutes per week. Soccer exposure = (training time x training intensity) + (game time x game intensity) and physical activity exposure = (exercise intensity x exercise time). Game intensity on a 1-10 RPE scale was a 10 (Ainsworth et al. 2000). Time was recorded in minutes while training intensity and physical activity intensity was measured in METs using Ainsworth et al. (2000). Training intensity was paired with a specific MET value (Table 1). The questionnaire administered had been developed by the principal investigator and had not been deemed valid. Test-retest reliability measures were conducted on ten occasions to determine the reliability of the questionnaire.

Table 1. Training intensity and corresponding MET value

Training intensity	1-2	3-4	5-6	7-8	9-10
MET value	4	5	6	8	10

Procedures

General

The study was a 12 week longitudinal design beginning in September and ending in late November. The subjects were asked to come into the laboratory once every other week for the duration of the study. There were a total of six data collection sessions. All sessions were conducted between 8:30-10:30 am. Subjects refrained from exercise 12 hours before the session. During the initial session, the subjects read and signed the informed consent and

medical history forms. The initial session also included the first data collection which consisted of the soccer exposure questionnaire. A blood draw from the antecubital vein also took place. The remaining five bi-weekly sessions consisted of the soccer exposure questionnaire as well as a blood draw from the antecubital vein.

Measurements

At the beginning of each session the subject completed the soccer exposure questionnaire. Upon completion of the questionnaire, 15 minutes of rest in a supine position took place. This ensured posture is accounted for and true resting values were recorded. After the rest period, a five mL blood sample was taken from the antecubital vein. All blood draws were taken by a trained individual and standard sterile procedures were used. After obtaining the blood sample the participant was allowed to ask any questions and was scheduled for the next session. Once this was completed, the subject was free to leave the laboratory. A follow-up email 24 hours after each visit was sent to ensure subject concern and safety was being upheld. Blood samples were placed in EDTA vacutainers and cold centrifuged (4 °C) for 15 minutes to separate plasma. The plasma was then removed from the vacutainer via a transfer pipette and placed into a small container and labeled. The plasma was then stored at -50 °C until all data collection was completed.

After the sixth data collection, all frozen samples were thawed and then analyzed. ELISA techniques were used to determine the resting values of CRP. Soccer exposure, physical activity exposure and total exercise exposure were calculated for each data collection period.

Analysis

Due to the exploratory nature of the study, the following data analysis was conducted ($\alpha = 0.05$):

A one-way (1 x 6) repeated measures ANOVA was used to compare resting CRP levels across the soccer season.

A one-way (1 x 6) repeated measures ANOVA was used to compare soccer exposure across the entire soccer season.

A one-way (1 x 6) repeated measures ANOVA was used to compare total exercise exposure across the entire soccer season.

A bar-line graph was used to examine trend of resting CRP levels and number of infections across the entire soccer season.

A bar-line graph was used to examine trend of resting CRP levels and soccer exposure across the entire soccer season.

A bar-line graph was used to examine trend of resting CRP levels and total exercise exposure across the entire soccer season.

An independent samples t-test was used to compare the two halves of the season in relation to soccer exposure, physical activity exposure, total exercise exposure and CRP levels

CHAPTER IV

RESULTS

The purpose of the study was to determine if a relationship exists between CRP and the incidence of URTI during a soccer season. To this end, 11 subjects were recruited for the study, which well represented the entire team. Of the 11 participants, there were three defenders, five midfielders, three strikers and no goalies. The average age of the subjects was 20.9 ± 1.5 years. The average height of the subjects was 164.3 ± 3.8 cm (5 feet, 4 inches). The average weight of the subjects was 57.3 ± 5.4 kg (126.2 lbs). The average BMI for the subjects was 21.2 ± 1.9 which is classified as normal. The amount of soccer exposure, physical activity exposure and total exercise exposure over the 12 weeks of study is presented in table 2.

Table 2. Amount of soccer exposure, physical activity exposure, total exercise exposure over 12 weeks of the study

Weeks	**Soccer Exposure* (MET-mins/wk) Mean ± SD**	**Physical Activity Exposure (MET-mins/wk) Mean ± SD**	**Total Exercise Exposure (MET-mins/wk) Mean ± SD**
1-2	2118 ± 535	2119 ± 1226	4237 ± 1288
3-4	1261 ± 504	2370 ± 1344	3631 ± 1431
5-6	1892 ± 607	1955 ± 1263	3848 ± 1225
7-8	901 ± 316	1803 ± 1178	2704 ± 1317
9-10	1203 ± 511	1808 ± 1223	3011 ± 1332
11-12	795 ± 697	2024 ± 1003	2819 ± 750

*$p < 0.05$ between weeks, explained below

Soccer Exposure

The results of the one-way repeated measures ANOVA show significant differences throughout the season (Table 2). Weeks 1-2 had the highest amount of soccer exposure at just over 2100 MET-mins/wk. Soccer exposure was significantly higher during this period compared to all other time periods except for weeks 5-6 ($p < 0.05$). Weeks 5-6 had the second highest amount of soccer exposure at approximately 1900 MET-mins/wk. Soccer exposure during this time frame was significantly higher ($p < 0.05$) than the lowest two collection periods, weeks 7-8 and weeks 11-12, but not any of the other weeks. The last time period (weeks 11-12) corresponded with the lowest soccer exposure throughout the season (~ 800 MET-mins/wk). The time period was significantly less ($p < 0.05$) than the two periods with the highest amount of soccer exposure (weeks 1-2, 5-6) but not to any other time period. Weeks 7-8 had the second lowest soccer exposure at approximately 900 MET-mins/wk. This time period was also significantly less ($p < 0.05$) than weeks 1-2 and weeks 5-6. Weeks 3-4 and weeks 9-10 had exhibited a similar amount of soccer exposure (around 1200 MET-mins/wk). These two time periods were only significantly different from the highest soccer exposure weeks.

Throughout the season, the amount of soccer exposure decreased. The highest recorded levels of soccer exposure all occurred in the first half of the season. The lowest recorded levels of soccer exposure corresponded with the second half of the season. The mean soccer exposure for the first half of the season (weeks 1-6) was 1757 MET-mins/wk. The mean soccer exposure for the second half of the season (weeks 7-12) was 966 MET-mins/wk. An independent samples t-test was used to determine that a significant difference ($p < 0.05$) existed between the first and second half of the season.

Physical Activity Exposure

The results of the one-way repeated measures ANOVA indicate that physical activity exposure (MET-mins/wk) undertaken throughout the season was not significantly ($p > 0.05$) different between weeks (see Table 1). In contrast to soccer exposure, physical activity exposure did not change comparing the two halves of the season ($p > 0.05$)

Total Exercise Exposure

The results of the one-way repeated measures ANOVA indicate that total exercise exposure (MET-mins/wk) undertaken throughout the season was not significantly ($p > 0.05$) different between weeks (see Table 1). The highest amount of total exercise exposure occurred in weeks 1-2, which was around 4000 MET-mins/wk. The lowest amount of total exercise exposure occurred in weeks 7-8 and was approximately 2700 MET-mins/wk. In a similar trend as soccer exposure, total exercise exposure decreased as the season progressed. Total exercise exposure was significantly higher during the first half of the season ($p <0.05$), while the lowest amounts of total exercise exposure occurred in the second half of the season. The mean total exercise exposure for the first half of the season (weeks 1-6) was 3905 ± 307 MET-minutes/week. The mean total exercise exposure for the second half of the season (weeks 7-12) was 2844 ± 155 MET-minutes/week.

CRP

The results of the one-way repeated measures ANOVA indicate that CRP levels were not statistically different ($p > 0.05$) throughout the season (Figure 1). CRP levels for weeks 1-2 were 487 ± 382 ng/mL. This was the lowest CRP level of the season. In weeks 3-4 CRP levels increased to 775 ± 1121 ng/mL before declining to 719 ± 486 ng/ mL in weeks 5-6. CRP increased in weeks 7-8 to 877 ± 1683 ng/mL. This was the highest mean CRP level

found. In weeks 9-10 CRP decreased slightly to 831 ± 1463 ng/mL and then decreased again to 655 ± 784 ng/mL in weeks 11-12. Across the entire season, CRP increased from 487 ng/mL to 655 ng/mL but this increase was not statistically significant. The mean CRP for the first half of the season (weeks 1-6) was 660 ± 727 ng/mL and the mean CRP for the second half of the season (weeks 7-12) was 788 ± 1325 ng/mL and this was also not statistically significant. The high degree of standard error can be attributed to several outliers in the data. As these outliers exhibited similar trend throughout the study as the other subjects, their results were retained.

Incidence of URTI

Overall there were a total of 16 URTIs documented throughout the season (Figure 1). In weeks 1-2, there were two infections. Weeks 3-4 saw an increase in URTIs to five. In weeks 5-6 the total number of infections reported decreased to one. There were two infections reported in weeks 7-8. The last month of the season (weeks 9-10 and 11-12) saw a total of six infections, three per collection period. In the first half of the season there were a total of eight infections. There were also eight infections reported in the second half of the season. In total eight out of the eleven subjects got sick at least once. Of those eight subjects who reported an URTI, five reported multiple URTIs across the season.

Figure 1 shows trend between CRP and the number of URTI reported. Due to the exploratory nature of the study, no statistical procedures were used to determine the significance of this relationship. The figure highlights that the first half of the season (weeks 1-6) showed greater variability in both CRP levels and the number of reported URTIs. The second half of the season showed smaller fluctuations in terms of both CRP levels and URTIs. Although the relationship was not consistent, it appears that at certain times

throughout the season, CRP and the number of URTIs responds in a similar fashion. There is a rise in both CRP and the URTIs from weeks 1-2 compared to weeks 3-4. CRP increased from a mean of 487 ng/mL to 775 ng/mL. During the same time span the number of infections reported increase from two to five. Weeks 5-6 show a decrease in both CRP and URTIs. CRP decreased slightly to a mean of 719 ng/mL while URTIs decreased to only one occurrence. In weeks 7-8 there was a rise in both CRP and URTIs. CRP increased to a mean of 878 ng/mL which was the highest reported CRP level of the study. Infections reported increased to two occurrences during this time. In the last two time period of the study, the number of URTIs reported was constant at three. CRP during the last month decreased to 655 ng/mL.

Figure 1 Mean CRP concentrations (bars) and the incidence of URTI (lines) across the 12 weeks of the study

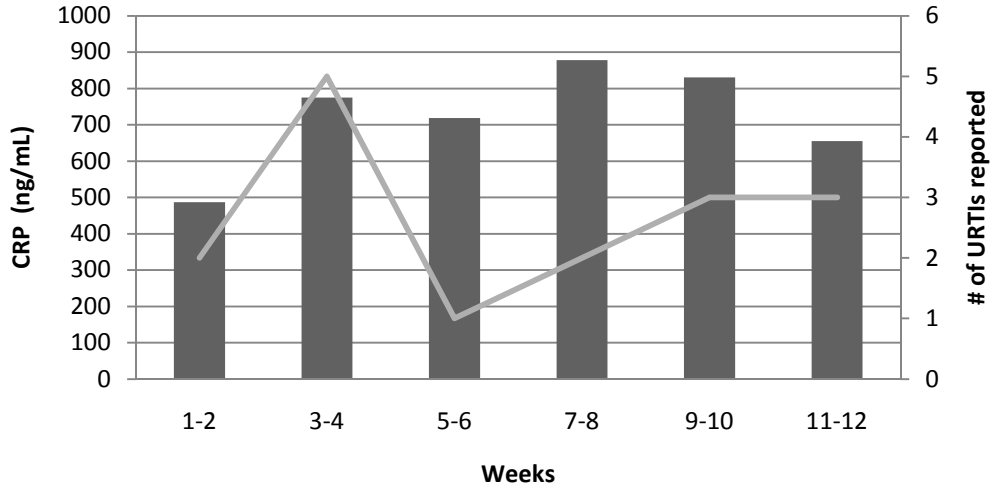


Figure 2 shows trend between CRP levels (ng/mL) and soccer exposure (MET-mins/wk). There appears to be little relationship or trend relating soccer exposure with CRP levels. Over time, soccer exposure decreased while CRP levels did not differ significantly.

Weeks 1-2 had the highest amount of soccer exposure recorded and decreased to the end of the season. Contrastingly, CRP levels did not follow the same trend. As previously discussed, the first half of the season exhibited a larger volume of soccer exposure compared to the second half. This was not reflected in CRP values at anytime during the 12 week study.

Figure 2. Comparison of mean CRP concentrations (bars) and soccer exposure (lines) across 12 weeks of the study

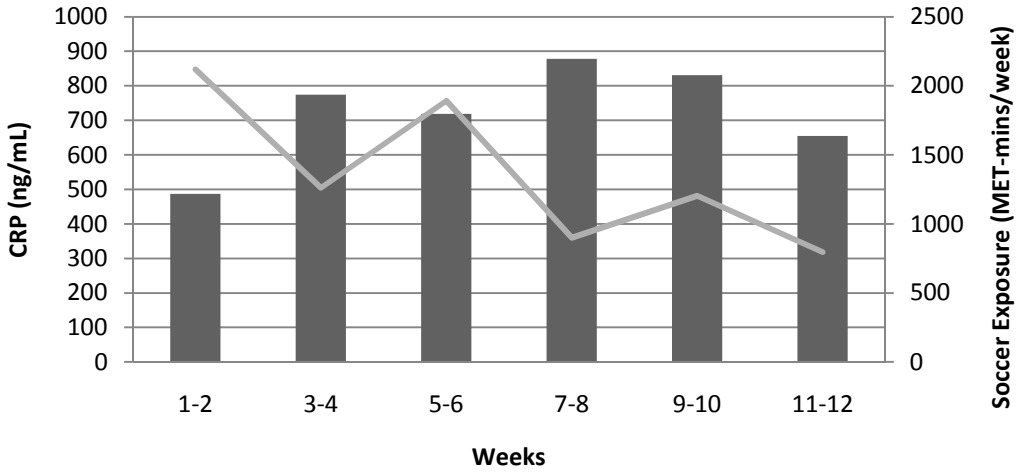
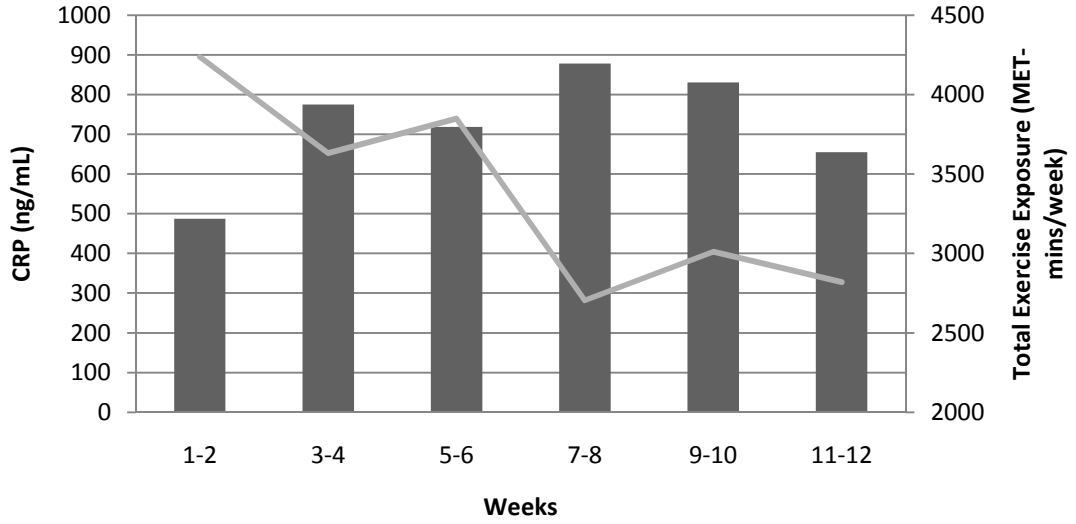


Figure 3 shows trend between CRP levels (ng/mL) and total exercise exposure (MET-mins/wk). Similarly to soccer exposure, total exercise exposure showed no trend in regards to CRP levels. The highest amount of total exercise exposure corresponded in weeks 1-2 and decreased until the end of the season. Contrastingly, CRP levels did not follow the same trend. As with soccer exposure, total exercise exposure was highest in the first half of the study. CRP levels showed no significant difference throughout the season.

Figure 3. Comparison of mean CRP concentrations (bars) and total exercise exposure (lines) across 12 weeks of the study



CHAPTER V

DISCUSSION

During a traditional soccer season, the majority of physical conditioning occurs preseason and at the beginning of the season (Ciccantelli, 1987) while the majority of important games occur at the end of the season. As a result, soccer exposure should be highest at the beginning of the season, decline throughout the majority of the season and then increase at the end of the season. In this study, there was a decline in soccer exposure throughout the season. In Weeks 1-2, physical conditioning was a regular part of practice. This time frame also corresponded with two full length soccer games played on consecutive days, and as a result exhibited the highest amount of soccer exposure of the entire season. In weeks 5-6 which had the second highest amount of soccer exposure, a three game tournament took place. The second half of the season saw a combination of minimal conditioning and fewer games per time period which led to a decrease in soccer exposure. As the majority of the subjects exhibited fairly similar physical activity routines outside of soccer commitments, the major reductions in total exercise exposure was a result of the changes that occur in soccer exposure. It would be valid to assume that at the early stages of the season soccer exposure is high and thus there is an increased risk for infection. Although not the case in this study, soccer exposure could also be elevated at the end of the season. The high intensity nature of both exercise conditioning and games could lead to a suppression of the immune system as proposed by Nieman (1994).

Exercise and incidence of URTI

There were a total of 16 incidences of URTI across the entire season. This is similar to the number of infections reported by previous studies examining immune function across a soccer season (Bury et al. 1998). Unlike the previous study, there were an equal number of infections reported during the first and second halves of the season. The colder months of the season (later stages of the study) did not produce any more infections, suggesting that weather had a little effect on the incidence of infection. The current study took place in North Carolina where mild weather conditions existed up until mid-November. This is in contrast to Bury et al. (1998) who reported that 77% of all URTI infections were diagnosed in the winter months, which took place in the relatively cooler climate of Belgium. No other studies reported URTIs.

Nieman (2000) suggested that for several weeks following intense exercise there is a corresponding increase in the incidence of URTI. Following a period of high soccer exposure and total exercise exposure in weeks 1-2 there was an increase in the number of URTIs recorded in weeks 3-4, some two weeks later. The total number of infections reported went from two incidences, up to five in this time period. Thus, the expected trend did occur. Weeks 5-6 saw the second highest period of soccer exposure. Although this period produced a high amount of soccer exposure and total exercise exposure, there was not the same increase in URTIs two weeks later (weeks 7-8). URTIs only increased from one infection reported to two and did not show the expected trend. One possible reason this trend wasn't evident is that the time period occurred later in the season when the athletes were better conditioned. The increased soccer exposure may not have provided enough additional strain on the adapted subjects to induce immune system suppression and increase the incidence of URTI.

Studies have reported that short term soccer games and training can alter the immune system (Malm et al. 2003; Malm et al. 2004; Ispirlidis; 2008). Malm et al. (2003) reported that following a five day soccer training camp, the number of URTIs increased 6-fold. The three weeks before the camp a total of two incidences of URTI were reported. The three weeks following the camp the total number of URTIs increased to 12 incidences. The exact timing of the infections was not reported but generally agrees with the increased number of URTI reported two weeks after intense training in this study. Malm et al. (2004) found that after two consecutive soccer games, immune system parameters produced varied responses. Although there was an increase in the number of B cells reported following the two soccer games, there was contrastingly, a decrease in the number of T cells. There was also a reduction NK cells which are involved in innate immunity, but these values returned to approximately normal within 48 hours. CRP values have also shown to increase 24 hours after a game (Ispirlidis et al. 2008), although these values returned to baseline within approximately 72-120 hours later. In the present study, consecutive soccer games and tournaments took place in the first several weeks of the study which could produce similar alterations to the immune system. This alteration may increase the susceptibility to infection and help explain the increased infections reported in weeks 3-4.

Previous research on the immune system across an entire soccer season has produced inconclusive results (Bury et al. 1998; Rebelo et al. 1998; Fallon et al. 2001). In these studies, certain immune parameters were either enhanced or depressed with highly individualized responses. Bury et al. (1998) reported a significant decrease in neutrophil function but no changes to the number or activity of NK cells across a competitive soccer season. Rebelo et al. (1998) found that throughout the course of a season total lymphocyte

populations did not change, but that there were some changes in lymphocyte subpopulations. These studies suggest that the immune function is highly varied amongst individuals and that further research on this area is needed. Fallon et al. (2001) tracked CRP across a soccer season and found that following either moderate or heavy training periods there was little evidence of an acute phase response. In fact, CRP levels actually declined compared to resting levels across the season. In the present study, CRP levels showed no relationship with either soccer exposure or total exercise exposure. The highest levels of soccer exposure corresponded with the lowest levels of CRP. This is in agreement with Fallon et al. (2001) in which periods of moderate and heavy training produced lower CRP values. The results of the study indicate that no acute phase response was present following heavy workloads. CRP levels did however increase across the season which disagrees with Fallon et al. (2001).

Exercise and CRP

CRP, the immune parameter measured in the study, exhibited typical values for the healthy, athletic, adult population. The baseline CRP values found are similar to another reported study (Dufaux et al. 1984). Dufaux et al. examined baseline CRP levels in over 450 athletes and found that the median CRP value for soccer players was 660 ng/mL. In the current study, the mean CRP for the first half of the season was 660 ng/mL and 788 ng/mL in the second half of the season. Thus the resting CRP values obtained are in a normal range for soccer players.

The general conclusion regarding CRP is that chronic exercise produces an anti-inflammatory effect, although the exact mechanism behind this reduction is still unknown. The majority of studies have found that a reduced CRP response is evident either at resting (Lakka et al. 2005; Mattusch et al. 2000; Stewart et al. 2007) or immediately after exercise

(Liesen et al. 1977) following a period of long term training. The current study did not find any significant difference between CRP levels across the season. There was however, an increase in CRP during the second half of the season. CRP at the beginning of the season was approximately 480 ng/mL and increased to 650 ng/mL, although not statistically or clinically significant. These findings are contrasting to previous literature reported. Although increased across the season, CRP levels were still within normal limits such that an inflammatory response was not evident.

Previous literature was conducted using mainly aerobic type activities such as swimming, cycling and running. Soccer is an impact based sport in which a high degree of physical contact and micro-trauma occurs. The risk of injury is also much greater compared to other aerobic based activities. Injuries have the potential to impact the results of the study. Acute or chronic injury has the potential to cause inflammation leading to an increased acute phase response. CRP levels will be elevated during this response. Injuries were documented by the subjects during the study. Overall there were a total of nine different injuries that occurred across the season ranging from mild concussions to sprained ankles. The resting CRP values showed no consistent response from the injured participants. There is however, a potential that injuries did alter the resting CRP values and could have therefore impacted the results of the study. This may help to explain why CRP did not follow a similar reduction as in previous research.

CRP did not follow any consistent trend with either soccer exposure, total exercise exposure or the incidence of URTI. There are several possible reasons as to why. Although soccer conditioning took place, two practices per week may not have been sufficient to, a)

increase CRP levels through an acute phase response, or b) suppress the immune system to increase risk of URTI. Overall the subjects were not only healthy before beginning the season, but remained relatively healthy throughout. The healthy nature of the subjects doesn't necessarily allow for a meaningful relationship to be exhibited. On an individual basis, CRP levels showed no consistent response when infections were reported. It is very possible that the two week window between data collection periods allowed the CRP to return to normal levels. Although this is the first study to profile the immune system bi-weekly across a season, the two-week sampling time frame may be too long, such that an increase in CRP due to an infectious event may be missed. The CRP changes induced by soccer exposure and total exercise exposure may also be transient and as a result may have returned to baseline levels before blood collection occurred.

Limitations

There are several important limitations to this study which need to be addressed. The small sample size of 11 may have contributed to the lack of results. A larger sample size is needed to produce meaningful conclusions based upon both resting CRP values and the incidences of infection. In addition to this, there are several uncontrollable factors that have the potential to confound the results. Over a prolonged study of this nature, several factors may compromise the immune system. The stresses and rigors of being a university student can increase stress on the immune system. Several factors such as sleep, academics, extracurricular activities and overall stress levels, were not taken into account. To evaluate whether acute sleep deprivation occurred in the subjects, the hours slept on the previous night was recorded prior to each blood draw. The subjects did not document that acute sleep deprivation occurred during the study. However, research has shown that moderate sleep

disturbances can impact the immune system (Irwin et al. 1996; Meier-Ewert et al. 2004). Even sleep deprivation for several hours on one night has the potential to impact on the immune system by reducing NK cells and potentially increasing the likelihood of infection (Irwin et al. 1996). Meier-Ewert et al. (2004) also found that both acute and short-term partial sleep deprivation causes an increase in CRP concentrations at rest. The amount of hours slept by the subjects was not controlled and as a result had the potential to impact the immune system, CRP and the findings of the study. Any additional and unwanted psychological stress whether it is prolonged or acute also has the potential to alter the immune system. Previous research (Dugué et al. 1993) suggest that this psychological stress although less significant, still can alter the immune system. CRP levels have shown to increase or stay the same during times of psychological stress. Although less conclusive, psychological stress may have been a factor that impacted on the CRP levels of the subjects. Overall these factors that have the potential to compromise the immune system and as a result are potential limitations to the study.

It is also important to note that the present study involved members of a university's club soccer team. The majority of studies involving soccer and the immune system have used elite/professional athletes. The level of training volume and intensity is significantly less in the present study compared to previous research. It is therefore difficult to draw meaningful comparisons and conclusions based on this. Further, the majority of studies have focused on male subjects, with only one previous study using females. This fact also makes drawing comparisons with previous research more difficult.

Strengths

There were strengths of the study which need to be highlighted. The subjects that volunteered for the study represented the entire team. There was an adequate sample of defenders, midfielders and forwards, as well as starters and non-starters. Another strength of the study was that the data collection period occurred every two weeks. In previous immune system studies examining an entire sports season, immune function was only examined preseason, midseason and postseason. Every two weeks gives the opportunity to profile the immune system and more accurately examine the relationship between exercise exposure and incidence of infection. It has been noted that potentially reducing the time in between blood samples further is ideal, but this is still the first study to attempt to profile the immune system in college athletes. The use of soccer exposure, physical activity exposure and total exercise exposure helped to identify exercise related stresses of each subject. Pairing this information with CRP levels and the incidence of URTI provided the opportunity to gain important information about how individuals respond to exercise. As mentioned previously, in only one other study examining immune function across a sports season have females been the population used. This present study has increased the literature available on immunology of the female population across a sports season.

Conclusions

For coaches and players, it is important to understand at what times during the season an athlete is most vulnerable to infection. By examining soccer exposure, physical activity exposure and total exercise exposure with incidence of URTI this study has provided the opportunity to do this. The results of the study indicate that immune function may be highly individualized, but that general trends of exercise exposure do occur. Soccer exposure is

going to be highest during the preseason/beginning of the season and generally at the end of the season when an increase in the number of important games occurs. Adequate rest and proper maintenance programs during the season will help to ensure athletes stay healthier on the field and therefore perform better.

The results of the current study suggest several interpretations. First, CRP is a highly individualized measurement of immune function and that resting levels are not significantly altered throughout the season. Second, there was no consistent trend and as such, the validity of using CRP as a marker of immune function for chronic exercise may be called in to question. Third, CRP did not show any relationship with soccer exposure, physical activity exposure or total exercise exposure across the season. Fourth, the two weeks in between blood samples may be too long to profile immune responses. Shorter time increments may be needed to capture either an acute phase response or any reductions in immune function. No research to date has followed the immune system on a weekly basis, but may be needed.

Recommendations

There are several recommendations needed to be given. First, reduce the time in between blood samples. This will increase the likelihood of capturing an infectious episode in the blood. Second, use an alternative immune marker to CRP. Third, attempt to reduce other variables such as additional physical activity, stress levels and sleep patterns. Fourth, use elite athletes as a subject pool. The increased volume and intensity of training may further suppress the immune system and give a better indication of the risks associated with elite athletics. Fifth, as coaches it is important to understand that the early stages of the season may produce the greatest risk of infection.

APPENDIX I

SOCCER EXPOSURE QUESTIONNAIRE

ID:_____

Session #:_____

1) What is your position? (circle one)

Goalie Defense Midfield Striker

2) How many total hours in the last two weeks did you practice soccer? _____ hrs
   a. On a scale of 1 to 10, 1 being very easy and 10 being the hardest practice you can imagine, how hard were the practices?_____
3) Did you have a match in the past two weeks? Yes No
   a). If yes, how many? _____
   b). If so, how much did you actually play during the match? (circle one)

   0-10 min 10-30 min 30-50 min 50-70 min 70-90 min

4) How many hours in the last two weeks did you spend weight lifting? _____
5) How many hours in the last two weeks did you spend doing any other physical activities?_____
   a. What were those activities?_____
6) In the past two weeks have you been injured? Yes No
   a. If so, please describe. _____
7) In the last two weeks have you gotten a cold? Yes No
   a. If so, how many times?_____
8) In the last two weeks have you gotten the flu? Yes No
   a. If so, how many times? _____
9) Have you had any other infections or illnesses during the past two weeks? Yes No
   a. If so, how many and what where they? _____

_____

10) Have you taken any anti-inflammatory medication in the last 48 hours? Yes No

a. If so, what was it? (e.g. ibuprofen, advil, motrin, nuprin, aleve) _____

11). On average how many hours of sleep do you get on a 'good' night? _____

12) In the past week how many hours of sleep (average) did you get each night? _____

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