PLASMA AND SALIVARY CYTOKINE RESPONSE TO TWO COMPETITIVE COLLEGIATE SOCCER GAMES

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2006

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ACKNOWLEDGEMENTS

I want to first thank Dr. Douglas Smith, it has been an honor and pleasure to work with you these past two years. I will always be in your debt for taking me in as your graduate student and providing me with the opportunity to succeed. I would also like to thank the rest of my committee: Dr. Steve Edwards, Dr. Frank Kulling, and Dr. James Breazile. I appreciate your guidance and patience during this process.

I want thank Dr. Melody Phillips who introduced me to the world of exercise immunology and helped make this study possible. I would also like to thank Thomas Buford for his assistance in data collection. I would like to thank my wife, Melanie, whose support and love has been unwavering. I love you and could not have done this without you. I also want to thank my family, Remo and Ellen Rossi; Bill, Linda, and Chris Hayes. Thank you for being so supportive and patient.

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CHAPTER I

INTRODUCTION

The immune system and its response to endurance exercise and sport has become a topic of interest to both health and sport professionals. Strenuous exercise training and competition associated with athletics induces a noticeable but mild systemic inflammatory response, which is similar to that of trauma, infection or sepsis (Starkie, Rolland, Angus, Anderson, & Febbraio, 2001). Exercise induced inflammation is illustrated, in part, by elevations in blood levels of inflammatory cytokines such as interleukin-6 (IL-6) and circulatinginterleukin -1 receptor antagonist (IL-1ra) (Moldoveanu, 2001; Smith, 2000). Cytokines are soluble peptides and proteins secreted by immune and non-immune cells which help coordinate communication between cells, organs, and organ systems of the body. Cytokines are important in the maintenance of physiological homeostasis and over production of inflammatory cytokines has been associated with cardiovascular disease and type II diabetes (Petersen & Pedersen, 2005). The initial inflammatory cytokine response to endurance exercise includes the secretion of pro-inflammatory tumor necrosis factor (TNF), interleukin-1βeta (IL-1β) , and "inflammatory responsive" IL-6 which is followed by the release of anti-inflammatory cytokines IL-10 and IL-1ra (Drenth, Van Hum, Van Deuren, Pesman, Van Der Ven-Jongekrijg, & Van Der Meer, 1995; Ostrowski, Rhode, Asp, Schjerling, & Pedersen, 1999; Weinstock, Konig, Harnischmacher, Keul, Berg, & Northoff, 1997).

Athletes participating in high intensity or prolonged exercise may be at greater risk for upper respiratory infections (URI) when compared to healthy, sedentary individuals (Nieman & Nehlsen-Cannarella, 1992; Nieman, Henson, Gusewitch, Warren, Dotson, Butterworth, & Nehlsen-Cannarella, 1993; Pyne, McDonald, Gleeson, Flanagan, Clancy, & Fricker, 1999). Weidner (1994) reported that 5% of athletes missed competition and 18% of athletes have missed practice due to URI symptoms during a single competitive season. Symptoms of URI reported by athletes include sore throat, runny nose, and cough (Mackinnon, Ginn, & Seymour 1993). Increased risk for URI symptoms may be related to exercise intensity and duration of training and competition. The "J" curve model presented by Nieman and Nehlsen-Cannarella (1992) illustrates moderate exercise may reduce the incidence of URI whereas intense or long duration exercise may suppress immune function. Following heavy exercise there appears to be a period of time or "open window" (3-72 hours post exercise) during which the immune system is suppressed and vulnerable to subsequent infection (Pedersen and Ullum, 1994). In one of the first published studies examining the incidence of URI, Peters and Bateman (1983) reported the incidence of URI occurred in 33.3% of the runners compared to 15.3% in matched controls two weeks following a 56 km race. One proposed mechanism for post exercise immune suppression is exercise-induced down-regulation of T-helper-1 cytokine production (Smith, 2003). T-helper-1 cytokines promote cell-mediated immunity (CMI) and T-helper-2 cytokines promote humoral immunity. The normal response to moderate exercise is a balanced Th-1/Th-2 cytokine response; however, intense exercise increases stress hormones, glucocorticoids (GC) and catecholamines

(CA) which restrain T-helper-1 cytokine secretion suppressing CMI and increasing the risk for post exercise infection (Smith, 2003).

Research examining the immune response in athletes participating in team sports is limited. A study similar to the current research observed the immune response in 10 semi-pro male soccer player's following two consecutive games separated by 20 hours. The authors reported a mixed response in immunological variables representing both enhancement and suppression of immune function following two soccer games (Malm, Ekblom, & Ekblom, 2004a). Decreases in natural killer (NK) cell number and activity have been reported in soccer players during a competitive season with normalization of these numbers following the season (Bury, Marechal, Mahieu, & Pirnay, 1998). Pool, Robson, Smith, Wyk, & Myburgh, (2002) reported higher resting blood levels of inflammatory IL-6 along with reduced in *vitro* endtoxin induced secretion of IL-6 at rest in rugby players versus age matched sedentary controls. These data suggest training and competition associated with team sport may induce a chronic suppression of the immune system and increase risk for infection.

Statement of the Problem

The inflammatory cytokine response following endurance exercise has been extensively studied (Ostrowski et al., 1998, 1999); yet, research examining systemic as well as salivary cytokine response to sportplay and training is lacking. Alterations in oral health or salivary immunoreactivity may indicate changes in both acute and chronic health status (Mobley and Saunders, 1997). Clinicians diagnosing and monitoring certain disease states measure salivary cytokine levels; however the use of salivary cytokine measures has not been fully explored in an exercise paradigm. Saliva sampling provides

an alluring technique for the collection of physiological data due to its simple and noninvasive nature. Changes in salivary cytokine levels may infer susceptibility to or a current infection (Puccetti, P., Romani, & Bistoni, 1995). This makes saliva a potentially effective biological tool to study the influence of exercise on cytokine response (Winkler, Hadnagy, & Idel, 2001).

Purpose of the Study

The purpose of this study was to measure the response of plasma and salivary cytokines IL-6, IL-10, TNF- α, and IFN- γ in soccer players competing in two consecutive collegiate soccer games. The second purpose was to compare plasma and salivary cytokine levels at rest and following exercise to determine if similarities exist.

Significance of the Problem

To our knowledge no report has compared plasma and salivary cytokine response following two competitive collegiate soccer games. The information obtained from this project would be beneficial to persons competing in, or coaching team sports, or those providing health care to individuals who participate in soccer or other team sport activity. These data will add to the current knowledge of biological influences of playing or competing in team sports such as soccer, especially during tournament play. We hope to assist in providing information necessary to prevent or to reduce the incidence of illness in athletes, thus reducing rate of occurrence of missed practice and competition and improving overall health.

Hypotheses

The following null hypotheses will be examined:

Ho1 = There will be no significant difference in salivary IL-6 levels relative to secretion rate between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

Ho2 = There will be no significant difference in absolute salivary IL-6 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

Ho3 = There will be no significant difference in plasma IL-6 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

Ho4 = There will be no significant relationship between salivary IL-6 levels and plasma IL-6 level 6 hours before the first soccer game.

Ho5 = There will be no significant relationship between salivary IL-6 levels and plasma IL-6 level 1 hour following the second soccer game.

Ho6 = There will be no significant relationship between salivary IL-6 levels and plasma IL-6 level 15 hours following the second soccer game.

Ho7 = There will be no significant difference in salivary IL-10 levels relative to secretion rate between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

Ho8 = There will be no significant difference in absolute salivary IL-10 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

Ho9 = There will be no significant difference in plasma IL-10 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

Ho10 = There will be no significant relationship between salivary IL-10 levels and plasma IL-10 level 6 hours before the first soccer game.

Ho11 = There will be no significant relationship between salivary IL-10 levels and plasma IL-10 level 1 hour following the second soccer game.

Ho12 = There will be no significant relationship between salivary IL-10 levels and plasma IL-10 level 15 hours following the second soccer game.

Ho13 = There will be no significant difference in salivary TNF-α levels relative to secretion rate between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

Ho14 = There will be no significant difference in absolute salivary TNF-α levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

Ho15 = There will be no significant difference in plasma TNF-α levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

Ho16 = There will be no significant relationship between salivary TNF-α levels and plasma TNF-α level 6 hours before the first soccer game.

Ho17 = There will be no significant relationship between salivary TNF-α levels and plasma TNF-α level 1 hour following the second soccer game.

Ho18 = There will be no significant relationship between salivary TNF-α levels and plasma TNF-α level 15 hours following the second soccer game.

Ho 19 = There will be no significant difference in salivary INF-γ levels relative to secretion rate between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

Ho20 = There will be no significant difference in absolute salivary INF-γ levels between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

Ho21 = There will be no significant difference in plasma INF-γ levels between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

Ho22 = There will be no significant relationship between salivary INF-γ levels and plasma INF-γ level 6 hours before the first soccer game.

Ho23 = There will be no significant relationship between salivary INF-γ levels and plasma INF-γ level 1 hour following the second soccer game.

Ho24 = There will be no significant relationship between salivary INF-γ levels and plasma INF-γ level 15 hours following the second soccer game.

Delimitation

This study will have the following delimitations:

1. All subjects were apparently healthy male varsity college soccer players from Oklahoma Baptist University.

2. Blood and salivary cytokine levels were measured with enzyme-linked immunosorbent assay (ELISA) kits.
3. The results of a study using college-aged male soccer athletes may not automatically generalize to female soccer athletes or athletes participating in different sports.

Limitations

The research may be limited by the following:

1. The subjects of this study were selected from a sample of convenience.
2. The limited subject sample size.
3. Distances covered and exercise intensity during the soccer games was not controlled.
4. The sensitivity of the ELISA kits used to measure plasma and salivary cytokine levels.
5. Blood and saliva were collected before and after exercise and not during exercise.

Assumptions

1. Medical history and physical examinations excluded persons with diseases, injuries or disorders that could have abnormal influences on cytokine levels.
2. All subjects were homogenous in their fitness levels as indicated by pre season fitness evaluations.
3. All subjects followed data collection instructions at each collection time point.
4. Data samples collected before exercise represent resting cytokine levels.
5. All subjects did not participate in any form of physical activity other than the two soccer games.

6. All laboratory equipment used in cytokine analysis was properly calibrated.

Operational Definitions

Antibody is an immunoglobulin that binds to antigens (Mackinnon, 1999).

Antigen is a protein that activates immune cells (Mackinnon, 1999).

Cell mediated immunity an immune response directed towards infected or damaged cells.

Chemokines are small proteins that attract cells of innate and specific immune system to sites of infected or injured tissue.

Humoral immunity is an immune response of soluble products circulating in bodily fluids (Mackinnon, 1999).

Interferon (IFN) is a group of antiviral cytokines (INF-α, INF-β, & INF-γ) produced by NK and T cells that activate macrophages and promote cell mediated immunity (Mackinnon, 1999).

Interleukin (IL) is a group of cytokines thatmediate communication between white blood cells (Mackinnon, 1999).

IL-1 a family of cytokines (IL-1α, IL-1β, & IL-1ra) that induce hypotension, fever, sleepiness, anorexia, deterioration and inflammation of joints, and lean tissue breakdown (Modoveanu, 2001).

IL-6 is an "inflammatory responsive" cytokine produced by various immune cells that is important in initiating the acute phase response and has some anti-inflammatory characteristics. Exercise induces a significant but temporary increase in IL-6 (Modoveanu, 2001).

IL-10 is an anti-inflammatory cytokine produced mainly by Th-2 cells that restrains production of specific pro-inflammatory cytokines and promotes humoral immunity.

IgA is a protein secreted by B cells that circulates in the blood and other fluids providing antibody defense (Mackinnon, 1999).

Mucosal immunity is the immune defense associated to the respiratory, uro-genital, and oronasal tract, gut, and salivary glands

T helper cells are cells which recognize antigens and secrete specific cytokines that activate immune cells (Mackinnon, 1999).

Tumor necrosis factor (TNF) is a strong tumor necrotising mediator primarily secreted by macrophages and Th cells thatinduces increased body temperature, activates immune cells.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The regulation of antigen presenting cells and lymphocytes is partially controlled by low molecular weight regulatory proteins called cytokines which mediate signals between various cells and systems of the body. Cytokines are generally classified as interleukins, interferon, and chemokines and over 80 cytokines have been identified. These various cytokines play an integral role in the activation and migration of immune cells to sites of tissue injury and promote resistance to infection. Physical activity of sufficient intensity may induce an inflammatory response. This inflammatory response is partially characterized by the release of pro- and anti-inflammatory cytokines. During and immediately following strenuous exercise pro-inflammatory cytokines TNF-α, IL-1β, and "inflammation-responsive" IL-6 are released followed by regulatory or anti-inflammatory cytokines IL-4, IL-10, and IL-1ra. Generally following exercise, pro-inflammatory cytokine levels are counterbalanced by anti-inflammatory cytokine levels promoting homeostasis; however if levels are unrestrained, incidences of post exercise infection may occur.

Influence of Exercise on Incidence of Illness

Nieman, Johanssen, & Lee (1989) examined the incidence of illness in runners (N=294) competing in a 5k, 10k, or half-marathon race. Subjects were asked to answer questions concerning demographics, training habits, race results, and incidence of injury and illness two months before and one week following the race. Subjects were grouped and analyzed by race (5 km & 10km or half-marathon) and by weekly training volume (less than 15 miles or 15 or miles). Overall, 30% of all runners reported at least one incidence of illness (12.8% colds and 16.1% flu) during the two months prior to the race. Thirty-one percent of the runners in the 5 & 10 km group reported at least one incidence of illness during pre-competition training opposed to 22.7% of the half marathoners. When runners were grouped by training volume, 25% of the runners training 15 miles or more a week reported at least one incidence of illness during pre-competition training versus 34% of the runners training less than 15 miles per week. When comparing the incidence of illness in all runners the week before and after the race, 9.9% reported being sick before the race and only 7.3% after the race. In conclusion, the authors speculated the more "serious" runners (trained 15 or more miles for the half-marathon) because of regular exercise were less likely to experience illness than recreational runners
In addition, the general stress of competing in a 5 km, 10 km, or half-marathon race did not increase the likelihood of infection.

In a second study, Nieman, Johanssen, Lee, & Arabatzis (1990) investigated the incidence of infection in 2,311 marathon runners participating in the 1987 Los Angeles Marathon. Eight days prior to the race, a questionnaire was sent to a group of randomly selected runners. Runners provided information concerning demographics, average

yearly training volume, training schedule, and the number of infectious episodes during the two months prior, 7 days before and 7 days after the race. Nieman et al. (1990) reported during the two months prior to the race, 40% of all runners suffered from at least one infection. The week following the race, 12.9% of the runners reported some type of infectious episode compared to 2.2% reported by matched controls that applied for but did not compete in the race. The authors found runners who trained more than 96 $km \cdot wk^{-1}$ were reported to double their odds of sickness compared to runners who trained less than 32 $km \cdot wk^{-1}$. These results indicate training for and participating in a marathon race increases the risk for infection and there may be a dose response relationship between training and incidence of illness. The results reported by Peters and Bateman (1983) and Nieman et al. (1989 & 1990) support the "J" curve model, individuals participating in heavy training or competition may be at greater risk for infection versus healthy sedentary and moderately trained individuals.

Influence of Endurance Exercise on Salivary IgA

The mucosal immune system plays a major role in defense against URI and the effectiveness of the mucosal immune system is frequently assessed by the measurement of the principal immunoglobulin in mucosal secretions, salivary IgA (sIgA)(Gleeson, 2000). Several studies have reported lower levels of salivary IgA in athletes following exercise and competition when compared to sedentary matched controls (Mackinnon & Hooper, 1994; Tomasi, Trudeau, Czerwinski, & Erredge, 1982). For example, Nieman et al. (2002) investigated the effect of carbohydrate, age, and gender on sIgA levels and the relationship between sIgA and URI in runners following a marathon race. Runners were randomly divided into either a carbohydrate (N = 48) or placebo (N = 50) group. Blood

and saliva samples were collected before, immediately after and 1.5 hours after the race. There were no significant differences in sIgA levels and sIgA secretion rate between groups following the race ($F [2,194] = 1.64$, $p = 0.1970$). As a whole, the runners sIgA levels dropped 46% immediately following the race ($p < 0.001$) and remained 30% below resting levels 1.5 hours post race ($p < 0.001$). Salivary IgA secretion rate was significantly decreased in all subjects immediately post race (34%, $p < 0.001$)) and 1.5 hours post race (25%, $p < 0.001$). Twenty seven percent of all runners reported at least one incidence of URI during the 15 days before the race. Differences did not exist between sIgA levels and sIgA secretion rate between runners reporting pre race URI and runners who did not. Sixteen runners (17%) from both the placebo (N=10) and carbohydrate (N=6) group reported at least one URI during the 15 days following the race. Salivary IgA levels immediately post race (201 ± 29 and 292 ± 30 $\mu g \cdot mg^{-1}$, respectively, $p = 0.018$) and 1.5 hours post race (254 ± 30 and 388 ± 26 $\mu g \cdot mg^{-1}$, respectively, $p = 0.002$) were significantly different between runners reporting URI and those who did not. Differences did not exist in training volume, cardiorespiratory fitness, race heart rate, and race time between those reporting URI's and those who did not. The results of the present study indicate that sIgA levels and sIgA secretion rate decreased significantly following a marathon race. The incidence of pre-race URI's was found to be unrelated to pre-race sIgA levels; however, post race sIgA levels and sIgA secretion rate were lower in runners reporting URI's versus those who did not. The authors concluded "the relationship between URI and sIgA levels does not appear to be clear and consistent".

Influence of Intermittent Exercise on Salivary IgA

The immune response in athletes participating in physical activity that requires intermittent bouts of exercise has not been thoroughly investigated. Intermittent exercise is described as intense physical activity interspersed with periods of low intense physical activity or rest ((Bishop, Blannin, Robson, Walsh, & Gleeson 1999). Walsh, Blannin, Clark, Cook, Robson, & Gleeson (1999) examined the influence of high intensity cycle exercise on salivary IgA. Eight male subjects were recruited to perform twenty 1 minute all out (100% $VO2_{max}$) sprints separated by 2 minutes of active rest (30% $VO2_{max}$) on a stationary bike. Saliva samples were collected 24 hours and 5 minutes before exercise, immediately post exercise, and 1, 2.5, 5, and 24 hours following exercise. Intermittent high intensity exercise did not significantly influence sIgA levels or sIgA secretion rate. The authors speculated the highly intense exercise protocol alone was not sufficient enough to induce changes in salivary IgA levels and sIgA secretion rate. Previous research suggest factors such as dehydration or breathing cold air contribute to changes in salivary IgA levels following exercise (Tomasi et al. 1983; Ford et al. 1997).

Bishop et al. (1999) chose to examine the effect of a simulated soccer-specific exercise protocol on sIgA levels and sIgA secretion rate. Eight university soccer players volunteered to participate in two randomized simulated soccer specific exercise protocols (carbohydrate and placebo). The simulated soccer protocol consisted of two 45 minute halves with a 15 minute half time. Each halve consisted of three bouts of exercise separated by 1.5 minutes of rest. During each bout of exercise subjects performed 2 minutes of dribbling a soccer ball through cones, backwards running, jogging, maximal sprints, and walking. Subjects provided saliva samples immediately before exercise, during half-time, and immediately following exercise, and 1 hour post exercise. There

were no significant changes in sIgA levels and sIgA secretion rate in both trials. Due to the lack of change in cortisol levels following both trials the investigators proposed the overall intensity of the simulated soccer exercise protocol was insufficient to stimulate an immune response. In addition, the authors stated the simulated soccer exercise protocol may not have induced the same psychological stress encountered during actual game play which could increase cortisol level.

Nieman, Kernodle, Henson, Sonnenfeld, & Morton (2000) chose to examine the effect of tennis drills on immune function. Ten male and female elite (14-18 years of age) tennis athletes performed 2 hours of intense tennis drills which included eight 15 minute sessions with a 4-5 minute break between sessions. Subjects provided saliva samples before, immediately following, and 1 hour following the tennis drills. The authors reported a significant decreases in sIgA secretion rate immediately following exercise when compared to pre exercise levels (275 ± 24 and 192 ± 21 $\mu g \cdot min^{-1}$, respectively, $p = 0.001$). Small but significant decreases in sIgA levels were reported immediately following exercise and remained suppressed 1 hour following exercise (459 ± 39, 346 ± 44, and 345 ± 43, respectively, $p = 0.014$). Nieman et al (2000) concluded the small change in sIgA levels following tennis drills may have been a result of the physical nature of tennis play which involves discontinuous bursts of intense physical activity.

Influence of Exercise Training on Salivary IgA

Research investigating sIgA response to exercise has primarily focused on acute exercise (Nieman et al., 2000; Bishop et al., 1999; Nieman et al., 2002; Tomasi et al., 1982); but, athletes train and exercise daily during a competitive season. Resting levels

of sIgA have been reported to be significantly lower in athletes during a competitive season when compared to sedentary matched controls (Tharp and Barnes, 1990; Gleeson, 2000).

Gleeson, McDonald, Cripps, Pyne, Clancy, & Fricker (1995) examined the impact of intense training over a 7 month period on sIgA levels in 26 elite swimmers and compared these results to 13 moderately active matched controls. Saliva samples were collected once per month before and after a training session in both swimmers and controls. Gleeson et al. (1995) reported a monthly decrease in both pre- and post-training sIgA levels during the training period in swimmers and no change in sIgA levels in matched controls. The authors reported a significant difference in pre- to post-training changes in sIgA level in swimmers versus controls following a training session ($p < 0.002$). The sIgA levels in swimmers generally decreased following each training session while sIgA levels in control subjects increased. Overall, the results of this study indicate exercise training may cause both an acute and chronic suppression of mucosal immunity.

In a similar study, Fahlman and Engels (2005) investigated the effect of a competitive American collegiate football season on sIgA. Saliva samples were provided by collegiate football players ($N = 75$) and age matched controls ($N = 25$) at 8 different time points over a 12 month period. The study revealed sIgA levels and sIgA secretion rate were lowest in football players during the months of heaviest training and months of competitive play ($F [1, 99] = 4.288$, $p = 0.005$). These levels were significantly lower when compared to matched controls ($F [1, 99] = 4.451$, $p = 0.004$). The highest percentage of URI's reported by football players were during the months of heavy training and competition ($F [1, 99] = 9.624$, $p = 0.000$) and these numbers were

significantly higher then those reported by matched controls ($F [1, 99] = 7.911$, $p = 0.001$). Fahlman and Engels (2005) concluded, decreases in sIgA levels and sIgA secretion rate along with increases in URI were related to heavy training and competition. The authors suggested monitoring of sIgA levels during seasonal training and competition may help reduce the incidences of illness in American football players.

Influence of Endurance Exercise on Circulating Inflammatory Cytokines

Systemic inflammation associated with endurance exercise is represented, partially, by elevations in blood concentrations of inflammatory cytokines such as TNF-α, IL-1β, and particularly IL-6 (Northoff, Weinstock, & Berg, 1994; Ostrowski, Rhode, Zacho, Asp, & Pedersen 1998; Ostrowski, Hermann, Bangash, Schjerling, Nis Nielsen, & Pedersen, 1998). It is believed that certain types of exercise requiring high metabolic workloads cause cell injury and the release of inflammatory cytokines (Drenth, Van Hum, Van Deuren, Pesman, Van Der Ven-Jongekrijg, & Van Der Meer, 1995; Ostrowski, Rhode, Asp, Schjerling, & Pedersen, 1999; Weinstock, Konig, Harnischmacher, Keul, Berg, & Northoff, 1997). Recently, reports have proposed intense exercise increases Th-2 cytokine production shifting the immune system towards humoral defense and increasing the risk for viral infection.

Ostrowski et al. (1998) examined the inflammatory cytokine response to a marathon race. Blood and muscle biopsy samples were collected from 16 male runners before and after a marathon race. Plasma IL-6 levels significantly increased from pre exercise levels immediately following exercise (1.5 ± 0.7 to 94.4 ± 12.6 pg$\cdot$ml^{-1}, $p < 0.001$) and declined 2 hours following the marathon race (22.1 ± 3.8 pg$\cdot$ml^{-1}). Pre exercise IL-1ra levels significantly increased from 123 ± 23 pg$\cdot$ml^{-1} to 2795 ± 551 pg$\cdot$ml^{-1}

immediately following exercise and continued to increase to 4119 ± 527 $pg \cdot ml^{-1}$ 2 hours following exercise ($p < 0.001$). Plasma levels of IL-1β and TNF-α increased immediately following exercise (1.5- and 2-fold, respectively, $p < 0$ .005). IL-6 mRNA was elevated in 5 of 8 muscle biopsies following exercise. IL-1ra mRNA was detected in 2 muscle biopsies and in 5 blood mononuclear cell (BMNC) samples following exercise. IL-1β mRNA was detected in 1 muscle biopsy and 4 BMNC samples following exercise. In conclusion, the results of this study illustrates marathon running increases circulating inflammatory cytokines with IL-6 primarily produced locally in skeletal muscle and IL-1ra and IL-1β produced by circulating BMNC.

Starkie et al. (2001) chose to examine the influence of strenuous exercise on stimulated cytokine production. The authors anticipated endurance exercise would reduce stimulated cytokine production from circulating monocytes. Five male subjects participating in a marathon race volunteered for the present study. Blood samples were collected before, immediately following, and 2 and 24 hours following the marathon race. Plasma IL-6 and TNF-α were significantly elevated immediately post and 2 hours post exercise ($p < 0.01$). The percentage of monocytes spontaneously producing TNF-α were significantly decreased immediately following exercise when compared to pre exercise percentages (18.7 ± 8.4 and 4.7 ± 2.1 %, respectively, $p < 0.05$). The amount of TNF-α per cell decreased immediately following exercise and 2 hours post exercise compared to pre exercise levels (27.9 ± 2.1, 16.2 ± 0.8, and 18.0 ± 1.6 αμ, respectively, $p < 0.05$). These results indicate that strenuous exercise reduced TNF- α production by new and previously producing monocytes. Spontaneous production of IL-1α (4.5 ± 1.4, 0.0 ± 0.0, and 0.1 ± 0.1 $x10^7$/L, respectively, $p < 0.05$) and IL-6 (6.7 ± 1.7, 2.6 ± 0.1, and 3.9 ± 1.1

$x10^7$/L, respectively, $p < 0.05$) was reported to be decreased immediately following the race when compared to pre race numbers. The percentage of cells producing IL-1α (12.1 ± 3.3, 0.0 ± 0.0, and 0.0 ± 0.0, respectively, $p < 0.05$) and IL-6 (18.1 ± 3.7, 3.2 ± 0.9, and 4.2 ± .9, respectively, $p < 0.05$) significantly decreased immediately and 2 hours following exercise compared to pre exercise values. The findings of the present study revealed the number and percentage of circulating monocytes producing specific cytokines decreases following a marathon race. These results, together with the significant increases in creatine kinase levels post exercise suggest circulating levels of inflammatory cytokines may be a result of skeletal muscle damage associated with running.

Ostrowski et al. (1998) investigated cytokine response during and following 2.5 hours of treadmill running. Ten male subjects ran at 75% of $VO2_{max}$ and blood was collected before exercise, every 30 minutes during, and every hour for 6 hours following exercise. Plasma IL-6 levels increased 4-fold above pre exercise levels following the first 30 minutes of exercise ($p < 0.001$) and peaked at the end of exercise (25-fold, $p < 0.001$). Plasma IL-6 levels decreased during the 6 hours following exercise but remained significantly elevated above pre exercise levels (6-fold, $p < 0.001$). Plasma IL-1ra levels significantly increased from pre exercise levels 1 hour following exercise (9-fold, $p < 0.001$) and peaked 2 hours post exercise (18-fold, $p < 0.001$). Both TNF-α, and IL-1β remained near pre exercise levels during and following exercise. The present study revealed IL-6 levels significantly increased following 30 minutes of running and IL-1ra levels significantly increased after exercise with no change in TNF α and IL-1β. The authors believe the early detection of plasma IL-6 levels during marathon running may

have been related to exercise-induced muscle damage (Bruunsgard, Galbo, Halkjaer-Kristensen, Johansen, MacLean, & Pedersen, 1997; Ostrowski et al., 1998).

Ostrowski et al. (1999) investigated the response of both pro-and anti-inflammatory cytokine levels following a marathon race. Blood samples were collected before, immediately following, and every 30 minutes for 4 hours after a marathon race in ten male subjects. Circulating inflammatory IL-6 peaked immediately following exercise with a 128-fold increase from pre-race levels ($p < 0$ .001). IL-6 levels declined during the 4 hours following exercise but remained significantly elevated from pre exercise levels ($p < 0.001$). Anti-inflammatory IL-1ra peaked 1 hour following exercise with a 39-fold increase from pre exercise values ($p < 0.001$). Anti-inflammatory IL-10 levels peaked immediately post-race with a 27-fold increase from pre exercise levels. Cytokine inhibitors sTNF-r1 and sTNF-r2 both peaked 1 hour following exercise and remained significantly elevated during the 4 hours following exercise (2.7 and 1.6 fold, respectively, $p < 0.05$). Plasma levels of pro-inflammatory TNF-α and IL-1β peaked immediately following exercise 2.1-and 2.3-fold, respectively, ($p < 0.05$) and remained elevated during the 4 hours following exercise. The present study revealed both a significant pro- and anti-inflammatory cytokine response following a marathon race.

Drenth et al., (1995) and Weinstock, König, Harnischmacher, Keul, Berg, & Northoff, (1997) examined lipopolysaccharide (LPS) *ex vivo* stimulated production of inflammatory cytokines as well as circulating cytokine response before and after exercise. Drenth and associates collected blood samples 24 hours before and immediately following exercise from 21 male athletes participating in a 6 hour run competition. The distance covered by the subjects during the race ranged from 51.7 – 86.2 km. Circulating

levels of plasma TNF-α and IL-1β were unchanged following exercise. Plasma levels of IL-1ra increased from pre exercise levels following exercise (188 ± 72 to 886 ± 395 $pg{\cdot}mL^{-1}$, $p < 0.0005$) along with circulating levels of plasma IL-6 (18.5 ± 4.2 to 71.5 ± 33.3 $pg{\cdot}mL^{-1}$, $p < 0.0001$). Production of LPS-stimulated IL-1β and TNF-α were significantly suppressed following exercise when compared to 24 hour pre exercise values ($p < 0.0005$), whereas IL-1ra remained unchanged. The results of the present study revealed a decreases in post exercise LPS-stimulated production of TNF-α and IL-1β indicating a possible exercise-induced suppression of immune function.

In a similar study, Weinstock et al. (1997) investigated the influence of a sprint triathlon (400 m swim, 25 km bike, and 4 km run) on serum, urine and LPS-stimulated production of inflammatory cytokines. Blood and urine samples were collected 24 hours before, 1 hour after, and 20 hours following the triathlon from 15 male subjects. Serum and urine IL-6 ($p < 0.01$) and TNF-α ($p < 0.05$) levels were significantly higher 1 hour following exercise when compared to pre exercise levels and returned to pre exercise values 20 hours following exercise. LPS-stimulated levels of TNF-α, IL-1β, and IL-6 were significantly lower 1 hour following exercise ($p < 0.05$) when compared to pre exercise levels and returned to pre exercise levels 20 hours following exercise. The significant decrease in LPS-stimulated production of inflammatory cytokines following the triathlon race implies a temporary suppression in immune function which returned to resting levels 20 hours following exercise. The results reported by Drenth et al. and Weinstock et al., in addition to previous data support the "open window" hypothesis which states following severe exercise there is a window of time (3-72 h post exercise)

the immune system may be suppressed and susceptible to infection (Pedersen and Ullum, 1994).

Peake, Suzuki, Hordern, Wilson, Nosaka, & Coombs, (2005b) choose to compare the effect of moderate and high intensity running and down hill running on plasma anti-inflammatory cytokine levels. The alterations in anti-inflammatory cytokine levels were compared to previously reported changes in IL-6 levels, stress hormone levels, and myoglobin levels. Ten male subjects completed three randomized exercise trials on separate occasions. Subjects ran on a treadmill (0% grade) for 1 hour at 60% of $VO2_{max}$ (moderate intensity) or at 85% $VO2_{max}$ (high-intensity). The third trial was performed running downhill (-10%) on a treadmill for 45 minutes at 60% $VO2_{max}$. Blood samples were collected immediately before, immediately following, and 1 hour after each exercise trial. Analysis of IL-6, stress hormones, and myoglobin were performed on the same blood samples used to assess anti-inflammatory cytokine levels. Plasma IL-1ra levels were significantly higher following the high intensity trial compared to the moderate and down hill running trial immediately following exercise and 1 hour following exercise ($p < 0.05$). Plasma IL-10 levels were significantly higher following the high intensity trial when compared to the moderate intensity and the down hill trial ($p < 0.05$). The results of the present investigation indicate high intensity running has a greater effect on plasma IL-1ra and IL-10 levels than moderate intensity or down hill running. Plasma levels of IL-6 (17- and 4-fold), cortisol (2.9- and 0.6-fold), norepinephrine (1.5- and 0.5-fold), and epinephrine (2.5- and 1.1-fold) were reported to be higher in subjects following the high intensity trial versus the down hill trial, respectively (Peake, Suzuki, Hordern, Wilson, Nosaka, & Coombs, 2005a). Myoglobin levels were reported to be highest following the

down hill trial (11-fold) when compared to the high intensity trial (3-fold). Percent change in IL-1ra and IL-10 following exercise were reported to be significantly correlated with percent change in IL-6, cortisol, and norepinephrine. Changes in myoglobin and anti-inflammatory cytokine level following exercise were reported to be unrelated. The authors proposed the release of stress hormones during 1 hour of treadmill running may have a greater influence on plasma levels of IL-1ra and IL-10 versus exercise induced muscle damage

The Influence of Intermittent Exercise on Circulating Inflammatory Cytokines

The inflammatory cytokine response to team sport play and training is limited (Nieman et al., 2000; Nemet, Rose-Grottron, Mills, & Cooper, 2003). Previous research has primarily focused on the inflammatory cytokine response following endurance type exercise. However, the cytokine response in athletes participating in team sport may differ from those participating in endurance type exercise.

Nieman et al. (2000) examined the immune response in teenage tennis athletes following 2 hours of tennis drills. Twenty subjects (10 male and 10 female) performed eight 15 minute sessions of tennis drills with a 4-5 minute break between sessions. Blood samples were collected from the subjects immediately before exercise, immediately after and 1 hour following exercise. Serum levels of IL-6 did not significantly change following 2 hours of tennis drills. Serum IL-1ra levels significantly increased immediately following exercise (173 ± 12 to 212 ± 13, $p = 0.011$). The authors speculated the low cytokine levels observed in the present study were a result of the relatively low degree of stress associated with tennis drills

In a similar study Nemet et al. (2003) investigated the cytokine response to 1.5 hours of water polo training in ten female high school athletes. The water polo training session included a warm-up (20 minutes), conditioning drills (20 minutes), passing and shooting drills (40 minutes), and game drills (10 minutes) performed in the pool. Blood samples were collected immediately before and after exercise. Significant increases in serum levels of IL-6 (1.95 ± 0.45 to 6.72 ± 1.23 pg·mL^{-1}, $p < 0.05$) and IL-1ra (290 ± 58 to 464 ± 60 pg·mL^{-1}, $p < 0.05$) where observed following exercise. Decreases in serum levels of TNF-α were reported following exercise (2.58 ± 0.28 to 2.4 ± 0.25 pg·mL^{-1}, $p < 0.05$). Pre exercise serum IL-1β levels did not change following exercise. Nemet et al. concluded 1.5 hours of water polo training induces an acute inflammatory response similar to that reported following endurance exercise; however, the authors note the biological implications of the acute inflammatory response to water polo is unclear.

The Influence of Exercise on Salivary Inflammatory Cytokines

The measurement of salivary IgA levels following exercise has been extensively researched as discussed earlier, however; the measurement of salivary cytokine levels to assess immune function or inflammation in athletes following exercise has not been thoroughly examined. Saliva collection is simple, non invasive, and inexpensive and could potentially be an effective biological tool to study the influence of physical stress and exercise on cytokine and/or inflammatory response (Winkler et al., 2001).

Rossi, Phillips, Dannenbaum, Shepard, Glass, Conrad & Bullard, (2004) investigated the influence of a competitive collegiate American football season and a single American football game on salivary inflammatory cytokine response. Sixteen NCAA Division I football athletes volunteered to provide saliva samples before and after

a competitive collegiate football season, and 24 hours before and 1 hour after a single competitive collegiate football game. After rinsing their mouth with water, athletes were instructed to allow saliva to naturally accumulate in their mouth and then "drool" into sterile tubes for 10 minutes. Saliva samples were analyzed for IFN-γ and TNF-α. Subjects were grouped by number of downs to assess the influence of a single game on salivary cytokines (Grp1 = 0, Grp 2 = 1-10, Grp 3 = 11+ downs). No differences existed among groups at any time point for salivary IFN-γ and TNF-α; therefore groups were collapsed for further statistical analysis. Participation in a competitive football game had no influence on salivary TNF-α (N = 12, T2 = 4.3 ± .92, T3 = 5.9 ± 2.6 pg·mL-1; $p > 0.05$); however, there was a tendency for an increase in salivary IFN-γ levels (N = 13, T2 = 14.9 ± 3.7, T3 = 32.6 ± 8.8 pg·mL-1; $p = 0.07$). When cytokine concentrations were expressed per total protein, participation in a competitive football game had no influence on TNF-α (N = 12, T2 = 6.1 ± 2.2, T3 = 6.2 ± 3 pg·mg-1; $p > 0.05$); however, salivary IFN-γ concentrations per total protein significantly increased (N = 11, T2 = 11.8 ± 2.5, T3 = 32.5 ± 7.9 pg·mg-1; $p = 0.007$). Resting salivary TNF-α concentrations tended to increase over a competitive collegiate football season (N = 15, T1 = 3.68 ± 0.64, T4 = 7.9 ± 2.1 pg·mL-1, $p = 0.058$); whereas resting salivary IFN-γ levels were significantly elevated after the competitive season (N = 16, T1 = 15 ± 2.8, T4 = 40.5 ± 7.5 pg·mL-1; $p = 0.006$). Conversely, when resting salivary cytokine concentrations were expressed per total protein, significant increases were not observed over a competitive season in either TNF-α (N = 13, T1 = 5.4 ± 1.3, T4 = 7 ± 1.1 pg·mg-1; $p > 0.05$) or IFN-γ (N = 14, T1 = 23.8 ± 6.2, T4 = 37.6 ± 7.7 pg·mg-1; $p > 0.05$). The increase in salivary IFN-γ levels may indicate an acute systemic or oral inflammation in response to a competitive collegiate

football game. It appears that cytokine production in the oral environment mimics that observed in blood resultant to intense exercise.

Phillips, Rossi, Flores, Stewart, Stewart, Bunce, & Flynn, (2003) evaluated the influence of a long course triathlon on salivary levels of inflammatory IL-6, TNF-α, and sTNF-r1. Saliva samples were collected from 11 triathletes 48 and 24 hours before and immediately following a long course triathlon. Salivary IL-6 levels significantly increased from resting levels following the long course triathlon (N = 11, 25.1 ± 1.6 to 40.5 ± 3.3 pg.ml-1, p = 0.002). Pre exercise TNF-α levels were elevated following the long course triathlon (N =11; 1.24 ± 0.3 to 2.3 ± 0.6 pg ml-1, p = 0.051). Soluble-TNF-r1 levels following the long course triathlon were not significantly different from resting levels. The present data indicate participation in a long course triathlon significantly increases salivary IL-6 levels and a tendency for an increase in salivary TNF -α. Cytokine production in the oral environment appears to follow that reported in circulation following prolonged exercise

Minetto, Rainoldi, Gazzoni, Terzalo, Borrione, Termine, Saba, Dovio, Angeli, & Paccotti (2005) chose to compare salivary and serum IL-6 response to strenuous exercise. Seven subjects participated in 3 hours of submaximal cycling on stationary bikes. Blood and saliva samples were collected before and after exercise. A second group of ten subjects performed four sets of 20 repetitions of bilateral maximal isokinetic contractions ($180° s^{-1}$) of the knee flexor and extensor muscles. Subjects rested 30 seconds between each set and 3 minutes between each leg. Blood and saliva samples were collected before and immediately following exercise and 7, 15, 30, 45, 60, 90, and 120 minutes following exercise. Serum IL-6 levels were significantly elevated from pre exercise levels

following 3 hours of submaximal cycling (1.75 ± 1.06 to 11.07 ± 8.07 $pg \cdot mL^{-1}$, $p = 0.01$). Salivary IL-6 levels were also significantly elevated following exercise (0.79 ± 0.63 to 2.62 ± 2.67 $pg \cdot mL^{-1}$, $p = 0.046$). No significant correlation was reported between serum and salivary IL-6 levels, both before and after the cycling exercise. The maximal isokinetic exercise test did not induce significant changes in either serum or salivary IL-6 levels. No significant correlation was reported between serum and salivary IL-6 levels before and after the maximal isokinetic exercise test.

The study by Minetto et al. (2005) which is very similar to the current research project is the only study to our knowledge to compare salivary cytokine levels to blood cytokine levels following exercise. The significant increase in serum IL-6 levels following 3 hours of cycling was believed to be due to both metabolic and skeletal muscle fatigue. An increase in salivary IL-6 following cycling was suggested to be a result of a decrease in saliva flow rate associated with dehydration. The author propose the uncorrelated response of serum and salivary IL-6 levels before and after exercise is mostly likely due to the independent mechanisms associated with salivary and systemic release of IL-6. In conclusion, the authors stated prolonged submaximal cycling and maximal isokinetic exercise induces separate and independent responses of salivary and circulating IL-6.

Summary

Systemic inflammation associated with exercise is represented, partially, by elevations in plasma concentrations of inflammatory-related cytokines such as TNF-α, IL-1β, and IL-6 (Ostrowski et al., 1998; Moldoveanu, Shepard, & Shek, 2000; Smith, 2000). Elevated levels of systemic inflammatory cytokines following exercise may be a

result of exercise-induced muscle damage and /or increased stress hormone levels. Furthermore, LPS-stimulated cytokine production is decreased following intense exercise indicating a decrease in immune function.

The use of salivary cytokine measures in assessing immune function or inflammation has not been thoroughly explored in an exercise paradigm (Slavinsky 2002). The influence of team sport play and training on plasma and salivary inflammatory cytokines is unclear. Preliminary studies report mixed results concerning blood cytokine response following sport play but significant increases in salivary cytokine levels have been observed in response to team sport as well as endurance exercise. Saliva sampling provides an alluring technique for the collection of physiological data due to its simple and noninvasive nature.

CHAPTER III

METHODOLOGY

Subjects

The Institutional Review Board (IRB) of Oklahoma State University along with the athletic directors of Oklahoma Baptist University approved this study (Appendix D & E). A total of 19 male soccer players were recruited from Oklahoma Baptist University (9 varsity and 10 junior varsity players, 19 ± 1.7 years of age). All subjects were apparently healthy individuals as indicated by pre-season physical examinations conducted by Oklahoma Baptist University medical staff. Subjects were asked to refrain from alcohol consumption 24 hours prior to sample collection and to report any dental work done during the previous 2 weeks. Subjects were instructed to refrain from brushing teeth 3 hours prior to saliva collection and to refrain from eating 60 minutes prior to collection. Subjects were instructed to drink only water during both soccer games.

Table I
Data Collection Time Points

Preliminary Procedures	
Paper work	
Data Collection Procedures	
DAY 1	
8:00am	Blood and Saliva Collection
4:00pm	First Soccer Game
DAY 2	
3:00pm	Second Soccer Game
5:00pm	Blood and Saliva Collection
DAY 3	
8:00am	Blood and Saliva Collection

Preliminary Procedures

Oklahoma BaptistUniversity varsity and junior varsity soccer players were recruited during pre-season physicals. Junior varsity soccer athletes acted as age-matched controls. Subjects were selected from a group of 30 male soccer athletes. All subjects read and signed the IRB approved informed consent form and completed a medical history questionnaire to screen for contraindications that would exclude them from participation in the study. Subjects were excluded based on the following criteria: acute infections, recent oral surgery, injuries, and certain medications known to influence cytokine production such as non-steroidal anti-inflammatory medications or medical conditions that would interfere with saliva samples, such as sores in the mouth.

Blood and Saliva Collection Procedures and Exercise Protocol

Subjects reported to the soccer training complex 8 hours fasted for blood and saliva collection the morning (7:00-8:00 a.m.) of the first soccer game and the morning following the second soccer game. Subjects rested for 10 minutes and blood samples (20mL) were collected from an antecubital vein into pre-chilled evacuated tubes treated

with EDTA. Whole unstimulated saliva samples were collected from all subjects. After rinsing their mouth with water, subjects were instructed to allow saliva to naturally accumulate in their mouth and then expectorate into sterile tubes for 10 minutes. Collection time and volume of saliva was recorded to calculate saliva flow rate and cytokine levels were adjusted for secretion rate (pg•min). Blood and saliva samples were stored on ice and transported to Oklahoma State University for processing.

Soccer games consisted of two 45 minute halves with a 15 minute half time. Starters played an average of 76 minutes per game and non starters acting as age matched controls averaged no more than 2.8 minutes per game. Blood and saliva samples were collected from starters and non starters within 1 hour following the second soccer game.

Plasma and Salivary Cytokine Analysis

Plasma and salivary cytokine levels (TNF-α, IFN-γ, IL-6, IL-10) were determined using PeliKine compact enzyme-linked immuno-sorbent assay (ELISA) kits (Sanquin, Amsterdam, Netherlands). This assay method employs the quantitative "sandwich" enzyme immunoassay technique. Plasma and saliva were diluted 1:2 in dilution buffer before analysis. Diluted samples were added to microtiter plates coated with monoclonal antibody specific for the cytokine according to manufacturer's instructions. Cytokine present in the sample was bound to the immobilized antibody and after washing, a biotinylated antibody specific for the cytokine is added. Following another wash to remove any unbound antibody was the addition of a polymer of horseradish peroxidase conjugated to streptavidin (HRP). The microtiter plate was washed again to remove unbound HRP. A substrate solution containing tetramethlybenzidine (TMB) and hydrogen peroxide was added causing a color development. The color development was

proportional to the amount of cytokine in the sample. The color development was stopped after 30 minutes with sulphuric acid and the absorbance was measured within 30 minutes with a Universal Microplate Reader (BIO-TEK Instruments, Winooski, VT). Cytokine level was determined by comparing the optical density (490 nm) of each sample to a standard curve determined using standards for each cytokine. The minimum detectable concentration of plasma IL-6 was 0.2 $pg{\cdot}mL^{-1}$, and 1 $pg{\cdot}mL^{-1}$for IL-10, TNF-α, and IFN-γ. Values below the minimum detectable level were extrapolated using a proper software program for this purpose. Plasma and salivary cytokine levels were expressed were expressed as $pg{\cdot}mL^{-1}$. Salivary cytokine levels were also adjusted for secretion rate (pg•min).

Statistical Analysis

This study employed a 2 x 3 (group x time) repeated measures analysis of variance (ANOVA) with group at two levels (VAR vs. JV) and time at three levels (6 hours prior to the first soccer game, 1 hour immediately following, and 15 hours following the second soccer game). An analysis was conducted for each of the following dependent variables: plasma and salivary IL-6, plasma and salivary IL-10, plasma and salivary TNF-α, and plasma and salivary INF-γ. Pearson correlations were calculated to determine if significant relationships existed among baseline plasma and salivary cytokine levels for both starters and non starters (n = 19) and to the absolute change from baseline levels in starters 1 and 15 hours post game two. Histogram and frequency analysis were performed on all cytokine data to determine normal distributions. A square root transformation was performed on all cytokine data. The transformed data were used

in statistical analysis. The level of significance was set at $p < 0.05$. Data were analyzed with SPSS 12.0.

-CHAPTER IV

RESULTS AND DISCUSSION

Introduction

This chapter reports the analysis for the dependent variables in male college soccer players. The first aim of the this study was to measure the response of plasma and salivary cytokines IL-6, IL-10, TNF- α, and IFN- γ in soccer players competing in two consecutive collegiate soccer games. The second aim was to compare plasma and salivary cytokine levels at rest and following exercise to determine if similarities exist between plasma and salivary cytokine levels. Subjects were grouped by starters, players who played and non starters, players who did not play.

Descriptive Statistics

Descriptive information for all soccer players were collected during pre season physical examinations conducted at Oklahoma Baptist University. A total of 19 subjects (10 starters & 9 non starters) volunteered for the study. The starters played an average of 76 minutes per game and the non starters played an average of 2.8 minutes per game. The combined mean values and standard deviations for both starters and non starters for age, weight, height, and percent body fat were 19.53 ± 1.31 years, 76.97 ± 6.84 kg, 178.47 ± 2.86 cm, and 13.23 ± 4.83 percent, respectively (Table II). The mean values and standard deviations for the starter's age, weight, height, and percent body fat were 20.3 ± 1.34 years, 76.94 ± 8.22 kg, 178.31 ± 3.33 cm, and 11.45 ± 3.51 percent,

respectively (Table III). The mean values and standard deviations for the non starter's age, weight, height, and percent body fat were 18.67 ± 0.5 years, 77.0 ± 5.40 kg, 178.6 ± 2.45 cm, and 15.21 ± 5.50 percent, respectively (Table IV).

Table II
Combined Descriptive Statistics

	N	Mean	Std. Deviation
AGE (y)	19	19.5263	1.30675
WEIGHT (kg)	19	76.9684	6.83919
HEIGHT (cm)	19	178.4682	2.86438
BODY FAT (%)	19	13.2316	4.82713

Table III
Descriptive Statistics of Starters

	N	Mean	Std. Deviation
AGE (y)	10	20.3000	1.33749
WEIGHT (kg)	10	76.9400	8.22168
HEIGHT (cm)	10	178.3080	3.32666
BODY FAT (%)	10	11.4500	3.50785

Table IV
Descriptive Statistics of Non Starters

	N	Mean	Std. Deviation
AGE (y)	9	18.6667	.50000
WEIGHT (kg)	9	77.0000	5.40324
HEIGHT (cm)	9	178.6000	2.44949
BODY FAT (%)	9	15.2111	5.49624

Hypotheses

Data in the current study were analyzed with a 2 x 3 (group x time) repeated measures analysis of variance (ANOVA) to determine if there were significant differences between and within groups. Pearson correlations were calculated to determine if significant relationships existed among plasma and salivary cytokine levels at rest and following exercise. The level of significance was set at $p < 0.05$.

Hypothesis 1

Ho1 = There will be no significant difference in salivary IL-6 levels adjusted for secretion rate between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in relative salivary IL-6 levels between starters and non starters among all time points [$F(1, 11) = 1.001$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 22) = 0.280$; $p > 0.05$]. The main effects of time were revealed to be significant [$F(2, 22) = 4.812$; $p = 0.018$] (Table V & Figure 1). Based on the current results the null hypothesis will be accepted.

Table V
Relative Salivary IL-6 Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	1.774	1.0017	7
	Non starters	1.461	.5457	6
	Total	1.630	.8075	13
1h post game 2	Starters	2.625	1.2309	7
	Non starters	1.992	.9352	6
	Total	2.333	1.1090	13
15h post game 2	Starters	1.804	.8795	7
	Non starters	1.500	.6051	6
	Total	1.664	.7511	13

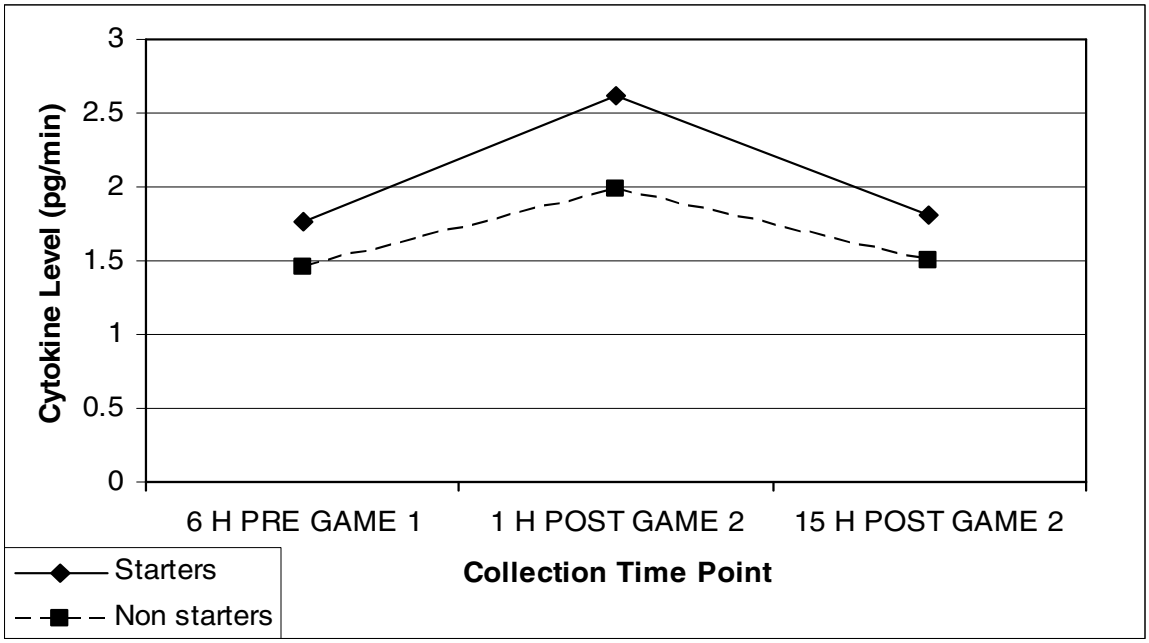

Figure 1. Relative Salivary IL-6 Level.

Hypothesis 2

Ho2 = There will be no significant difference in absolute salivary IL-6 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in absolute salivary IL-6 levels between starters and non starters among all time points [$F(1, 11) = 0.314$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 22) = 0.052$; $p > 0.05$]. The main effects of time were revealed to be significant [$F(2, 22) = 1.089$; $p > 0.05$] (Table VI & Figure 2). Based on the current results the null hypothesis will be accepted.

Table VI
Absolute Salivary IL-6 Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	3.783	2.6565	7
	Non starters	3.159	1.0897	6
	Total	3.495	2.0318	13
1h post game 1	Starters	4.121	1.7319	7
	Non starters	3.715	1.0928	6
	Total	3.934	1.4289	13
15h post game 2	Starters	3.532	1.8692	7
	Non starters	3.147	1.1306	6
	Total	3.354	1.5230	13

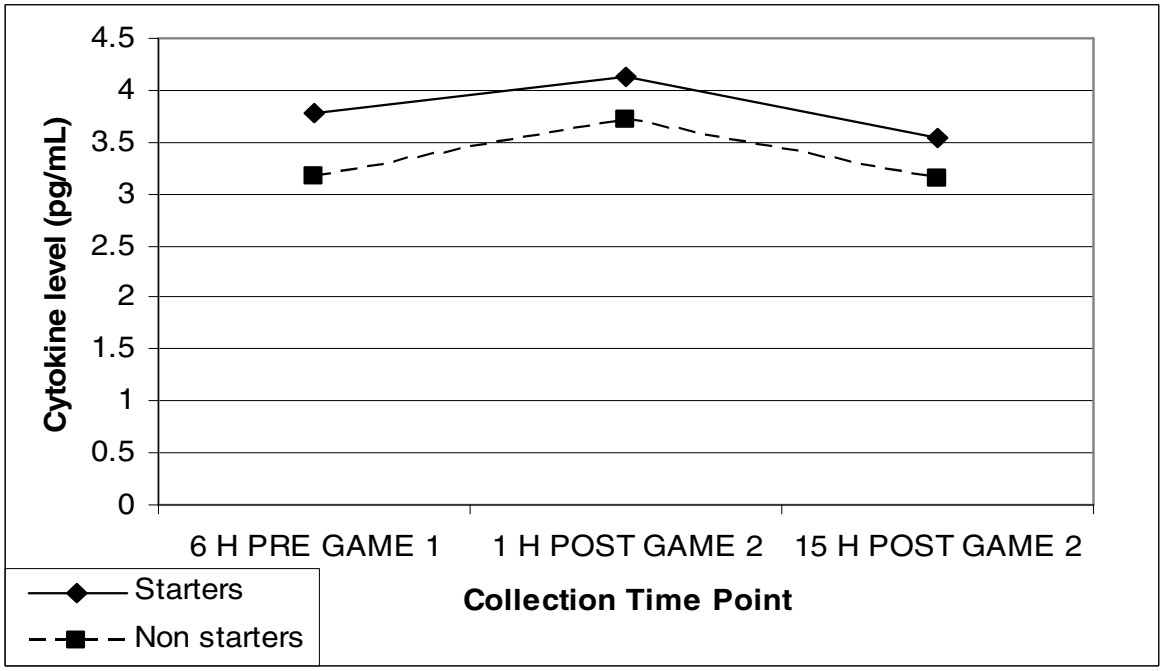

Figure 2. Absolute Salivary IL-6 Level.

Hypothesis 3

Ho3 = There will be no significant difference in plasma IL-6 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in plasma IL-6 levels between starters and non starters among all time points

[F (1, 8) = 0.925; p > 0.05]. There were no significant within-groups interaction across time x group [F (2, 16) = 0.835; p > 0.05]. The main effects of time were revealed to be statistically non significant [F (2, 16) = 0.450; p > 0.05] (Table VII & Figure 3). Based on the current results the null hypothesis will be accepted.

Table VII
Plasma IL-6 Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	2.083	.2730	6
	Non starters	1.798	.8177	4
	Total	1.969	.5347	10
1h post game 2	Starters	2.578	.4766	6
	Non starters	1.749	.6973	4
	Total	2.246	.6864	10
15h post game 2	Starters	2.242	1.5184	6
	Non starters	2.186	.2493	4
	Total	2.220	1.1413	10

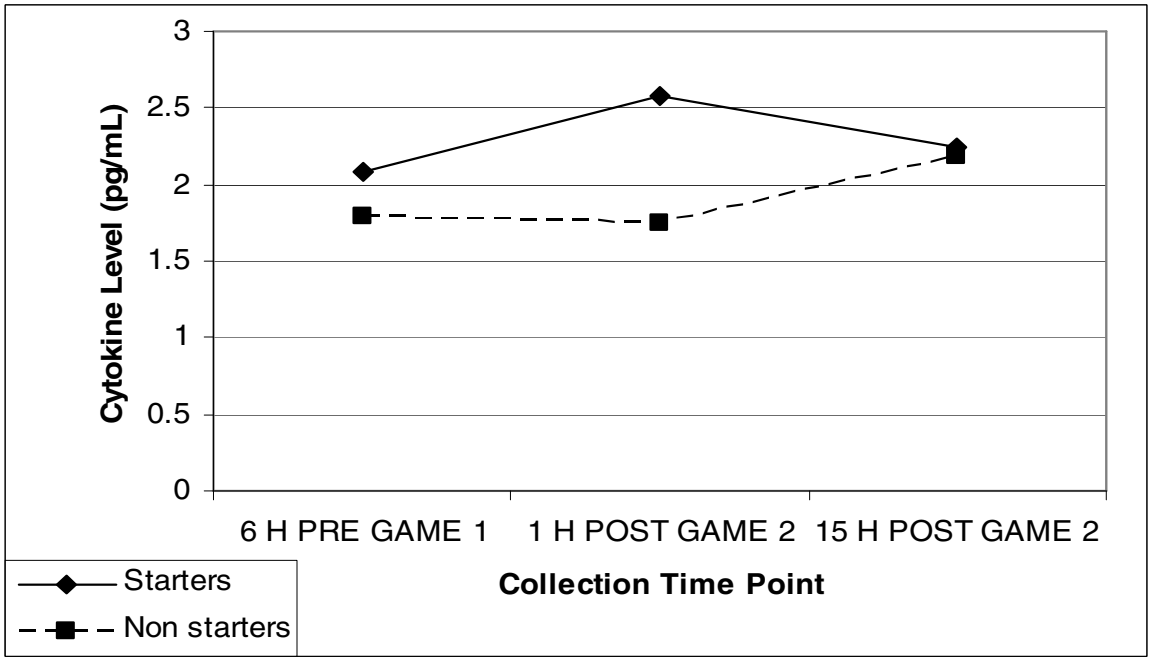

Figure 3. Plasma IL-6 Level.

Hypothesis 4

Ho4 = There will be a non significant relationship between salivary IL-6 levels and plasma IL-6 level 6 hours before the first soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary IL-6 levels adjusted for secretion rate and plasma IL-6 levels before the first soccer game ($r = 0.15$, $n = 13$; $p > 0.05$). A non significant relationship existed between pre exercise relative salivary IL-6 level and plasma IL-6 level ($r = -0.179$, $n = 13$; $p > 0.05$) (Table VIII). Based on the current results the null hypothesis will be accepted

Table VIII
Baseline IL-6 Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	1.631	.8693	16
Saliva (pg/ml)	3.555	1.8284	16
Plasma (pg/ml)	2.170	.5730	16

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.658**	.015
	Sig. (2-tailed)	.	.006	.961
	N	16	16	13
Saliva (pg/ml)	Pearson Correlation	.658**	1	-.179
	Sig. (2-tailed)	.006	.	.559
	N	16	16	13
Plasma (pg/ml)	Pearson Correlation	.015	-.179	1
	Sig. (2-tailed)	.961	.559	.
	N	13	13	16

**. Correlation is significant at the 0.01 level (2-tailed).

Hypothesis 5

Ho5 = There will be a non significant relationship between salivary IL-6 levels and plasma IL-6 level 1 hour following the second soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary IL-6 levels adjusted for secretion rate and plasma IL-6 levels 1 hour

post the second soccer game (r = -0.116, n = 6; p > 0.05). A non significant relationship existed between absolute salivary IL-6 level and plasma IL-6 level 1 hour post the second soccer game (r = -0.536, n = 6; p > 0.05) (Table IX). Based on the current results the null hypothesis will be accepted.

Table IX
1 Hour Post IL-6 Pearson Correlations

	Mean	Std. Deviation	N
Saliva (pg/min)	.8681	.97051	8
Saliva (pg/ml)	.4399	2.19947	8
Plasma (pg/ml)	.7274	.66772	7

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.703	-.116
	Sig. (2-tailed)	.	.052	.827
	N	8	8	6
Saliva (pg/ml)	Pearson Correlation	.703	1	-.536
	Sig. (2-tailed)	.052	.	.273
	N	8	8	6
Plasma (pg/ml)	Pearson Correlation	-.116	-.536	1
	Sig. (2-tailed)	.827	.273	.
	N	6	6	7

Hypothesis 6

Ho6 = There will be a non significant relationship between salivary IL-6 levels and plasma IL-6 level 15 hours following the second soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary IL-6 levels adjusted for secretion rate and plasma IL-6 levels 15 hours following the second soccer game (r = -0.029, n = 5; p > 0.05). A non significant relationship existed between absolute salivary IL-6 levels and plasma IL-6 levels 15 hours post game two (r = 0.245, n = 5; p > 0.05) (Table X). Based on the current results the null hypothesis will be accepted.

Table X
15 Hours Post IL-6 Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	.0887	.88773	8
Saliva (pg/ml)	-.3349	1.60952	8
Plasma (pg/ml)	.1597	1.39623	6

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.812*	-.029
	Sig. (2-tailed)	.	.014	.963
	N	8	8	5
Saliva (pg/ml)	Pearson Correlation	.812*	1	.245
	Sig. (2-tailed)	.014	.	.691
	N	8	8	5
Plasma (pg/ml)	Pearson Correlation	-.029	.245	1
	Sig. (2-tailed)	.963	.691	.
	N	5	5	6

*. Correlation is significant at the 0.05 level (2-tailed).

Hypothesis 7

Ho7 = There will be no significant difference in salivary IL-10 levels adjusted for secretion rate between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in salivary IL-10 levels adjusted for secretion rate between starters and non starters [$F(1, 6) = 1.478$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 12) = 0.443$; $p > 0.05$]. The main effects of time were revealed to be statistically non significant [$F(2, 12) = 0.460$; $p > 0.05$] (Table XI & Figure 4). Based on the current results the null hypothesis will be accepted.

Table XI
Relative Salivary IL-10 Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	1.646	1.0661	5
	Non starters	1.725	.8575	3
	Total	1.676	.9280	8
1h post game 2	Starters	1.994	1.0868	5
	Non starters	1.322	1.1081	3
	Total	1.742	1.0708	8
15h post game 2	Starters	1.655	.8810	5
	Non starters	.848	.3500	3
	Total	1.352	.8082	8

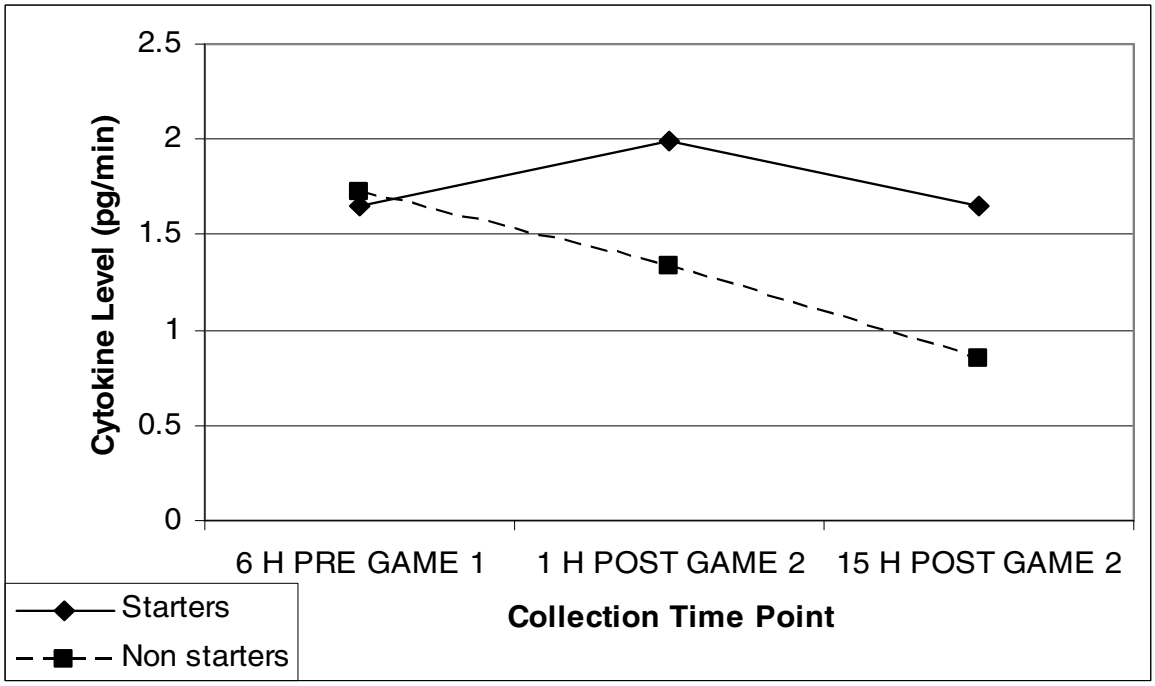

Figure 4. Relative Salivary IL-10 Level.

Hypothesis 8

Ho8 = There will be no significant difference in absolute salivary IL-10 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in absolute salivary IL-6 levels between starters and non starters among all

time points[F (1, 6) = 0.281; p > 0.05]. There were no significant within-groups interaction across time x group [F (2, 12) = 1.249; p > 0.05]. The main effects of time were revealed to be statistically non significant [F (2, 22) = 0.027; p > 0.05] (Table XII & Figure 5). Based on the current results the null hypothesis will be accepted.

Table XII
Absolute Salivary IL-10 Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	starters	2.415	1.0169	5
	nonstarters	2.525	.1902	3
	Total	2.456	.7775	8
1h post game 2	starters	2.762	1.3834	5
	nonstarters	1.944	.9103	3
	Total	2.455	1.2285	8
15h post game 2	starters	3.157	1.3374	5
	nonstarters	1.682	1.3252	3
	Total	2.604	1.4515	8

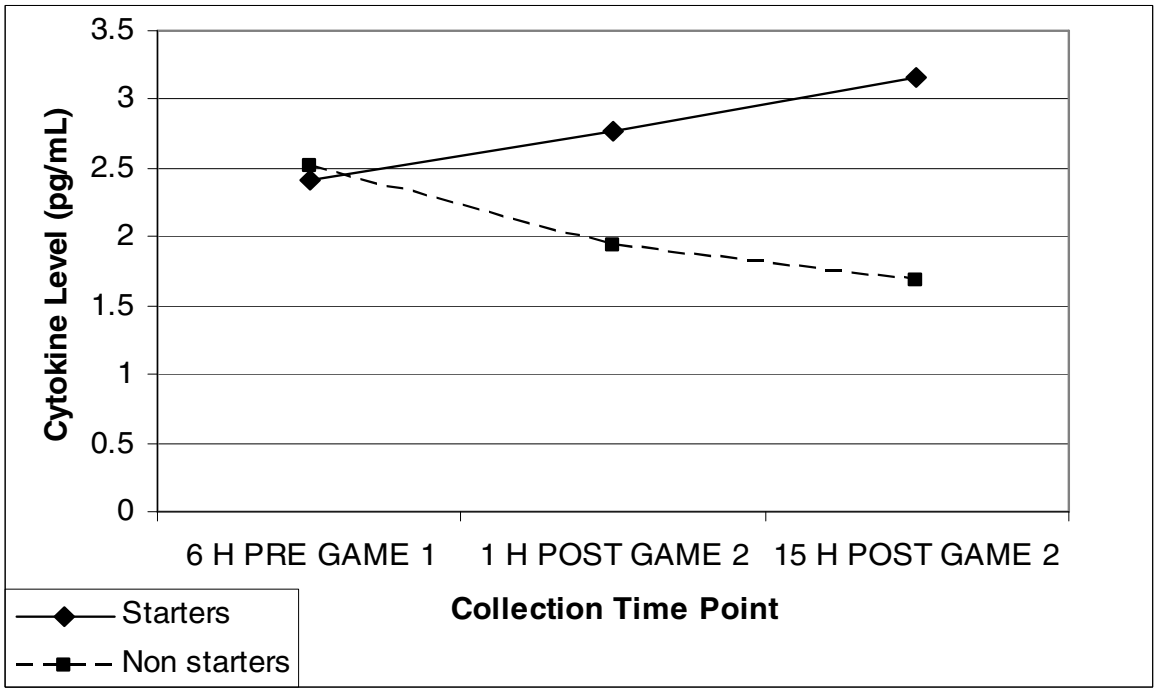

Figure 5. Absolute Salivary IL-10 Level.

Hypothesis 9

Ho9 = There will be no significant difference in plasma IL-10 levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in plasma IL-6 levels between starters and non starters among all time points [$F(1, 8) = 1.088$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 16) = 1.006$; $p > 0.05$]. The main effects of time were revealed to be statistically non significant [$F(2, 16) = 0.331$; $p > 0.05$] (Table XIII & Figure 6). Based on the results the null hypothesis will be accepted.

Table XIII
Plasma IL-10 Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	1.480	.6916	4
	Non starters	2.109	1.2963	6
	Total	1.858	1.0948	10
1h post game 2	Starters	1.988	.8159	4
	Non starters	2.074	1.0856	6
	Total	2.039	.9373	10
15h post game 2	Starters	1.508	.6850	4
	Non starters	2.444	.8942	6
	Total	2.070	.9136	10

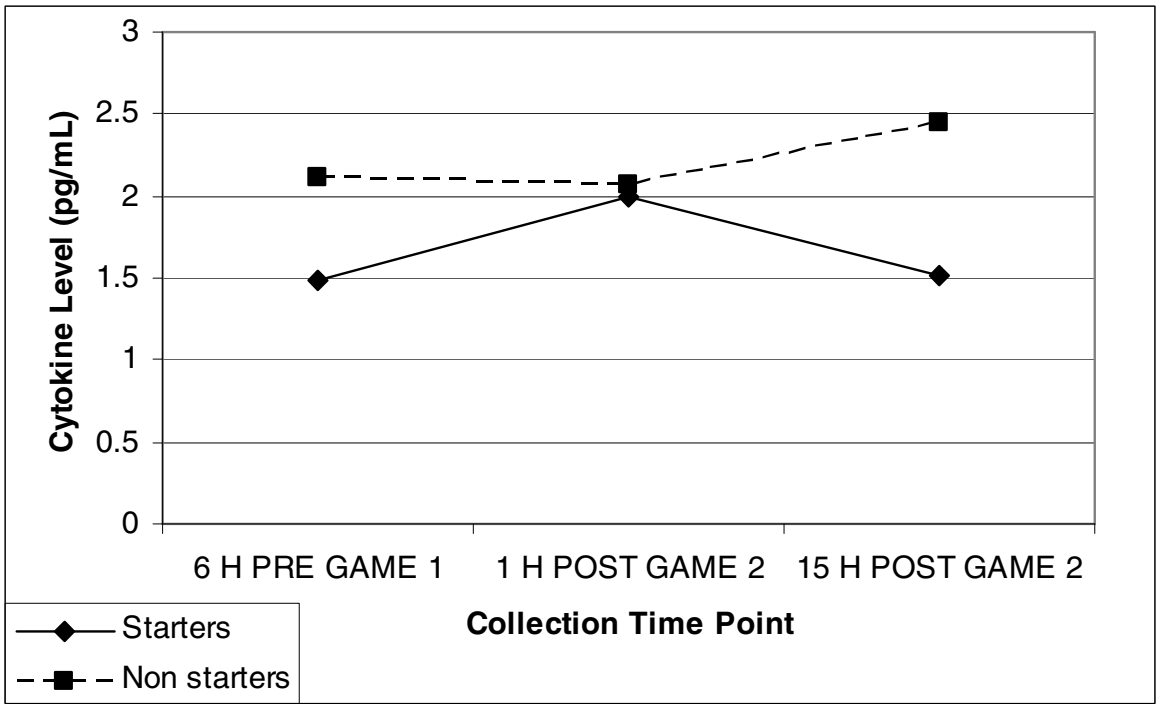

Figure 6. Plasma IL-10 Level.

Hypothesis 10

Ho10 = There will be a non significant relationship between salivary IŁ 10 levels and plasma IL-10 level 6 hours before the first soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary IL-10 levels adjusted for secretion rate and plasma IL-10 levels ($r = 0.167$, $n = 9$; $p > 0.05$). A non significant relationship existed between pre exercise absolute salivary IL-10 level and plasma IL-10 level ($r = 0.463$, $n = 9$; $p > 0.05$) (Table XIV). Based on the current results the null hypothesis will be accepted.

Table XIV
Baseline IL-10 Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	1.475	.7861	13
Saliva (pg/ml)	2.450	.6298	13
Plasma (pg/ml)	2.026	1.0201	14

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.738**	.167
	Sig. (2-tailed)	.	.004	.668
	N	13	13	9
Saliva (pg/ml)	Pearson Correlation	.738**	1	.463
	Sig. (2-tailed)	.004	.	.210
	N	13	13	9
Plasma (pg/ml)	Pearson Correlation	.167	.463	1
	Sig. (2-tailed)	.668	.210	.
	N	9	9	14

**. Correlation is significant at the 0.01 level (2-tailed).

Hypothesis 11

Ho11 = There will be a non significant relationship between salivary IL-10 levels and plasma IL-10 level 1 hour following the second soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary IL-10 levels adjusted for secretion rate and plasma IL-10 levels 1 hour following the second game ($r = 0.819$, $n = 4$; $p > 0.05$). A statistically significant relationship existed between absolute salivary IL-10 level and plasma IL-10 level 1 hour post the second game ($r = 0.957$, $n = 4$; $p = 0.043$) 1 hour following the second soccer game (Table XV & Figure 7). Based on the current results the null hypothesis will be accepted.

Table XV
1 Hour Post IL-10 Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	.3073	1.36708	7
Saliva (pg/ml)	.1393	1.57497	7
Plasma (pg/ml)	.2672	1.41053	5

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.951**	.819
	Sig. (2-tailed)	.	.001	.181
	N	7	7	4
Saliva (pg/ml)	Pearson Correlation	.951**	1	.957*
	Sig. (2-tailed)	.001	.	.043
	N	7	7	4
Plasma (pg/ml)	Pearson Correlation	.819	.957*	1
	Sig. (2-tailed)	.181	.043	.
	N	4	4	5

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

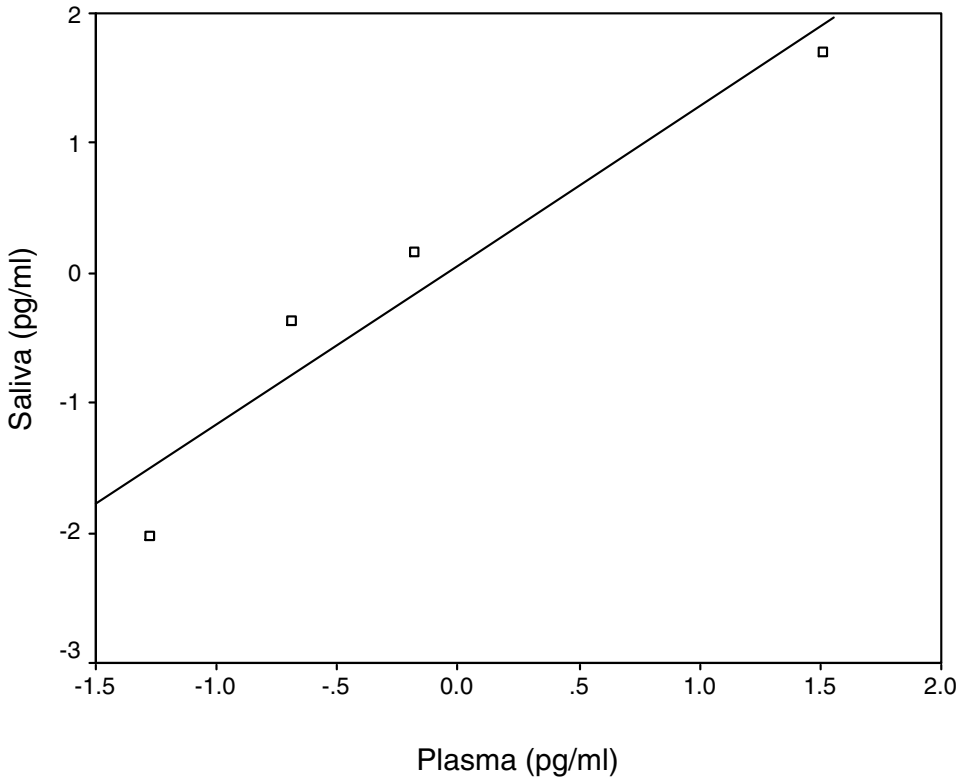

Figure 7. Significant correlation between absolute salivary IL-10 and plasma IL-10 levels in starters 1h post game two. (r = 0.957, n = 4; p = 0.043)

Hypothesis 12

Ho12 = There will be a non significant relationship between salivary IL-10 levels and plasma IL-10 level 15 hours following the second soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary IL-10 levels adjusted for secretion rate and plasma IL-10 levels 15 hours following the second soccer game ($r = -0.567$, $n = 3$; $p > 0.05$). A non significant relationship existed between relative salivary IL-10 level and plasma IL-10 15 hours post game two ($r = -0.611$, $n = 3$; $p > 0.05$) 15 hours following the second soccer game (Table XVI). Based on the current results the null hypothesis will be accepted.

Table XVI
15 Hour Post IL-10 Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	.0086	1.74723	5
Saliva (pg/ml)	.7423	1.71060	5
Plasma (pg/ml)	.0274	.15462	4

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.959**	-.567
	Sig. (2-tailed)	.	.010	.616
	N	5	5	3
Saliva (pg/ml)	Pearson Correlation	.959**	1	-.611
	Sig. (2-tailed)	.010	.	.582
	N	5	5	3
Plasma (pg/ml)	Pearson Correlation	-.567	-.611	1
	Sig. (2-tailed)	.616	.582	.
	N	3	3	4

**. Correlation is significant at the 0.01 level (2-tailed).

Hypothesis 13

Ho13 = There will be no significant difference in salivary TNF-α levels adjusted for secretion rate between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in salivary TNF-α levels adjusted for secretion rate between starters and non starters among all time points [$F(1, 8) = 2.093$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 16) = 1.068$; $p > 0.05$]. The main effects of time were revealed to be statistically non significant [$F(2, 16) = 1.843$; $p > 0.05$] (Table XVII & Figure 8). Based on the current results the null hypothesis will be accepted.

Table XVII
Relative Salivary TNF-α Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	2.789	2.1164	5
	Non starters	2.191	.7910	5
	Total	2.490	1.5389	10
1h post game	Starters	6.131	3.5898	5
	Non starters	2.793	3.0446	5
	Total	4.462	3.5976	10
15h post game 2	Starters	3.819	3.2811	5
	Non starters	2.925	1.3157	5
	Total	3.372	2.4034	10

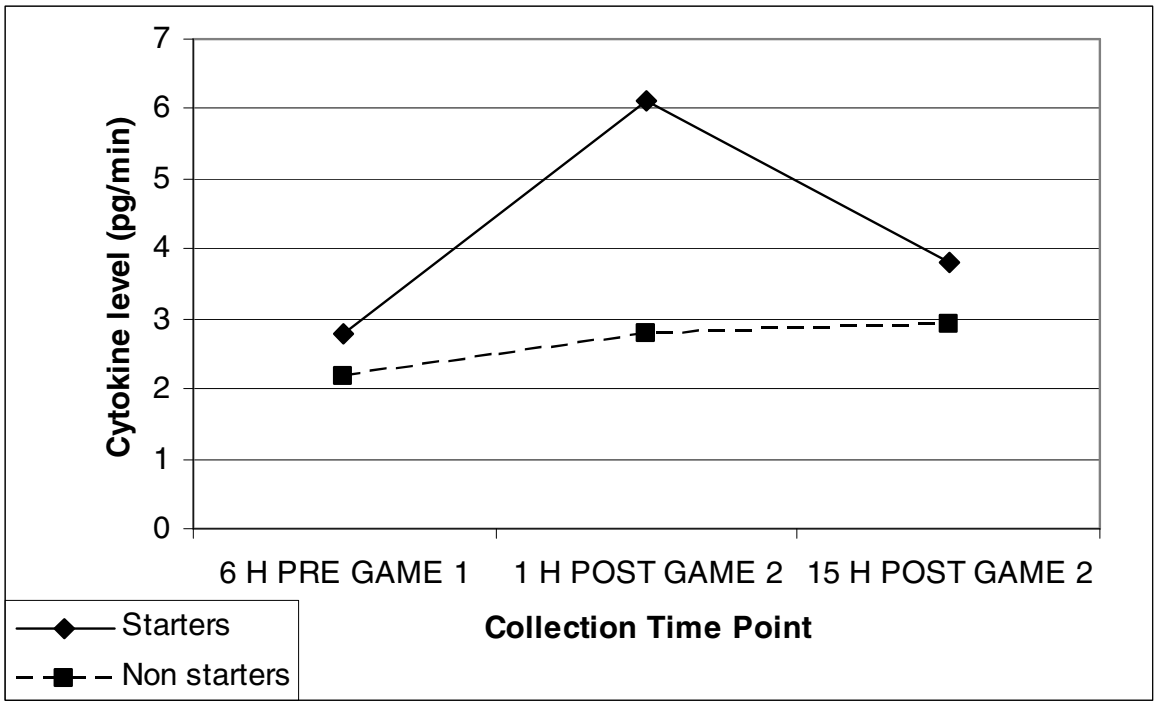

Figure 8. Relative Salivary TNF-α Level.

Hypothesis 14

Ho14 = There will be no significant difference in absolute salivary TNF-α levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in absolute salivary TNF-α levels between starters and non starters among all time points [$F(1, 8) = 0.710$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 16) = 0.970$; $p > 0.05$]. The main effects of time were revealed to be statistically non significant [$F(2, 16) = 1.149$; $p > 0.05$] (Table XVIII & Figure 9). Based on the current results the null hypothesis will be accepted.

Table XVIII
Absolute Salivary TNF-α Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	5.150	1.7371	5
	Non starters	5.278	2.1457	5
	Total	5.214	1.8417	10
1h post game 2	Starters	9.460	5.7136	5
	Non starters	5.459	3.8246	5
	Total	7.459	5.0455	10
15h post game 2	Starters	7.367	5.7153	5
	Non starters	6.704	3.1972	5
	Total	7.035	4.3798	10

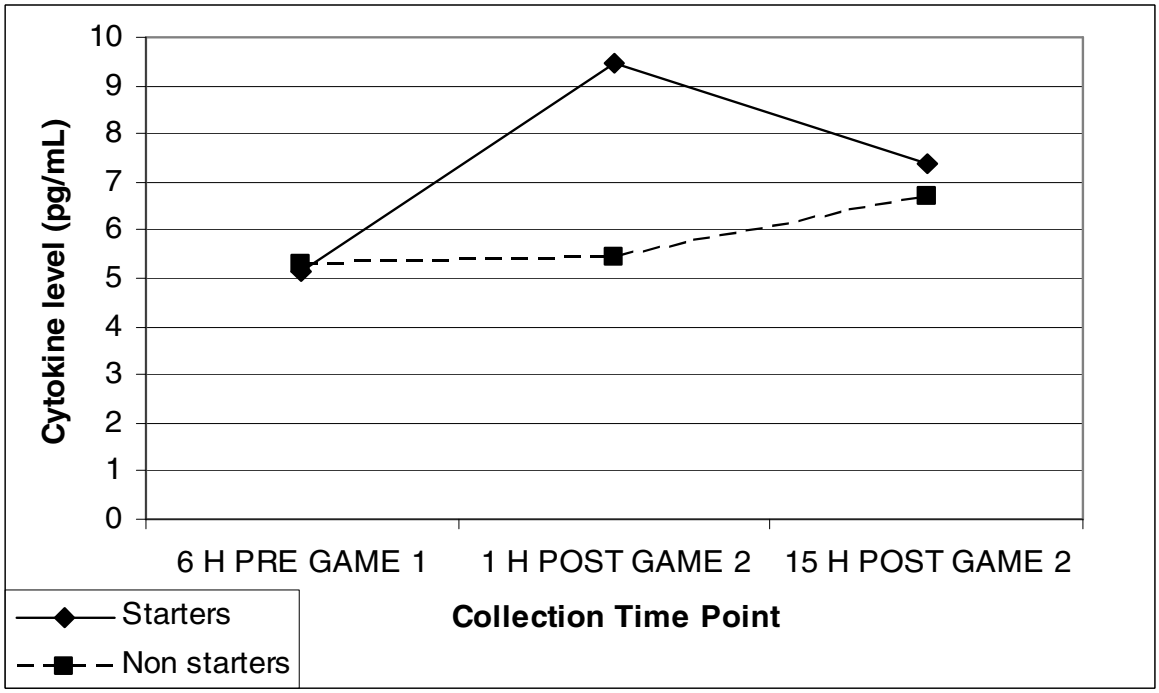

Figure 9. Absolute Salivary TNF-α Level.

Hypothesis 15

Ho15 = There will be no significant difference in plasma TNF-α levels between starters and nonstarters among all time points, which include 6 hours before the first game and 1 and 15 hours following the second soccer game.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in plasma TNF-α levels between starters and non starters among all time points

[F (1, 8) = 0.001; p > 0.05]. There were no significant within-groups interaction across time x group [F (2, 16) = 0.597; p > 0.05]. The main effects of time were revealed to be statistically non significant [F (2, 16) = .086; p > 0.05] (Table XIX & Figure 10). Based on the current results the null hypothesis will be accepted.

Table XIX
Plasma TNF-α Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	2.986	.5872	4
	Non starters	3.428	1.2006	6
	Total	3.251	.9837	10
1h post game 2	Starters	3.241	.4785	4
	Non starters	3.160	1.4813	6
	Total	3.193	1.1389	10
15h post game 2	Starters	3.232	1.3676	4
	Non starters	2.923	.6074	6
	Total	3.047	.9241	10

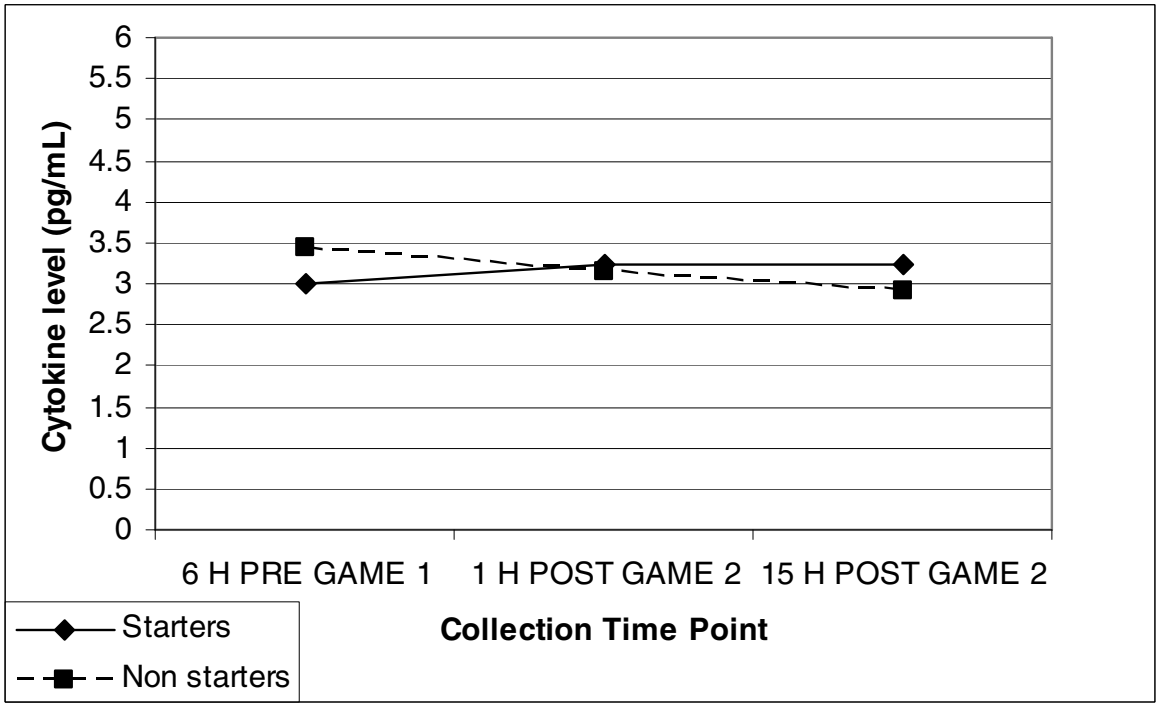

Figure 10. Plasma TNF-α Level.

Hypothesis 16

Ho16 = There will be a non significant relationship between salivary TNF-α levels and plasma TNF-α level 6 hours before the first soccer game.

The Pearson moment correlation revealed a non significant relationship between baseline salivary TNF-α levels adjusted for secretion rate and baseline plasma TNF-α ($r = -0.280$, $n = 10$; $p > 0.05$). A non significant relationship existed between pre exercise absolute salivary TNF-α level and plasma TNF-α level ($r = -0.409$, $n = 10$; $p > 0.05$) (Table XX). Based on the current results the null hypothesis will be accepted.

Table XX
Baseline TNF-α Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	2.558	1.2998	14
Saliva (pg/ml)	5.270	1.8426	14
Plasma (pg/ml)	3.018	.9725	14

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.659*	-.280
	Sig. (2-tailed)	.	.010	.433
	N	14	14	10
Saliva (pg/ml)	Pearson Correlation	.659*	1	-.409
	Sig. (2-tailed)	.010	.	.241
	N	14	14	10
Plasma (pg/ml)	Pearson Correlation	-.280	-.409	1
	Sig. (2-tailed)	.433	.241	.
	N	10	10	14

*. Correlation is significant at the 0.05 level (2-tailed).

Hypothesis 17

Ho17 = There will be a non significant relationship between salivary TNF-α levels and plasma TNF-α level 1 hour following the second soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary TNF-α levels adjusted for secretion rate and plasma TNF-α levels 1 hour post the

second soccer game (r = -0.733, n = 4; p > 0.05). A statistically significant relationship existed between absolute salivary TNF-α levels and plasma TNF-α levels 1 hour post the second soccer game (r = -0.997, n = 4; p = 0.003) (Table XXI & Figure 11). Based on the current results the null hypothesis will be accepted.

Table XXII
1 Hour Post TNF-α Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	2.1958	4.16874	7
Saliva (pg/ml)	4.1220	5.55658	7
Plasma (pg/ml)	.2332	.77651	5

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.797*	-.733
	Sig. (2-tailed)	.	.032	.267
	N	7	7	4
Saliva (pg/ml)	Pearson Correlation	.797*	1	-.997**
	Sig. (2-tailed)	.032	.	.003
	N	7	7	4
Plasma (pg/ml)	Pearson Correlation	-.733	-.997**	1
	Sig. (2-tailed)	.267	.003	.
	N	4	4	5

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

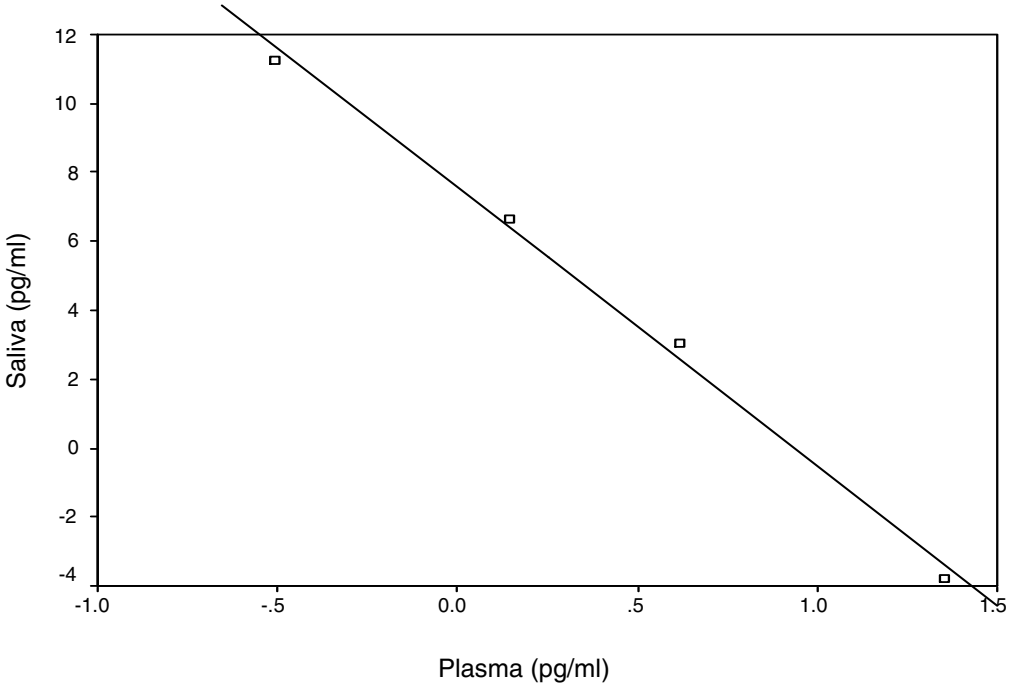

Figure 11. Significant negative correlation between absolute salivary TNF-α levels and plasma TNF-α levels in starters 1 hour post game two(r = -.997, n = 4, p = 0.003).

Hypothesis 18

Ho18 = There will be a non significant relationship between salivary TNF-α levels and plasma TNF-α level 15 hours following the second soccer game.

The Pearson moment correlation revealed a non significant relationship between salivary TNF-α levels adjusted for secretion rate and plasma TNF-α levels 15 hours following the second soccer game ($r = -0.913$, $n = 3$; $p > 0.05$). A non significant relationship existed between absolute salivary TNF-α level and plasma TNF-α level 15 hours post game two ($r = -0.760$, $n = 3$; $p > 0.05$) (Table XXIII). Based on the current results the null hypothesis will be accepted.

Table XXII
15 Hour Post TNF-α Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	1.0307	3.84305	5
Saliva (pg/ml)	2.2164	5.98609	5
Plasma (pg/ml)	.2462	1.67404	4

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/ml)	Pearson Correlation	1	.981**	-.913
	Sig. (2-tailed)	.	.003	.267
	N	5	5	3
Saliva (pg/ml)	Pearson Correlation	.981**	1	-.760
	Sig. (2-tailed)	.003	.	.451
	N	5	5	3
Plasma (pg/ml)	Pearson Correlation	-.913	-.760	1
	Sig. (2-tailed)	.267	.451	.
	N	3	3	4

**. Correlation is significant at the 0.01 level (2-tailed).

Hypothesis 19

Ho 19 = There will be no significant difference in salivary INF γ levels adjusted for secretion rate between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in relative salivary INF-γ levels between starters and non starters among all time points [$F(1, 12) = 2.692$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 24) = 3.046$; $p = 0.066$]. The main effects of time were revealed to be statistically non significant [$F(2, 24) = 3.267$; $p = 0.056$] (Table XXIII & Figure 12). Based on the current results the null hypothesis will be accepted

Table XXIII
Relative Salivary INF-γ Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	4.828	3.7687	8
	Non starters	3.770	2.9947	6
	Total	4.375	3.3752	14
1h post game 2	Starters	8.254	4.9398	8
	Non starters	3.555	2.1221	6
	Total	6.240	4.5492	14
15h post game 2	Starters	5.293	2.9979	8
	Non starters	3.068	1.8675	6
	Total	4.339	2.7360	14

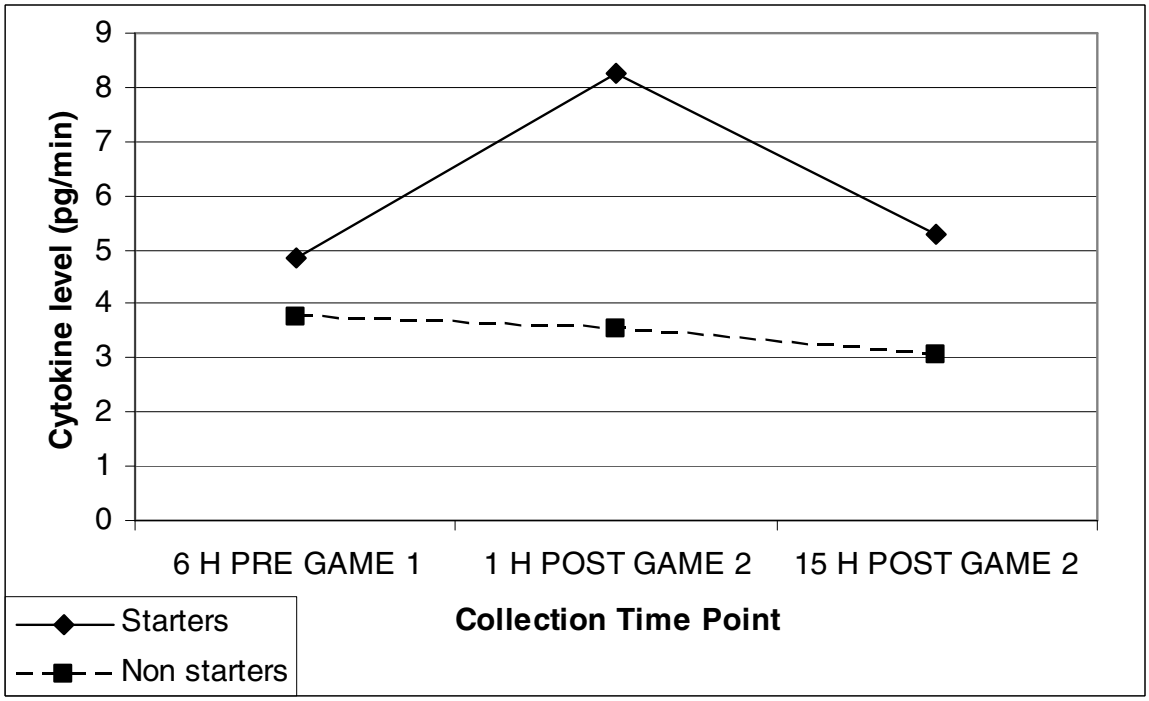

Figure 12. Relative Salivary INF-γ Level.

Hypothesis 20

Ho20 = There will be no significant difference in absolute salivary INF-γ levels between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in absolute salivary INF-γ levels between starters and non starters 6 hours

before the first game and 1 and 15 hours following the second soccer game [F (1, 12) = 2.683; p > 0.05]. There were no significant within-groups interaction across time x group [F (2, 24) = 3.259; p = 0.056]. The main effects of time were revealed to be statistically non significant [F (2, 24) = 0.546; p > 0.05] (Table XXIV & Figure 13). Based on the current results the null hypothesis will be accepted.

Table XXIV
Absolute Salivary INF-γ Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	9.304	5.7722	8
	Non starters	7.940	4.4787	6
	Total	8.719	5.1133	14
1h post game 2	Starters	12.419	6.1914	8
	Non starters	6.201	3.3022	6
	Total	9.754	5.9188	14
15h post game 2	Starters	10.441	4.4880	8
	Non starters	6.238	3.5421	6
	Total	8.640	4.5089	14

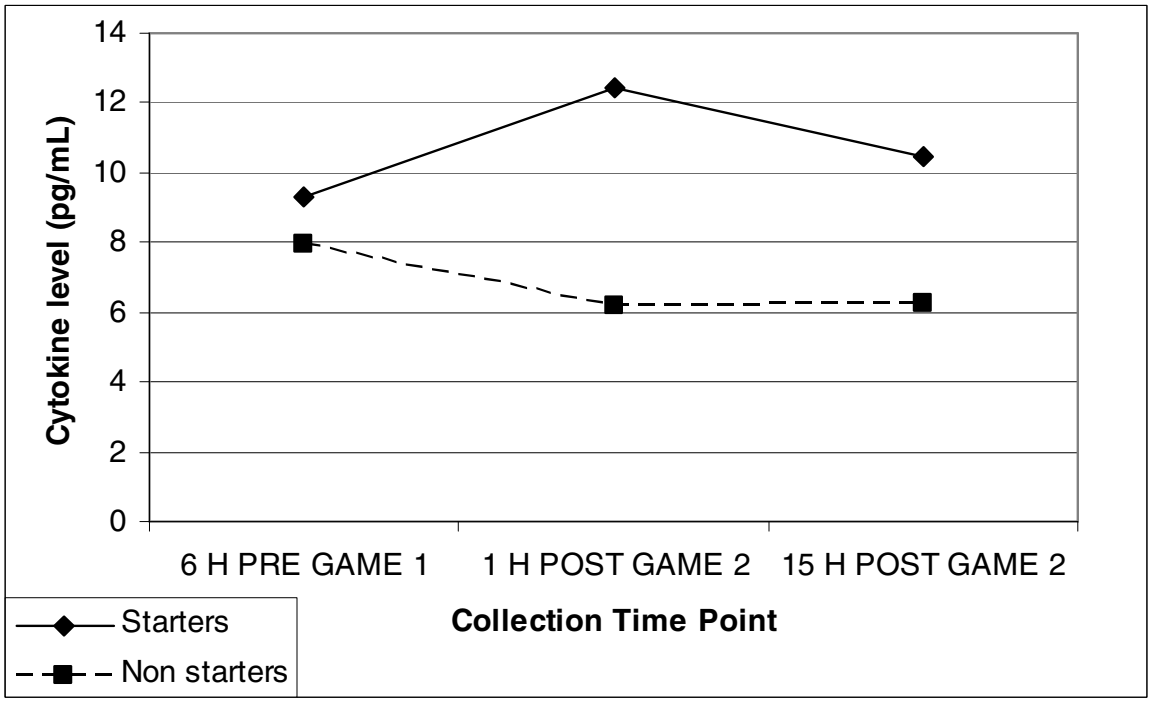

Figure 13. Absolute Salivary INF-γ Level.

Hypothesis 21

Ho21 = There will be no significant difference in plasma INF-γ levels between starters and nonstarters among all time points, which include 6 hours before, 1 hour following, and 15 hours following two soccer games.

The 2 X 3 repeated measure of ANOVA indicated statistically no significant difference in plasma INF-γ levels between starters and non starters among all time points [$F(1, 11) = 0.099$; $p > 0.05$]. There were no significant within-groups interaction across time x group [$F(2, 22) = 0.768$; $p > 0.05$]. The main effects of time were revealed to be statistically non significant [$F(2, 22) = 0.009$; $p > 0.05$] (Table XXV & Figure 14). Based on the current results the null hypothesis will be accepted.

Table XXV
Plasma Salivary INF-γ Means

	Groups	Mean	Std. Deviation	N
6h pre game 1	Starters	3.074	1.2514	5
	Non starters	3.242	1.6526	8
	Total	3.177	1.4569	13
1h post game 2	Starters	3.431	1.3625	5
	Non starters	2.964	1.7751	8
	Total	3.144	1.5852	13
15h post game 2	Starters	3.423	1.7416	5
	Non starters	2.927	1.5829	8
	Total	3.118	1.5923	13

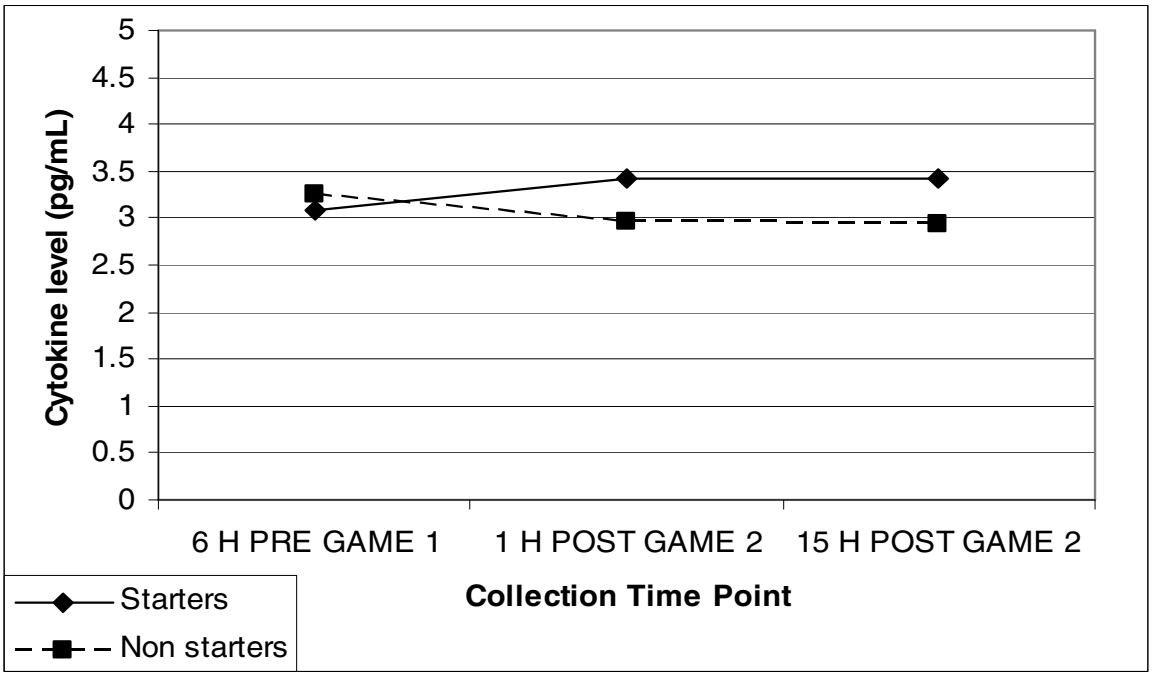

Figure 14. Plasma INF-γ Level.

Hypothesis 22

Ho22 = There will be a non significant relationship between salivary INF-γ levels and plasma INF-γ level 6 hours before the first soccer game.

The Pearson moment correlation revealed a non significant relationship between baseline salivary INF-γ levels adjusted for secretion rate and baseline plasma INF-γ ($r = 0.359$, $n = 12$; $p = 0.013$). A statistically significant relationship existed between pre exercise absolute salivary INF-γ level and plasma INF-γ level ($r = 0.690$, $n = 12$; $p = 0.013$) (Table XXVI & Figure 15). Based on the current results the null hypothesis will be accepted.

Table XXVI
Baseline INF-γ Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	4.375	3.3752	14
Saliva (pg/ml)	8.719	5.1133	14
Plasma (pg/ml)	2.951	1.4746	15

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.788**	.359
	Sig. (2-tailed)	.	.001	.252
	N	14	14	12
Saliva (pg/ml)	Pearson Correlation	.788**	1	.690*
	Sig. (2-tailed)	.001	.	.013
	N	14	14	12
Plasma (pg/ml)	Pearson Correlation	.359	.690*	1
	Sig. (2-tailed)	.252	.013	.
	N	12	12	15

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

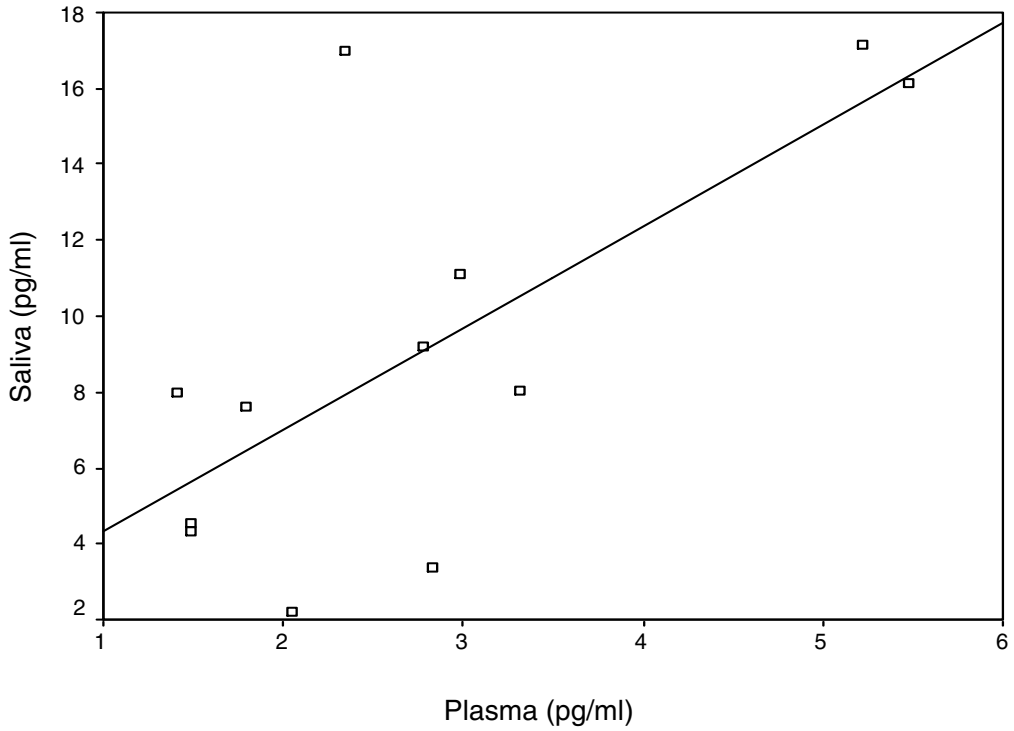

Figure 15. Significant correlation between absolute salivary IFN-γ and plasma IFN-γ levels in starters and non starters at baseline. (r = 0.690, n = 12; p = 0.013)

Hypothesis 23

Ho23 = There will be a non significant relationship between salivary INF-γ levels and plasma INF-γ level 1 hour following the second soccer game.

The Pearson moment correlation revealed a no significant relationship between salivary INF-γ levels adjusted for secretion rate and plasma INF-γ levels ($r = 0.723$, $n = 6$; $p > 0.05$) 1 hour post the second game. A non significant relationship existed between absolute salivary INF-γ level and plasma INF-γ level 1 hour post the second game ($r = 0.229$, $n = 6$; $p > 0.05$) (Table XXVII). Based on the current results the null hypothesis will be accepted.

Table XXVII
1 Hour Post INF-γ Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	3.4260	3.35087	8
Saliva (pg/ml)	3.1149	4.89272	8
Plasma (pg/ml)	.2551	.39704	6

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	.834*	.723
	Sig. (2-tailed)	.	.010	.105
	N	8	8	6
Saliva (pg/ml)	Pearson Correlation	.834*	1	.229
	Sig. (2-tailed)	.010	.	.663
	N	8	8	6
Plasma (pg/ml)	Pearson Correlation	.723	.229	1
	Sig. (2-tailed)	.105	.663	.
	N	6	6	6

*. Correlation is significant at the 0.05 level (2-tailed).

Hypothesis 24

Ho24 = There will be a non significant relationship between salivary INF-γ levels and plasma INF-γ level 15 hours following the second soccer game.

The Pearson moment correlation revealed statistically a significant relationship between salivary INF-γ levels adjusted for secretion rate and plasma INF-γ levels 15

hours post game two (r = -0.710, n = 5; p > 0.05). A significant relationship existed between relative salivary INF-γ level and plasma INF-γ level 15 hours post game two (r = -0.089, n = 5; p > 0.05) (Table XXVIII). Based on the current results the null hypothesis will be accepted.

Table XXVIII
15 Hour Post INF-γ Pearson Correlation

	Mean	Std. Deviation	N
Saliva (pg/min)	.4649	2.19732	8
Saliva (pg/ml)	-1.7753	3.71761	9
Plasma (pg/ml)	.3489	1.61455	5

		Saliva (pg/min)	Saliva (pg/ml)	Plasma (pg/ml)
Saliva (pg/min)	Pearson Correlation	1	-.609	-.710
	Sig. (2-tailed)	.	.109	.179
	N	8	8	5
Saliva (pg/ml)	Pearson Correlation	-.609	1	-.089
	Sig. (2-tailed)	.109	.	.887
	N	8	9	5
Plasma (pg/ml)	Pearson Correlation	-.710	-.089	1
	Sig. (2-tailed)	.179	.887	.
	N	5	5	5

Discussion

The first purpose of the current investigation was to explore the influence of two competitive collegiate soccer games on the production of plasma and salivary inflammatory cytokine levels of IL-6, IL-10, TNF-α and INF-γ in male collegiate soccer players. The second purpose of this investigation was to examine the relationship between plasma and salivary cytokine levels before and after exercise. The following

sections will discuss the details of the present findings and compare and contrast them with previously reported research on inflammatory cytokine response to exercise.

Plasma Cytokine Response

The results of the present study indicate a non significant change in plasma levels of IL-6, IL-10, TNF-α , and INF-γ following two competitive collegiate soccer games in starters and nonstarters (Figures 3, 6, 10, & 14). Although considered statistically insignificant plasma IL-6 levels in starters increased 24% 1 hour post exercise from pre exercise levels and remained slightly elevated 15 hours post exercise (Table VII & Figure 3). Plasma IL-10 levels peaked 1 hour post exercise in starters with a 34% increase from resting levels and returned to pre exercise levels 15 hours post exercise (Table XIII & Figure 6). Pre exercise levels of plasma TNF-α and IFN-γ were small or non detectable with relatively no change following the two soccer games (Figure 10 & 14).

The results of the present study are in contrast with previous studies reporting significant elevations in blood levels of inflammatory cytokines following endurance exercise (Nieman et al., 2001; Starkie et al., 2001; Weinstock et al., 1997; Ostrowski et al., 1999). Nieman et al. (2001)) reported a significant increase in plasma levels of IL-6 and IL-10 in runners following a marathon race. Plasma levels of TNF-α were reported to be elevated post race but the degree of change was low. Small or non detectable levels of plasma INF-γ were reported in all runners with no significant change following the race. The authors proposed the low level of pro-inflammatory cytokines observed following the marathon race may have been a result of elevated levels of IL-6, IL-10 and cortisol, which together help regulate the systemic inflammatory response. Peake et al. (2005) reported plasma IL-10 levels significantly increased following 1 hour of high

intensity (85%) running. The percent change in plasma IL-10 levels from pre to post exercise was reported to be significantly correlated to percent change in plasma cortisol and IL-6 levels. The above studies allude to the fact that post exercise cortisol and anti-inflammatory cytokine levels may contribute to the regulation of pro-inflammatory cytokine levels and the overall systemic inflammatory response following intense exercise. The present finding of a non significant increase in plasma levels of pro-inflammatory TNF-α and INF-γ following exercise may be a result of the insignificant increases in IL-10 and IL-6 levels. Cortisol levels in soccer players have been reported to be significantly elevated following two competitive soccer games and significantly related to alterations in immune signaling and adhesion molecules and immune cells (Malm et al., 2004). Even though cortisol was not directly measured in the present study possible increases could have contributed to the post exercise levels of pro-inflammatory TNF-α and IFN-γ observed in the present study.

The present study is an agreement with the finding of Nieman et al. (2000) who reported a non significant change in plasma IL-6 following 2 hours of tennis drills in male and female tennis athletes. The authors speculated the physical activity associated with tennis play was not stressful enough to produce an inflammatory response. In contrast, Nemet et al. did report a significant increase in serum IL-6 levels following a 1.5 hour water polo practice in ten high school female athletes. Possible explanations for discrepancies in IL-6 response include differences in exercise mode, intensity, fitness level. The nature of tennis and soccer play involves intermittent bursts of high intensity exercise followed by periods of low to moderate intensity exercise. Therefore, the overall stress associated with acute tennis and soccer play may have been less than water

polo practice. In addition, previous studies have reported decreased cytokine levels in trained individuals after acute exercise following a period of exercise training (Rhind et al., 1995). Malm et al. (2004) reported alterations in immune signaling and adhesion molecules in 10 elite male soccer players following two soccer games were negatively correlated with V02max. The subjects in the current study and Nieman et al. (2000) were highly trained elite athletes. The average $VO2_{max}$ for the soccer athletes in the present study were 50.1 $ml/min^{-1}/kg^{-1}$ and in the tennis athletes in the Nieman et al. study were reported to be 52.7 $ml/min^{-1}/kg^{-1}$ for boys and 43.3 $ml/min^{-1}/kg^{-1}$ for girls. Nemet et al. (2003) reported the average peak $V02_{max}$ in water polo players to be 30.5 ± 1.9 $ml/min^{-1}/kg^{-1}$. Fitness level may contribute significantly to the immunological response to exercise.

Salivary Cytokine Response

The present study revealed there were no significant differences in absolute and relative levels of salivary IL-6, IL-10, TNF-α , and INF-γ between starters and nonstarters following two competitive collegiate soccer games (Figures 1, 2, 4, 5, 8, 9, 12, & 13). Repeated ANOVA revealed a significant overall time effect for salivary IL-6 levels adjusted for secretion rate ($p = 0.018$). Pre exercise salivary IL-6 levels adjusted for secretion rate increased 48% 1 hour post game 2 and returned to pre exercise levels 15 hours following the second soccer game in starters (Table V). The observed increase in salivary IL-6 in the present study is supported by Phillips et al. (2003) who reported a 61% increase in absolute salivary IL-6 levels from resting levels following a long course triathlon ($N = 11$, 25.1 ± 1.6 to 40.5 ± 3.3 $pg{\cdot}ml^{-1}$, $p = 0.002$). In support of Phillips et al. (2003), and those of the present study, Minetto et al. (2005) reported salivary IL-6 levels

increased 232% from pre exercise levels following 3 hours of submaximal cycling (N = 7, 0.79 ± 0.63 to 2.62 ± 2.67 pg·mL^{-1}, p = 0.046).

In the present study, pre exercise salivary TNF-α levels adjusted for secretion rate increased 120% immediately post exercise and remained elevated 15 hours following the second soccer game in starters (Table XVII). Pre exercise absolute salivary TNF-α level increased 84% immediately post exercise and remained elevated 15 hours following the second soccer game in starters (Table XVIII). In support of the present study, Phillips et al. (2003) reported absolute levels of salivary TNF-α increased 106% from resting levels following a long course triathlon (N =11; 1.24 ± 0.3 to 2.3 ± 0.6 pg ml-1, p = 0.051).

Repeated ANOVA revealed a near significant overall time effect for INF- γ levels adjusted for secretion rate (p = 0.056) and within-groups interaction for INF- γ levels adjusted for secretion rate (p = 0.066) and absolute IFN-γ levels (p = 0.056). Pre exercise INF-γ salivary levels adjusted for secretion rate increased 71% 1 hour post game 2 and remained elevated 15 hours following the second soccer game in starters (Table XXIII). Pre exercise absolute salivary INF-γ levels increased 33% immediately post exercise and remained elevated 15 hours following the second soccer game in starters (Table XIV). The observed increase in salivary INF-γ levels in the present study is supported by Rossi et al. (2003) who reported a 119% increase in pre exercise levels of absolute salivary IFN- γ (N = 13, T2 = 14.9 ± 3.7, T3 = 32.6 ± 8.8 pg·mL-1; p = 0.07) following a competitive collegiate football game. When cytokine concentration was expressed per total protein, salivary IFN-γ concentrations significantly increased 175% following the competitive football game. The results of the present study along with previous studies indicate cytokine production in the oral environment mimics that observed in blood

resultant to intense exercise. Tendencies for increases in salivary IL-6 (48%), TNF-α (120%), IFN-γ (71%) levels adjusted for secretion rate and absolute salivary levels of TNF-α (84%) and IFN-γ (33%) may represent acute systemic or oral inflammation in response to competitive collegiate soccer play; however definitive conclusions can not be made with our small sample sizes.

Relationship between Plasma and Salivary Cytokine Levels

The result of the present study revealed a non significant relationship between absolute salivary IL-6 levels and salivary IL-6 levels adjusted for secretion rate (pg·min) and plasma IL-6 levels before and after two competitive collegiate soccer games (Table VIII, IX, & X). In agreement with the present study, Minetto et al. (2005) reported no significant correlation between plasma and absolute salivary IL-6 levels before and after 3 hours of submaximal cycling and before and after 20 maximal bilateral isokinetic contractions of the knee flexor and extensor muscles. The authors state the mechanisms responsible for increases in plasma IL-6 levels are most likely independent of those responsible for increases in salivary IL-6 levels. Minetto et al. (2005) explained the salivary cytokine response to exercise is a local response of the salivary glands where, systemic cytokine response "reflects mechanisms associated with muscle contraction and repair". Furthermore the authors state " it is unlikely that the molecule diffuses through acinar cells of the salivary glands from the blood stream into saliva, and that serum levels can influence the salivary concentration, as occurs for steroid hormones".

The present study reported a significant positive relationship between absolute salivary IL-10 levels and plasma IL-10 levels 1 hour post the second soccer game (Table XV & Figure 7). A significant negative correlation was reported between absolute

salivary TNF- α levels and plasma TNF-α level 1hour post the second soccer game (Table XXI & Figure 11). A significant positive correlation was reported between baseline absolute salivary IFN-γ levels and plasma INF-γ levels in starters (Table XXVI & Figure 15). The research comparing plasma and salivary cytokine levels before and after exercise is limited. To our knowledge, the study by Minetto et al. (2005) and the present study are the only studies comparing salivary and plasma cytokine levels before and after exercise. Preliminary data report mixed results when comparing the relationship between plasma and salivary cytokines levels; further research is needed to clarify the relationship between plasma and salivary cytokine levels following exercise and its biological implications.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECCOMENDATIONS

Summary

The present study revealed participation in two competitive collegiate soccer games statistically did not influence post exercise levels of plasma and salivary IL-6, IL-10, TNF-α, and IFN-γ. The trend for increase in plasma IL-6 and IL-10 and similar tendencies in salivary IŁ 6, TNF-α and INF-γ may represent an acute systemic and/or oral inflammatory response to two competitive collegiate soccer games which was undetectable with the small sample size in the present study. A significant negative correlation was reported between absolute salivary TNF-α and plasma TNF-α level 1 hour post the second soccer game. A significant positive correlation was observed between baseline absolute salivary IFN-γ levels and plasma IFN-γ levels and between absolute salivary and plasma IL-10 levels 1 hour post exercise. The results of the present study indicate plasma and salivary cytokine levels before and after exercise are most likely unrelated; further studies are necessary to determine the relationship between plasma and salivary cytokine levels.

Conclusions

Preliminary data suggest plasma and salivary cytokine levels are increased as a result of soccer play; however, the relationship between plasma and salivary cytokine levels before and after exercise appear to be independent and unrelated. The results of the present study along with previous research indicate cytokine production in the oral environment mimics that observed in blood resultant to intense exercise. Saliva sampling offers an inexpensive, non invasive and simple collection of physiological. This makes saliva a potentially effective tool for health professionals to monitor the physiological cost of training and participating in sport. This information would be beneficial in preventing and reducing the incidences of illness during a competitive season. Future studies are needed to fully understand the salivary cytokine response to exercise and its immunological implications.

Recommendations

1. A more random and larger sample size is recommended. The increased numbers would improve recognizing significant changes in dependent variables.
2. Include female soccer athletes and soccer athletes with varying skill and fitness levels.
3. Measurement of stress hormones would improve identification of underlying mechanisms associated with the immune systems response to soccer play.
4. Include the measurement of *in vitro* mitogen stimulated cytokine production and measure cytokine levels in tissue to assess the overall effect of soccer play on cytokine production.

5. Measure plasma and salivary cytokine levels throughout an entire season to assess cytokine response to various training volumes, competitive play, travel, and conference and national tournament play.
6. Record incidences of illness, injury and psychological stress associated with life, physical training, and competition to investigate other factors that may contribute to cytokine production.

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APPENDICES

APPENDEX A

RESEARCH PARTICIPANT CONSENT FORM
BLOOD AND SALIVARY IMMUNE RESPOSNE TO A COMPETITIVE COLLEGIATE SOCCER TOURNAMENT

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Oklahoma State University
Department of Health, Leisure, and Human Performance

The present research project will examine the effect of a collegiate soccer tournament on the presence of immune markers present in blood and saliva. You have been selected because you are an Oklahoma Baptist University varsity soccer athlete, or age-matched junior varsity soccer athlete who will be involved in the soccer tournament hosted by OBU on September 23rd-25th. You will be asked to provide data samples based on playing time in the tournament games on the 23rd, 24th, and 25th of September. You will receive a copy of this consent form to keep for your records.

Purpose of Research

The purpose of this project is to assess the influence of a competitive collegiate soccer tournament on blood and salivary immune markers.

Requirements of Participants

1. Received a physical examination and cleared to participate in competitive sports.
2. Complete medical and informed consent forms.
3. Height, weight, and skinfold measurement will be performed during in-season and at the end of the playing season.
4. Complete a graded treadmill test to determine aerobic fitness (maximal oxygen consumption). You will perform each test twice, once during your in-season and the second at the end of your season. Blood samples will be collected via finger stick during the graded treadmill test to further assess endurance performance. There is a small risk associated with participation in graded treadmill testing. All treadmill tests will be administered and monitored by CPR/AED and first aid certified technicians. In the unlikely event of adverse events medical assistance will be obtained.
5. Complete a strength test during in-season and after season to assess the strength of the thigh muscles. You will perform three maximal isometric or isokinectic muscle actions with each leg on a Cybex 6000 dynamometer. Electrical activity and vibrations of the thigh muscles will be assessed using electrodes placed on the thigh. There is a slight risk associated with participation in a strength test. Strength tests will be administered and monitored by experienced technicians and medical assistance will be provided in the unlikely case of adverse events.
6. Complete the Bangsbo soccer specific field test and 1.5 mile run test during in-season and after season to predict aerobic fitness. There is a small risk associated with participation in cardiorespiratory field testing. All tests will be administered and monitored by CPR/AED and first aid certified technicians.

7. Provide blood and saliva samples that will be taken the morning (7:00-8:00 am) of the first game, within 1 hour after the second game, and the morning following the second game (7:00-8:00 am); or at a corresponding time points if you are a control subject.
8. You will be asked to report any injuries in the mouth due to abrasive brushing, poor oral health, etc. before saliva sample collection. Also, we ask that you do not brush your teeth within 3 hours prior to sample collection. Dental work should not be performed within 24 hours prior to sample collection. In addition, do not eat a meal within 60 minutes and avoid alcohol 24 hours prior to sample collection. With blood draws some individuals have described moderate pain during needle insertion, while others describe it as a quick prick or stinging sensations.
9. Control subjects will be asked to refrain from strenuous exercise during the sampling period.
10. Subjects will be asked to complete, on a daily basis, a written questionnaire to assess URTI symptoms and their severity. Subjects will be provided with a semi-quantitative list of respiratory symptoms including: cough, sneezing, nasal discharge, stuffy nose, sore throat, headache, malaise, chilliness, shaking chills, fever, hoarseness, aching muscles or joints, and watery or burning eyes. Participants will rate each symptom with an impact score A (not present), B (mild), C (moderate), D (severe) (5). In addition, participants will be asked to complete a daily training diary. Subjects will report mode, time and intensity of training. A weekly e-mail survey, which summarizes each subject's URTI infection/severity and training information, will be completed and sent to the researchers via an attached document. At the end of the 3-week recording period, all participants in the study will be asked to mail their packet of daily assessment forms to the research lab. Subjects without e-mail, but wishing to participate will receive and send weekly surveys via U.S. mail.

Duration of Participation

A total of 30 days of participation will be required of each subject. These include filling out medical history and informed consent, tests for maximal oxygen consumption and prediction of aerobic fitness, strength testing of thigh muscles, donation of blood and saliva samples the morning (7:00-8:00 am) of the first game, within 1 hour after the second game, and the following morning (7:00-8:00 am), and filling out email and written questionnaires. The maximal oxygen consumption test will require approximately 45 minutes. Tests for prediction of aerobic fitness will require 1 hour. Strength testing of the thigh muscles will take approximately 1 hour. Time for sample collection will take approximately 15 minutes. The daily written survey will take 2-4 minutes to complete resulting in a maximum of 1.5 hours spent completing the survey during the 3 week period. The weekly e-mail survey will take approximately 5-10 minutes to fill out, totaling 30 minutes to complete and send e-mail form. Total project time commitment is no more than 180-195 minutes.

Benefits to the Individual

Benefits to the individual include the maximal test for oxygen consumption, which is the gold standard for measuring aerobic fitness, measurement of muscle strength, and information on how your blood and saliva immune proteins (IgA and cytokines) respond to participation in a competitive collegiate soccer tournament or normal activity (controls). Blood and salivary cytokines have been utilized as markers of the body's immune response to exercise. In addition, salivary IgA is commonly employed to assess the effectiveness of the secretory immune system. The results of this study will help to clarify the response of immune system to a competitive collegiate soccer tournament. We hope to assist in providing information necessary to prevent or reduce the incidence of illness in athletes, thus improving their overall health. In addition, provide information that may aid in the development of a scientifically based training program. You will be presented with the general finding upon completion of the analysis.

Risks to the Individual

There are no known risks involved with saliva donation. Risks associated with venipuncture blood draw are minimal and include bleeding, feeling light-headed, hematoma (collection of blood under the skin), infection (small risk any time skin is broken), and multiple punctures to locate vein. Risks associated with finger stick for capillary blood collection are minimal and include soreness accompanied by inflammation. Risks associated with maximaltreadmill test, field tests for prediction of aerobic fitness, and muscle strength are minimal in a healthy population, but include lightheadedness, fainting, muscle soreness, and the remote risk of cardiovascular complication or death. Measurement of thigh muscle strength also includes the possibility of infection and soreness from abrasions due to electrode placement. Risks will be minimized by following established American College of Sports Medicine guidelines for exercise testing and utilization of antibiotic cream at electrode sites to decrease infection and soreness. Personnel administering tests are certified in CPR and first aid.

Medical liability

I understand the risks associated with this study and voluntarily choose to participate. I understand that in case of injury or illness resulting from this study, emergency medical care is available through community health care providers by dialing 911. I understand that no funds have been set aside by Oklahoma State University to compensate me in the event of illness or injury.

Confidentiality

All scientific and personal data collected on subjects will be kept confidential and stored in a locked room. This information will be available only to the Principal investigator and student advisor. All subjects will be assigned a subject number and all samples being analyzed by lab technicians will be labeled with these subject numbers to ensure anonymity. Information concerning assigned subject numbers will be kept separate from confidential material. All data will be reported as means and standard errors. E-mail communications will be kept strictly confidential and access to the completed

questionnaires will be limited to A.B. Harrison Human Performance Lab personnel and hard copy forms will be coded to ensure anonymity. Once hard copies are printed, e-mails will be deleted.

Voluntary Nature of Participation

Participation in this research project is voluntary and you can withdraw your involvement in the project at any time without penalty.

Human Subject Statement

For any questions you may have about this research project, contact Stephen J Rossi, M.S. (405) 269-6552. If you have any concerns dealing with subjects' rights, contact Dr Carol Olson, IRB chair, 415 Whitehurst Hall, Oklahoma State University, 405-744-1676.

I HAVE READ AND FULLY UNDERSTAND THE CONSENT FORM. I SIGN IT FREELY AND VOLUNTARILY. A COPY OF THIS FORM HAS BEEN GIVEN TO ME.

_____	_____	_____
Signature of Participant	Date of birth	Date

I certify that I have personally explained this document before requesting that the participant sign it.

_____	_____
Signature of Researcher	Date

APPENDEX B

OKLAHOMA STATE UNIVERSITY
HARRISON HUMAN PERFORMANCE LABORATORY
Personal Medical History Survey

Complete the front and back of this form.

Name:_____ Date:_____

Address:_____ City/State:_____ Zip:_____

Phone:_____ E-mail Adress: _____

Age:_____Sex:_____ Weight:_____ Height:_____

1. Have you ever been diagnosed as having: (check all that apply)

	Never	**Past**	**Presently**
A. Heart disease	_____	_____	_____
B. Rheumatic fever	_____	_____	_____
C. High blood pressure	_____	_____	_____
D. Other vascular disorders	_____	_____	_____
E. Diabetes	_____	_____	_____
F. Kidney disease	_____	_____	_____
G. Asthma	_____	_____	
H. Allergies	_____	_____	_____
I. Chronic bronchitis	_____	_____	_____
J. Other respiratory illness	_____	_____	_____
K. High serum lipids (cholesterol)	_____	_____	_____
L. Anemia	_____	_____	_____
M. Low blood sugar	_____	_____	_____
N. Neuro-musculo-skeletal disease	_____	_____	_____
O. Sores in mouth	_____	_____	_____
P. Cavities in teeth	_____	_____	_____
Q. Gum disease	_____	_____	_____
R. "Strep" throat	_____	_____	_____
S. Other oral infections	_____	_____	_____

2. Please indicate any surgery that you have undergone and the approximate date(s).

_____

_____

3. Please indicate recent illnesses or major injuries that you have had. Also list approximate dates.

_____

_____

4. Do you smoke? _____ Packs per day? _____

Do you use smokeless tobacco (chew or dip)? _____ How often?_____

5. Please list all medications or supplements (prescription and non-prescription) that you are presently taking.

Medication	Dosage	Duration

6. Describe exercise or activity program during the last 2 months (excluding your taper). (Please include: the activity, amount per day, days per week, and length of time you have been exercising at this level)

Activity	minutes/day	days/week	weeks of exercise

7. How long was your taper? _____ days
8.How many long course distance races have you completed in the past? _____
9.When did you start training for this triathlon?_____

10. Have you smoked any tobacco product in the last 2 hours? No Yes
When?_____

11. Used any smokeless tobacco product in the last 2 hours? No Yes
When?_____

12. Have you chewed gum in the last 2 hours? No Yes
When?_____

13. When did you last eat? (meal or snack; anything) _____

14. When did you last brush your teeth? _____

15. Have you exercised today? If so, what did you do? _____

_____ _____
Signature Date

APPENDEX C

GROUP	PRSAIFN	POSALIFN	PPOSAIFN	PRSALIFN	POSAIFN
1	23.7	31.7	38.7	84.9	158.5
1	23.2	91.0	38.0	123.9	157.4
1	31.6	17.3	12.2	98.1	48.0
1	1.7	114.4	14.0	4.9	261.1
1	178.3	348.9	119.9	289.0	344.1
1	14.7	74.7	53.7	294.0	473.0
1	9.9	21.0	2.7	12.3	21.0
1	.0	.0	.0	.0	76.0
1	2.8	16.8	7.7	18.6	39.0
1	.0	.0	.0	.0	.0
2	10.0	58.7	18.0	57.9	120.6
2	10.1	8.4	14.1	64.5	22.2
2	.0	.0	.0	.0	.0
2	93.3	11.7	30.8	261.0	96.3
2	.0	.0	.0	.0	.0
2	.0	.0	.0	.0	.0
2	1.1	3.4	1.6	11.3	19.6
2	9.4	4.2	9.1	20.4	14.7
2	6.2	11.8	.3	63.5	11.8

GROUP	PPOSLIFN	PRPLAIFN	POPLAIFN	POSPLIFN	PRSALIL6
1	156.0	7.7	6.5	5.8	1.8
1	122.6	8.9	11.2	2.5	2.2
1	55.8	.0	19.0	8.0	2.8
1	36.3	4.2	6.2	6.5	1.7
1	278.2	5.5	8.9	28.0	4.9
1	278.2	27.2	33.5	27.9	.4
1	45.0	.0	4.6	6.4	9.5
1	73.3	.0	.0	.0	.0
1	41.0	2.2	1.5	.0	14.3
1	.0	.0	.0	.0	.2
2	83.9	3.2	4.3	2.1	1.7
2	61.1	11.0	7.4	8.0	.9
2	.0	2.9	1.9	12.7	.0
2	114.4	30.0	33.5	21.8	2.5
2	.0	14.0	7.5	4.4	.0
2	.0	32.1	32.1	32.1	.8
2	9.1	8.0	3.2	2.0	2.7
2	24.5	2.2	.0	.0	5.7
2	3.2	2.0	2.4	3.0	1.8

GROUP	POSALIL6	PPOSLIL6	PRSAIL6	POSAIL6	PPOSAL6
1	5.8	2.7	6.6	29.1	10.9
1	19.5	10.6	11.6	33.7	34.1
1	9.0	1.5	8.7	24.9	7.0
1	2.1	2.0	4.7	4.8	5.3
1	5.7	1.6	8.0	5.6	3.8
1	.9	.9	7.9	5.4	4.7
1	16.6	.0	11.8	21.0	.0
1	.0	2.2	.0	.0	13.8
1	14.4	8.0	95.0	33.4	42.5
1	.0	1.0	14.1	.0	8.0
2	11.0	1.1	9.7	22.6	5.2
2	6.9	2.3	6.0	18.2	9.8
2	1.6	1.7	.0	19.4	27.2
2	.5	1.4	7.1	4.2	5.3
2	5.8	4.5	.0	7.4	12.1
2	1.6	.6	4.3	7.2	5.4
2	3.6	4.8	26.3	20.5	26.3
2	4.6	5.1	12.4	16.1	13.8
2	.0	3.6	18.2	.0	36.4

GROUP	PREPLIL6	POPLAIL6	PPLIL6	PRSALTNF	POSALTNF
1	5.3	10.9	26.3	4.8	47.6
1	5.0	8.4	5.3	5.6	145.7
1	.0	11.2	7.1	7.3	10.4
1	3.7	5.3	1.0	.2	18.4
1	2.9	4.0	3.9	38.9	17.4
1	3.7	5.2	1.0	.0	12.4
1	.0	28.4	11.0	5.6	.7
1	5.8	7.2	4.2	.0	.0
1	4.0	17.0	.0	6.6	7.6
1	5.5	.0	.0	.0	.0
2	4.3	4.4	3.7	12.7	57.5
2	5.5	4.7	5.9	2.3	3.9
2	7.5	6.3	.0	.0	.0
2	4.8	4.1	4.1	3.8	.1
2	4.1	3.7	.0	.0	79.9
2	.0	4.5	4.3	3.9	14.5
2	.3	.5	5.6	3.8	.1
2	11.2	.0	.0	10.9	2.9
2	6.6	.0	7.2	7.2	.0

GROUP	PPOSLTNF	PRSATNF	POSATNF	PPOSATMF	PREPLTNF
1	32.0	17.2	238.0	129.0	10.3
1	73.8	30.1	252.0	238.0	.0
1	2.5	22.8	28.9	11.5	.0
1	.3	11.6	41.9	6.3	6.3
1	7.4	63.0	17.2	17.2	6.3
1	6.1	.0	78.3	31.6	13.8
1	.0	7.1	10.9	.0	.0
1	2.4	.0	.0	14.8	.0
1	.0	44.0	177.0	.0	3.9
1	14.1	.0	.0	38.1	5.2
2	1.1	74.0	118.0	5.1	7.9
2	13.9	14.6	10.3	60.0	27.5
2	.0	.0	.0	.0	10.7
2	6.7	10.7	6.3	24.8	19.6
2	46.0	.0	103.8	124.6	8.2
2	7.4	20.9	66.3	61.9	.0
2	20.6	37.5	6.6	113.8	3.8
2	.0	23.8	10.2	.0	3.8
2	15.1	55.6	.0	151.4	12.5

GROUP	POPLATNF	PPOPLTNF	PSALTEN	POSALTEN	PPOSATEN
1	7.3	6.8	1.9	1.5	3.0
1	5.6	12.0	.9	13.9	9.0
1	7.2	11.5	1.4	1.1	.0
1	9.8	4.9	.2	2.7	2.0
1	14.9	27.5	5.8	1.1	2.5
1	10.7	8.2	.0	.8	1.0
1	7.1	5.2	9.3	5.4	.3
1	4.5	4.2	.0	.0	.0
1	4.5	.0	1.4	3.0	.0
1	.0	.0	.0	.0	.8
2	8.2	6.8	.0	2.3	.5
2	37.8	15.8	.8	1.9	.0
2	7.3	9.2	.0	.0	.0
2	4.9	6.3	1.9	.1	.2
2	6.9	9.8	.0	13.2	9.7
2	59.6	34.2	1.2	6.3	1.0
2	5.8	5.2	7.3	1.3	1.2
2	5.8	.0	3.0	1.3	.0
2	.0	.0	.6	.0	.0

GROUP	PRSATEN	POSATEN	PPOSATN	PRPLATEN	POPLATEN
1	6.8	7.7	12.0	3.1	2.5
1	5.0	24.1	28.9	.0	1.8
1	4.5	3.0	.0	.0	2.5
1	.6	6.1	5.1	1.0	6.3
1	9.4	1.1	5.8	5.4	1.1
1	.0	5.1	5.2	.7	7.9
1	11.5	6.8	5.2	.0	.0
1	.0	.0	.0	.0	4.1
1	9.1	7.0	.0	12.9	8.4
1	.0	.0	6.3	4.4	.0
2	.0	4.8	2.5	1.8	1.9
2	5.2	5.0	.0	4.1	3.6
2	.0	.0	.0	2.1	3.7
2	5.4	.8	.7	21.0	17.0
2	.0	17.1	26.2	1.0	1.0
2	6.5	6.3	1.0	.0	1.0
2	7.3	5.9	10.3	5.1	4.5
2	6.6	4.6	.0	4.1	.0
2	4.9	.0	.0	4.3	.0

GROUP	PPOPLTEN	IFNSQRT	IFNSQRT2	IFNSQRT3	IFNSQRT4
1	2.5	4.9	5.6	6.2	9.2
1	2.4	4.8	9.5	6.2	11.1
1	27.8	5.6	4.2	3.5	9.9
1	1.0	1.3	10.7	3.7	2.2
1	6.0	13.4	18.7	11.0	17.0
1	1.0	3.8	8.6	7.3	17.1
1	.0	3.2	4.6	1.7	3.5
1	4.2	.0	.0	.0	.0
1	.0	1.7	4.1	2.8	4.3
1	.0		.0	.0	.0
2	2.0	3.2	7.7	4.2	7.6
2	3.1	3.2	2.9	3.8	8.0
2	7.3	.0	.0	.0	.0
2	15.4	9.7	3.4	5.5	16.2
2	7.5	.0	.0	.0	.0
2	1.0	.0	.0	.0	.0
2	4.6	1.1	1.8	1.3	3.4
2	6.5	3.1	2.1	3.0	4.5
2	4.5	2.5	3.4	.6	8.0

GROUP	IFNSQRT5	IFNSQRT6	IFNSQRT7	IFNSQRT8	IFNSQRT9
1	12.6	12.5	2.8	2.5	2.4
1	12.5	11.1	3.0	3.3	1.6
1	6.9	7.5	.0	4.4	2.8
1	16.2	6.0	2.0	2.5	2.5
1	18.5	16.7	2.3	3.0	5.3
1	21.7	16.7	5.2	5.8	5.3
1	4.6	6.7	.0	2.1	2.5
1	8.7	8.6	.0	.0	.0
1	6.2	6.4	1.5	1.2	.0
1	.0	.0	.0	.0	.0
2	11.0	9.2	1.8	2.1	1.4
2	4.7	7.8	3.3	2.7	2.8
2	.0	.0	1.7	1.4	3.6
2	9.8	10.7	5.5	5.8	4.7
2	.0	.0	3.7	2.7	2.1
2	.0	.0	5.7	5.7	5.7
2	4.4	3.0	2.8	1.8	1.4
2	3.8	4.9	1.5	.0	.0
2	3.4	1.8	1.4	1.5	1.7

GROUP	L6SQRT1	L6SQRT2	L6SQRT3	L6SQRT4	L6SQRT5
1	1.4	2.4	1.6	2.6	5.4
1	1.5	4.4	3.3	3.4	5.8
1	1.7	3.0	1.2	2.9	5.0
1	1.3	1.4	1.4	2.2	2.2
1	2.2	2.4	1.3	2.8	2.4
1	.6	.9	1.0	2.8	2.3
1	3.1	4.1	.0	3.4	4.6
1	.0	.0	1.5	.0	.0
1	3.8	3.8	2.8	9.7	5.8
1	.5	.0	1.0	3.8	.0
2	1.3	3.3	1.1	3.1	4.8
2	1.0	2.6	1.5	2.4	4.3
2	.0	1.3	1.3	.0	4.4
2	1.6	.7	1.2	2.7	2.0
2	.0	2.4	2.1	.0	2.7
2	.9	1.3	.8	2.1	2.7
2	1.6	1.9	2.2	5.1	4.5
2	2.4	2.1	2.3	3.5	4.0
2	1.3	.0	1.9	4.3	.0

GROUP	L6SQRT6	L6SQRT7	L6SQRT8	L6SQRT9	TNFSQRT1
1	3.3	2.3	3.3	5.1	2.2
1	5.8	2.2	2.9	2.3	2.4
1	2.6	.0	3.3	2.7	2.7
1	2.3	1.9	2.3	1.0	.4
1	1.9	1.7	2.0	2.0	6.2
1	2.2	1.9	2.3	1.0	.0
1	.0	.0	5.3	3.3	2.4
1	3.7	2.4	2.7	2.0	.0
1	6.5	2.0	4.1	.0	2.6
1	2.8	2.3	.0	.0	.0
2	2.3	2.1	2.1	1.9	3.6
2	3.1	2.3	2.2	2.4	1.5
2	5.2	2.7	2.5	.0	.0
2	2.3	2.2	2.0	2.0	1.9
2	3.5	2.0	1.9	.0	.0
2	2.3	.0	2.1	2.1	2.0
2	5.1	.6	.7	2.4	1.9
2	3.7	3.3	.0	.0	3.3
2	6.0	2.6	.0	2.7	2.7

GROUP	TNFSQRT2	TNFSQRT3	TNFSQRT4	TNFSQRT5	TNFSQRT6
1	6.9	5.7	4.1	15.4	11.4
1	12.1	8.6	5.5	15.9	15.4
1	3.2	1.6	4.8	5.4	3.4
1	4.3	.5	3.4	6.5	2.5
1	4.2	2.7	7.9	4.1	4.1
1	3.5	2.5	.0	8.8	5.6
1	.8	.0	2.7	3.3	.0
1	.0	1.5	.0	.0	3.8
1	2.8	.0	6.6	13.3	.0
1	.0	3.8	.0	.0	6.2
2	7.6	1.0	8.6	10.9	2.3
2	2.0	3.7	3.8	3.2	7.7
2	.0	.0	.0	.0	.0
2	.3	2.6	3.3	2.5	5.0
2	8.9	6.8	.0	10.2	11.2
2	3.8	2.7	4.6	8.1	7.9
2	.3	4.5	6.1	2.6	10.7
2	1.7	.0	4.9	3.2	.0
2	.0	3.9	7.5	.0	12.3

GROUP	TNFSQRT7	TNFSQRT8	TNFSQRT9	L10SQRT1	L10SQRT2
1	3.2	2.7	2.6	1.4	1.2
1	.0	2.4	3.5	.9	3.7
1	.0	2.7	3.4	1.2	1.0
1	2.5	3.1	2.2	.4	1.6
1	2.5	3.9	5.2	2.4	1.0
1	3.7	3.3	2.9	.0	.9
1	.0	2.7	2.3	3.0	2.3
1	.0	2.1	2.0	.0	.0
1	2.0	2.1	.0	1.2	1.7
1	2.3	.0	.0	.0	.0
2	2.8	2.9	2.6	.0	1.5
2	5.2	6.1	4.0	.9	1.4
2	3.3	2.7	3.0	.0	.0
2	4.4	2.2	2.5	1.4	.3
2	2.9	2.6	3.1	.0	3.6
2	.0	7.7	5.8	1.1	2.5
2	1.9	2.4	2.3	2.7	1.1
2	1.9	2.4	.0	1.7	1.1
2	3.5	.0	.0	.8	.0

GROUP	L10SQRT3	L10SQRT4	L10SQRT5	L10SQRT6	L10SQRT7
1	1.7	2.6	2.8	3.5	1.8
1	3.0	2.2	4.9	5.4	.0
1	.0	2.1	1.7	.0	.0
1	1.4	.8	2.5	2.3	1.0
1	1.6	3.1	1.0	2.4	2.3
1	1.0	.0	2.3	2.3	.8
1	.5	3.4	2.6	2.3	.0
1	.0	.0	.0	.0	.0
1	.0	3.0	2.6	.0	3.6
1	.9	.0	.0	2.5	2.1
2	.7	.0	2.2	1.6	1.3
2	.0	2.3	2.2	.0	2.0
2	.0	.0	.0	.0	1.4
2	.4	2.3	.9	.8	4.6
2	3.1	.0	4.1	5.1	1.0
2	1.0	2.5	2.5	1.0	.0
2	1.1	2.7	2.4	3.2	2.3
2	.0	2.6	2.1	.0	2.0
2	.0	2.2	.0	.0	2.1

GROUP	L10SQRT8	L10SQRT9	AGE	BODYMASS	HEIGHT
1	1.6	1.6	18	97	74
1	1.3	1.5	20	73	68
1	1.6	5.3	21	78	69
1	2.5	1.0	21	69	64
1	1.0	2.4	18	70	72
1	2.8	1.0	21	82	72
1	.0	.0	21	69	67
1	2.0	2.0	21	77	74
1	2.9	.0	22	76	69
1	.0	.0	20	80	73
2	1.4	1.4	19	72	67
2	1.9	1.8	19	85	73
2	1.9	2.7	19	78	75
2	4.1	3.9	19	73	71
2	1.0	2.7	18	73	70
2	1.0	1.0	18	72	68
2	2.1	2.1	19	82	70
2	.0	2.5	18	73	70
2	.0	2.1	19	84	69

GROUP	BODYCOMP	L6CHAN2	L6CHAN3	L6CHAN5	L6CHAN6
1	18	1.06	.29	2.83	.73
1	11	2.94	1.78	2.40	2.43
1	12	1.32	-.44	2.04	-.30
1	14	.16	.14	.02	.13
1	11	.16	-.94	-.46	-.88
1	11	.29	.32	-.49	-.64
1	16	.99	.00	1.15	.00
1	7	.00	.00	.00	.00
1	9	.02	-.94	-3.97	-3.23
1	7	.00	.50	.00	-.93
2	22	2.03	-.24	1.64	-.83
2	12	1.66	.53	1.82	.68
2	19	.00	.00	.00	.00
2	11	-.88	-.40	-.62	-.36
2	11	.00	.00	.00	.00
2	7	.36	-.10	.61	.25
2	18	.26	.55	-.60	.00
2	16	-.24	-.12	.49	.19
2	22	.00	.57	.00	1.77

GROUP	L6CHAN8	L6CHAN9	L10CHAN2	L10CHAN3	L10CHAN5
1	1.00	2.83	-.15	.35	.17
1	.66	.07	2.78	2.05	2.67
1	.00	.00	-.13	.00	-.39
1	.38	-.92	1.20	.97	1.70
1	.30	.27	-1.36	-.83	-2.02
1	.36	-.92	.00	.00	.00
1	.00	.00	-.73	-2.50	-.78
1	.27	-.36	.00	.00	.00
1	2.12	.00	.55	.00	-.37
1	.00	.00	.00	.00	.00
2	.02	-.15	.00	.00	.00
2	-.18	.08	.48	.00	-.04
2	-.23	.00	.00	.00	.00
2	-.17	-.17	-1.06	-.93	-1.43
2	-.10	.00	.00	.00	.00
2	.00	.00	1.41	-.10	-.04
2	.12	1.78	-1.56	-1.61	-.27
2	.00	.00	-.59	.00	-.42
2	.00	.11	.00	.00	.00

GROUP	L10CHAN6	L10CHAN8	L10CHAN9	TNFCHAN2	TNFCHAN3
1	.86	-.18	-.18	4.71	3.47
1	3.14	.00	.00	9.70	6.22
1	.00	.00	.00	.52	-1.12
1	1.48	1.51	.00	3.84	.10
1	-.66	-1.27	.13	-2.07	-3.52
1	.00	1.97	.16	.00	.00
1	-1.11	.00	.00	-1.53	.00
1	.00	.00	.00	.00	.00
1	.00	-.69	.00	.19	.00
1	.00	.00	.00	.00	.00
2	.00	.04	.05	4.02	-2.51
2	.00	-.13	-.26	.46	2.21
2	.00	.47	1.25	.00	.00
2	-1.49	-.46	-.66	-1.67	.64
2	.00	.00	1.74	.00	.00
2	-1.55	.00	.00	1.83	.75
2	.51	-.14	-.11	-1.63	2.59
2	.00	.00	.52	-1.60	.00
2	.00	.00	.05	.00	1.20

GROUP	TNFCHAN5	TNFCHAN6	TNFCHAN8	TNFCHAN9	IFNCHAN2
1	11.28	7.21	-.51	-.60	.77
1	10.39	9.94	.00	.00	4.72
1	.60	-1.38	.00	.00	-1.46
1	3.07	-.90	.62	-.30	9.38
1	-3.79	-3.79	1.35	2.73	5.33
1	.00	.00	-.44	-.85	4.81
1	.64	.00	.00	.00	1.43
1	.00	.00	.00	.00	.00
1	6.67	.00	.15	.00	2.43
1	.00	.00	.00	.00	.00
2	2.26	-6.34	.05	-.20	4.51
2	-.61	3.92	.90	-1.27	-.28
2	.00	.00	-.57	-.24	.00
2	-.76	1.71	-2.21	-1.92	-6.23
2	.00	.00	-.24	.27	.00
2	3.57	3.30	.00	.00	.00
2	-3.55	4.54	.46	.33	.78
2	-1.68	.00	.46	.00	-1.01
2	.00	4.85	.00	.00	.94

GROUP	IFNCHAN3	IFNCHAN5	IFNCHAN6	IFNCHAN8	IFNCHAN9
1	1.36	3.38	-.10	-.23	-.37
1	1.35	1.41	-1.47	.36	-1.40
1	-2.12	-2.98	.54	.00	.00
1	2.43	13.94	-10.13	.44	.50
1	-2.40	1.55	-1.87	.64	2.95
1	3.49	4.60	-5.07	.57	.07
1	-1.49	1.08	2.13	.00	.00
1	.00	.00	-.16	.00	.00
1	1.11	1.93	.16	-.26	.00
1	.00	.00	.00	.00	.00
2	1.08	3.37	-1.82	.29	-.34
2	.57	-3.32	3.10	-.60	-.49
2	.00	.00	.00	-.32	1.86
2	-4.11	-6.34	.88	.31	-.81
2	.00	.00	.00	-1.00	-1.64
2	.00	.00	.00	.00	.00
2	.22	1.07	-1.41	-1.04	-1.41
2	-.04	-.68	1.12	.00	.00
2	-1.93	-4.53	-1.65	.13	.32

APPENDEX D

Oklahoma State University Institutional Review Board

Date: Wednesday, September 01, 2004

IRB Application No ED0513

Proposal Title: Comparison of Circulating and Salivary Cytokine Response to a Competitive Collegiate Soccer Tournament

Reviewed and Processed as: Expedited (Spec Pop)

Approval Status Recommended by Reviewer(s): Approved **Protocol Expires: 8/31/2005**

Principal Investigator(s):

Stephen J. Rossi
180 Colvin Center
Stillwater, OK 74078

Melody Phillips
180 Colvin
Stillwater, OK 74078

Douglas Smith
180 Colvin Center
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact me in 415 Whitehurst (phone: 405-744-1676, colson@okstate.edu).

Sincerely,

Carol Olson

Carol Olson, Chair
Institutional Review Board

APPENDEX E

OBU Oklahoma Baptist University
Division of Kinesiology, Leisure Studies and Athletics

405.878.2136

OBU Box 61171
500 West University
Shawnee, OK 74804
www.okbu.edu

Oklahoma State University Institutional Review Board

The athletic director and assistant athletic director are aware of the project "Comparison of circulating and salivary cytokine response to a competitive collegiate soccer tournament". We know the student athlete will have to complete a graded treadmill test to determine aerobic fitness and provide blood and saliva samples, we approve of the project.

Sincerely,

Mike Manlapig
Assistant Athletic Director
OBU Box 61171
500 W. University
Shawnee, Ok 74804
Phone: (405) 878-2140
Fax: (405) 878-2152
Mike.Manlapig@okbu.edu

VITA

Stephen Joseph Rossi

Candidate for the Degree of

Doctor of Philosophy

Thesis: PLASMA AND SALIVARY CYTOKINE RESPONSE TO TWO COMPETITIVE COLLEGIATE SOCCER GAMES

Major Field: Health, Leisure, and Human Performance

Biographical:

Personal Data: Born in Springfield, Massachusetts, on April 4, 1976 the son of Remo and Ellen Rossi. Married to the former Melanie Rossi, on July 6, 2002.

Education: Graduated from Woodbridge High School in Woodbridge, VA in May 1994; received a Bachelor of Arts in Physical Education from University of North Carolina at Wilmington, Wilmington, N.C. in December 1998. Completed the requirements for the Master of Science degree with a major in Exercise Science at Appalachian State University in May 2002. Completed the requirements for the Doctorate of Philosophy degree at Oklahoma State University in May 2006.

Experience: Oklahoma State University: Department of Health and Human Performance as a research and teaching graduate assistant, 2002 to 2006.

Professional Memberships: National Strength and Conditioning Association, American College of Sports Medicine, International Society of Exercise and Immunology , United States Weightlifting.

Name: Stephen Joseph Rossi Date of Degree: May, 2006

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: PLASMA AND SALIVARY CYTOKINE RESPONSE TO TWO COMPETITIVE COLLEGIATE SOCCER GAMES

Pages in Study: 104 Candidate for the Degree of Doctor of Philosophy

Major Field: Health, Leisure and Human Performance

ABSTRACT

PURPOSE**:** The first purpose of this study was to determine the influence of playing in two competitive collegiate soccer games on cytokine levels in college male soccer players. The second purpose of this study was to compare cytokine levels in plasma and saliva before and after exercise**.** METHODS: Blood and saliva were collected the morning of game 1, within one hour after game 2, and 15 hours after game 2 from starter's and non starters. Starters played an average of 76 minutes per game and nonstarters played an average of 2.8 minutes per game. Blood was collected into chilled EDTA tubes from an antecubital vein. After rinsing their mouths with water, athletes were instructed to expectorate into sterile tubes for 10 minutes. Saliva volume and time was recorded to calculate salivary cytokine levels adjusted for secretion rate (pg/min). Clarified saliva and plasma samples were analyzed for IL-6, IL-10, TNF-α, and IFN-γ levels using ELISA. RESULTS: TNF-α and IL-10 was largely undetectable Participation in two competitive collegiate soccer games statistically did not influence post exercise levels of plasma and salivary IL-6, IL-10, TNF-α, and IFN-γ; however, pre exercise plasma levels of IL-6 (24%) and IL-10 (34%) did increase in starters following two competitive soccer games. A non significant increase in salivary levels adjusted for secretion rate of IL-6 (48%), TNF-α (120%), and INF-γ (71%) along with increases in absolute TNF-α (87%) and IFN-γ (33%) was observed following two competitive soccer games. Plasma and salivary cytokine levels before and after exercise were determined to be unrelated**.** CONCLUSION**:** The trend for increases in plasma IL-6 and IL-10 and similar tendencies in salivary IL-6, TNF-α and INF-γ may represent an acute systemic and/or oral inflammatory response to two competitive collegiate soccer games which was undetectable with the small sample size in the present study. Further studies are necessary to determine the relationship between plasma and salivary cytokine levels.

ADVISER'S APPROVAL: Dr. Douglas B. Smith