Western Kentucky University
TopSCHOLAR®

Masters Theses & Specialist Projects | Graduate School

12-1-2010

Effects of a Simulated Tennis Match on Lymphocyte Subset Measurements

Holly Kell
Western Kentucky University, holly.kell@topper.wku.edu

Follow this and additional works at: http://digitalcommons.wku.edu/theses

Part of the Motor Control Commons, Other Immunology and Infectious Disease Commons, and the Sports Sciences Commons

Recommended Citation
Kell, Holly, "Effects of a Simulated Tennis Match on Lymphocyte Subset Measurements" (2010). *Masters Theses & Specialist Projects.* Paper 218.
http://digitalcommons.wku.edu/theses/218

This Thesis is brought to you for free and open access by TopSCHOLAR®. It has been accepted for inclusion in Masters Theses & Specialist Projects by an authorized administrator of TopSCHOLAR®. For more information, please contact connie.foster@wku.edu.

EFFECTS OF A SIMULATED TENNIS MATCH ON LYMPHOCYTE SUBSET MEASUREMENTS

A Thesis
Presented to
The Faculty of the Department of Kinesiology, Recreation and Sport
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Holly Kell
December 2010

EFFECTS OF A SIMULATED TENNIS MATCH ON LYMPHOCYTE SUBSET MEASUREMENTS

Date Recommended___December 2010_____

_____

Director of Thesis

_____

_____

_____ Jan 6, 2011

Dean, Graduate Studies and Research Date

Table of Contents

Abstract……iv

Chapter I- Introduction…….3

Chapter II- Review of Literature……..8

Immune System and Tennis……..8

Intensity and Match Play……..9

Fatigue and Match Play……9

Exercise Immunology……..…10

Chapter III- Methods……..18

Chapter IV- Results…….21

Chapter V- Discussion…….....25

Appendix I- Human Subjects Review Board…….…….39

Bibliography……58

Tables

Subject Characteristics…….21

Figures

Figure of Apoptosis in B Lymphocytes…….……...21

Figure of Cell Migration in Cytotoxic cells……..22

Figure of Decrease in Helper T cell Counts…….....23

EFFECTS OF A SIMULATED TENNIS MATCH ON LYMPHOCYTE SUBSET MEAUSUREMENTS

Name: Holly Kell Date: December 2010 Pages: 60

Directed By: James Navalta

Department of Kinesiology, Recreation and Sport Western Kentucky University

Research has shown that maximal exercise has a significant effect on cells of the immune system. Specifically, lymphocyte count increases during exercise and decreases to a value lower than baseline following an acute exhaustive bout of exercise. The overall lymphocyte response is well characterized, however, the ability of exercise to affect lymphocyte subfractions is unknown to our knowledge. The purpose of this study was to assess and evaluate the affects of a simulated tennis match across two sessions on lymphocyte subsets.

Initial measurements such as age, height, weight, skinfold analysis, and heart rate were recorded for each player, as well as blood samples being obtained by a finger prick before and after the tennis sessions. The tennis protocol started with five serves to the deuce court and five serves to the ad court, then individuals hit twenty-four forehands and twenty-four backhands against an oscillating ball machine. Each bout of serves and ground strokes were repeated ten times, with one minute rests in between each session. Before and immediately after completing the tennis trial, subjects were pricked with a lancet on the non dominant hand so to obtain at least two capillary tubes of blood. Whole

blood was then added to the antibody cocktail, which is mixed according to the antibodies that were tested, which were CD4, CD8, CD19, CD95, and CX_3CR1. Whole blood was added to red blood cell lysis buffer and fixation buffer, and the blood solution was incubated with antibodies specific to cell phenotype. The main results of this study indicated that there was a decrease in mainly post cell counts in pre and post CD19/CD95 measurements (P= .007), an increase in CD8/CX_3CR1 in pre counts and an increase then decrease in post counts without wearing the bionic glove (P= .042), and a decrease in CD4 in the post count measurement with the bionic tennis glove (P= .043). The study's can assist in making recommendations for after match treatment such as health and diet suggestions. Knowledge of prevention and treatment methods are low in the field of tennis and immune functions, so findings in this area could prevent elite athletes from contracting infections between matches.

Chapter I

INTRODUCTION

The awareness of prevention and treatment techniques in tennis are low. When the body is exercised to maximal exertion, there is an “open window” in which immunosuppresion makes it more likely to be susceptible to disease and infections. Thus, it is possible that tennis play may provide the physiological stimulus necessary to induce a similar immunosuppressive response that is seen with maximal exertion exercise. In addition, many tennis players participate in back to back matches through tournaments and may be open to a greater risk of infections due to low lymphocyte counts. It has been shown by Fernandez et al (2006) that during a match, there is a combination of periods of maximal and near maximal work, and longer periods of moderate and low intensity activity [6].

The purpose of this study is to determine the effects of a simulated tennis match on select parameters of the immune system. According to Nieman (2000), when an individual exercises intensely, up to 6 hours after exercise, natural killer cell activities are lowered 40-60% [2]. White blood cells play an important part in the immune system, and when cell counts are low, the body is open to disease and infection. Navalta et al (2010) found there was an increase in lymphocyte apoptosis in male rats with maximal and submaximal exercise [9]. Findings from this study also indicated an increase in lymphocyte apoptosis in female mice immediately and 24-h post exercise [9]. These results show that after maximal and even submaximal exercise in rats/mice, there is an

increase of apoptosis in white blood cells. It has been found that neutrophil counts increase during and after exercise, when lymphocyte counts increase while exercising and then decrease after [1]. This study will be researching lymphocytes, T cells, B cells, monocytes, neutrophils, CD95 (delayed apoptosis), and migration. Lymphocytes are a type of white blood cell that is important in the formation of antibodies, and function in the development of immunity and include T cells and B cells. T cells are a type of white blood cell that helps fight and protect the body from infections. B cells are a type of white blood cell that develops in the bone marrow and produces antibodies that help the immune system fight infection. CD95 is a cellular marker that has been utilized to determine the delayed onset of apoptosis. Migration refers to the movement of a population of cells from one place to another. According to the study performed by Neiman et al (2000), lymphocyte counts decreased 19% 1-hr post exercise ($P<.01$) [3].

The study by Nieman et al (2000) measured the response of the immune system of individuals who performed 2 hours of tennis drills outside between the ages of 14-18 years old. The investigation found no differences in lymphocyte counts between males and females [3]. In an investigation by Navalta et al (2007), there were similar exercise-induced apoptosis found between untrained males and menstruating females when performing a single VO_2 max test [11]. The study by Girard et al (2009) assessed that speed, vertical power abilities, and maximal strength in the dominant side were significantly correlated with tennis performance [8]. According to Navalta et al (2009), cycle ergometry stimulates lymphocyte apoptosis within endurance trained individuals [10]. The simulated tennis match in the current study limited participants to being one

handed on the forehand side and one or two handed on the backhand side so as to control for speed and power abilities on the dominant side.

STATEMENT OF PURPOSE

The purpose of this investigation is to determine if lymphocyte cell count, apoptosis, migration, and delayed apoptosis will be affected after a simulated tennis match. Results may allow recommendations to be made in relation to post match treatment.

STATEMENT OF HYPOTHESIS

Cell Count

Ho: There will be no change in lymphocyte counts before or after a simulated tennis match.

Ha1: There will be a change in overall lymphocyte counts before or after a simulated tennis match.

Migration

Ho: There will be no migration in lymphocytes before or after a simulated tennis match.

Ha1: There will be migration in lymphocytes before or after a simulated tennis match.

Delayed Apoptosis

Ho: There will not be delayed apoptosis in lymphocytes before or after a simulated tennis match.

Ha1: There will be delayed apoptosis in lymphocytes before or after a simulated tennis match.

DEFINITION OF TERMS

1. Lymphocyte (CD3): a type of white blood cell that is important to the formation of antibodies. They function in the development of immunity and include T cells and B cells.
2. T cell (CD4/CD8): a type of white blood cell that helps fight and protect the body against infection.
3. B cell (CD19): a type of white blood cell that develops in the bone marrow and produces antibodies that help the immune system fight infection.
4. Monocyte (CD16): a type of white blood cell that increases during a variety of conditions including severe infection. They remove debris and microorganisms.
5. Neutrophil (CD45): a type of white blood cell that is highly destructive of microorganisms.
6. Delayed apoptosis (CD95): an antibody that determines the delayed onset of apoptosis.
7. Migration (CX_3CR1): movement of a population of cells from one place to another.
8. Apoptosis (Annexin V): cell death/the elimination of cells without releasing harmful substances into the body.
9. Immune Function: protects the body against infection and disease.
10. Flow Cytometry: technique for examining and counting microscopic particles, such as cells.

LIMITATIONS

1. Potential loss of subjects due to scheduling conflicts.

2. Potential loss of subjects due to individual's lack of tennis experience.
3. Difficulty obtaining parental consent for minors.
4. Potential for equipment not functioning correctly.
5. Lack of equipment could lead to longer resting periods between testing sessions.
6. Equipment malfunction could lead to subjects not completing the hitting sessions.

DELIMITATIONS

- For the two tennis sessions, subjects will report to the Tennis Performance Institute (TPI) in Bowling Green, Kentucky to perform a simulated tennis match.
- The simulated tennis match will be performed with the Bionic™ Tennis Glove and without the Bionic™ Tennis Glove with at least one day between testing.
- Before starting the warm up, subjects needed to run around two tennis courts twice and perform any necessary stretching.
- The warm up consisted of hitting 5 serves to the deuce court, and 5 serves to the ad court. Subjects then hit 10 ground strokes to the deuce court, and 10 ground strokes to the ad court against the oscillating ball machine.
- The protocol begins with 5 serves to the deuce court and 5 serves to the ad court. Participants will then hit 24 crosscourt forehands and 24 crosscourt backhands against an oscillating ball machine. This procedure will be repeated 10 times with a 1 minute rest between each bout.
- Subjects will hit a total of 100 serves and 480 ground strokes.

Chapter II

REVIEW OF LITERATURE

Immune System and Tennis

This study by Nieman et al measured the response of the immune system to a 2hr intensive bout of tennis drills in adolescent male and female athletes. There were twenty participants, ten male and ten female, between the ages of 14-18 years old. Athletes reported to the tennis facility at 6:30 a.m. without food and exercise for nine hours. A 35 µ blood sample was taken from each participant before beginning the tennis regimen. Subjects took 4-5 minute breaks every fifteen minutes between drills to ingest a carbohydrate beverage, and measure HR and RPE. Tennis drills consisted of cross-court rallies, overheads, approach shorts, and drop shots. Immediately after the 2hr tennis drills, another 35 µ blood sample was taken, followed by a 1hr post exercise sample. Blood samples were obtained from an antecubital vein in the supine position. Immune and hormone values were analyzed using repeated measures analysis of variance. Changes from pre exercise to immediate and 1hr post exercise values were tested for significance using paired t-tests.

There were no significant changes shown between males and females. Neutrophil counts increased 77%, and monocyte counts increased 26% immediately post exercise while lymphocyte counts (primarily NK) decreased 19% 1hr post exercise. Tennis drills were comparable to those the athletes experienced during their 17-18 hour weeks of training. Immune changes following the tennis drills were of low magnitude and much smaller than those measured after 2-3hr bouts of intensive running and cycling. The

small changes could have been due to frequent resting periods, and the mixed aerobic and anaerobic exertion demands of tennis. This study provided no evidence that an intensive and prolonged bout of tennis drills causes excessive physiological stress to the immune system [3].

Intensity and Match Play

This review by Fernandez et al focuses on the characteristics of tennis players during match play and gives insight into the energy demands of tennis. Match play often lasts longer than one hour and in some cases longer than 5 hours. During a match, there is a combination of periods of maximal and near maximal work, and longer periods of moderate and low intensity activity. Match intensity depends greatly on the player's level, style, and sex, and is also influenced by the court surface and ball type. These factors have important influence over the training of a tennis player, which should resemble match play intensity, and include intensity training with appropriate work to rest ratios [6].

Fatigue and Match Play

This review by Mendez-Villanueva et al addresses metabolic, neural, mechanical and thermal alterations during tennis match play with specific focus on associations with fatigue. Several studies have provided a link between fatigue and the impairment of tennis skills. An important physiological requirement to succeed at a competitive level might be the player's ability to resist fatigue. The ability to maintain skilled on-court performance and/or optimal muscle function during a demanding match can be

compromised due to hypoglycemia, muscle damage, and hyperthermia. Research evidence on this topic is limited. It is unclear at what extent players experience fatigue during high-level tennis match play and what the physiological mechanisms are that are likely to contribute to the deterioration in performance [15].

Exercise Immunology

Marlee Gleeson explains that even though exercise immunology has been studied for over a century, it is still thought of as a moderately new area of study. Investigations on the effects of infection, exercise and blood cells has expanded and enhanced the world of sports medicine, sports science, and immunology. It is agreed that moderate levels of exercise increase immune function while decreasing the risk of infection, and that maximal levels of exercise can increase the risk of infection. As discussed in a different article, Nieman explains there is an “open window” for infection when an individual involves themselves in maximal exertion. A review performed by Laurel Mackinnon discussed the immune system’s effects from overtraining and showed an increased risk of infection. A review by Bente Pederson explained that eccentric and concentric exercises differ in terms of the cytokine reaction to exercise, being that eccentric exercises are linked to an increase and production of cytokines.

It is obvious that the maintenance of health and exercise is extremely important to all populations. Research regarding moderate and maximal exercise and the effects of immune systems are in the early stages and require further investigations so that exercise and the body’s response can be grasped and appreciated [14].

Many studies have found that moderate intensity training can enhance the body's immune system which will decrease susceptibility to infection. This overview by Roy J. Shepard covers the different investigations looking at upper respiratory infections (URI) and whether they are increased by intense exercise/physical activity. It is thought that previous studies give too little information on activity patterns, fitness levels, endurance levels, frequency, intensity, durations, and immune functions. There were seven studies which had the highest arbitrary ratings and three of these studies found that there was an increased susceptibility to disease when there was an increase in exercise. The remaining four studies in this group found either no or decreased infection susceptibility. It is thought that there is no evidence supporting that the quality of experiment design is related to an increase or decrease in being susceptible to infection.

URI evidence is based on objective criteria which has been recovered from medical records or a questionnaire based on various URI symptoms. Qualifications for determining respiratory infections are vague and inconsistent. Because of these discrepancies, results are hindered and false data is reported. Natural killer (NK) cells play an imperative role in protecting the body's immune system and in preventing viral infection. By performing repeated sessions of moderate intensity exercise, NK counts increase but are decreased 2-24 hours after strenuous exercise.

There is great need for further research to be conducted in the area of physical activity and upper respiratory infections. A way of improving future studies in this field of study could be to improve techniques of data collection which would in turn enhance

outcomes and results. It is well known by exercise immunologists that moderate intensity exercise increases the body's immune system which results in fighting infections where the opposite reaction happens when the body performs maximal levels of exercise. There are many exercise and URI studies that have found contradicting results which means there needs to be improved study designs that will allow clearer answers to the many questions that are still being asked [17].

Immunity and exercise has been researched since the 19th century, but not until the 1980's has it been studied more in depth. This paper by Nieman focuses on literature over the effects of extended and intense exercise on the immune system since 1990. A widespread thought among coaches and athletes is that prolonged endurance exercise and overtraining can lead to upper respiratory tract infections (URTI). It has been found by various studies that intense exercise can increase the risk of URTI for up to 1-2 weeks after the excessive training.

A study by Baj et al measured lymphocyte responses during high and low training volumes between 16 untrained males and 15 elite male cyclists. There were no major differences in immune system changes when the two groups cycled at low intensities, however, there were differences measured during the high training volumes.

Natural killer cell activity (NKCA) respond when neutrophil counts are low and when the immune system responds differently to intensive exercise. The majority of cross sectional studies have demonstrated increased NKCA measurements in endurance athletes as opposed to non athletes. In endurance athletes, neutrophil counts have been found to be normal, except during high intensity exercise, when they are lowered.

Nieman (2000) refers to a change in the immune system after intense exercise as an “open window.” To current knowledge, this theory has not been completely investigated or proven that there are extreme immune system deficits 1-2 weeks after heavy and prolonged exercise. When an individual exercises intensely, up to 6 hours after exercise, NKCA are lowered 40-60%. It is not clear on whether this finding is due to migration or where the NK cells go or if it is linked to URTI.

There are multiple precautions for endurance athletes to take in order to protect the body’s immune system against foreign pathogens which are limiting stress, well balanced diet, avoid overtraining, proper sleep, limit hand/eye contact, no rapid weight loss, avoid sick people, and get proper vaccinations [2].

The overview by Phaneuf and Leeuwenburgh covers programmed cell death (apoptosis) and its relation to exercise. It is apparent that apoptosis and necrosis happens not only with exercise but in different ways from one another. Necrosis happens when there is injury to a cell and is described as a cell that has swelling, inflammation, and rupturing, and a characteristic of this type of injury to a cell is delayed-onset muscle soreness. Apoptosis occurs when the cell shrinks, and when there is membrane blebbing and chromatin condensation.

Previous studies have found that with exercise, there is lymphocyte and skeletal muscle apoptosis. Different stresses on the body such as heat, anxiety, and stress to the body can induce immune system changes. A very important finding in apoptosis research is there is apoptosis in lymphocytes which takes place immediately post and 24 hours post intensive exercise.

Apoptosis has been shown to occur in skeletal muscles after exercise and it is still unknown if the cells are completely dead or are marked as going through the apoptotic process. Intensive or eccentric exercise may be a source of mechanical injury, and inflammation which leads to necrosis and apoptosis. There are future studies which need to be conducted in this area of apoptosis and necrosis during intensive exercise. Further investigations should concentrate on how the cells are being lost, where they go, and when exactly they are being lost [18].

The article by Mars et al explains Lymphocytosis happens with exercise and T cells, B cells, and Natural Killer (NK) cells are recruited, and it has been demonstrated that there is a significantly larger increase in B cells rather than T cells. Lymphocyte counts decrease after exercise and with maximal exercise, lymphocyte functions are reduced and there is a 50% decrease in mitogen stimulated lymphocyte proliferation.

The first study conducted had eleven males with a mean age of 29.6 years old, all of who participated regularly in endurance training at least three times a week. The treadmill protocol consisted of a 5 minute warm up at 8km/h at 3% gradient, then the speed and gradient was increased 1km/h and 1% each minute until the subjects reached 12km/h. Heart rate, oxygen consumption, and blood samples were taken before, immediately after, 24h and 48h post treadmill test. Blood samples were analyzed on a glass microscopic slide and then viewed with a fluorescent microscope. There was 25 cells viewed on each slide and two slides per sample were viewed. Identification of lymphocytes was done according to size and nuclear shape.

The second study included 3 athletes, all of who completed the treadmill test explained in the previous study. Blood samples were taken before, immediately after, 24h and 48h post treadmill test. Samples were read using Flow Cytometry.

Lymphocytes with central nuclei were greatly decreased in all of the post exercise measurements ($P<.0005$). There was an increase in peripheral aggregation immediately post exercise ($P=.004$), and polar clumping was also increased in all of the blood samples post exercise.

It is apparent that necrosis and apoptosis differ in many ways being that apoptosis is referred to as "cell suicide." Apoptosis is extremely important in lymphocyte development and maintenance, and the apoptotic process can be triggered by different stresses to the body as mentioned before. This study found that microscopy indicates that 50% of lymphocytes have nuclear differences 24h after exercise and that flow cytometry indicates that over 80% of lymphocytes have already gone through the apoptotic process at this time point. This outcome suggests that apoptosis is still taking place many hours after exercise. All of the participants had an increase in lymphocyte apoptosis immediately post exercise by 31-48%, and 24h post was 83-88%. Lymphocyte apoptosis is shown in this study and could be an important part in decreased immunity associated with high intensity exercise [13].

The study by Park et al examined the effects leukocytes, neutrophils, lymphocytes, monocytes, immunoglobulins, IL-6, CK, and CRP in elite and amateur triathletes. Triathlons are endurance based and consist of swimming, cycling, and running. The Olympic triathlon includes a 1.5k swim, 40k cycle, and 10km run.

Endurance events such as triathlons take an extreme toll on the body and immune system, causing muscle damage, inflammation, metabolic changes, and immune system changes.

The investigation included 7 elites, and 8 amateur triathletes who participated in the 2006 TONG YUNG international triathlon. The average age of the elite subjects was 23.5 years old, and average age of the amateur subjects was 37.2 years old. Pre measurement blood samples were taken three days before the race, and post measurement samples were taken immediately after the race, and 2 hours post and 7 days post samples were taken as well.

Leukocyte measurements immediately post triathlon and in the recovery period greatly increased in both the elite and amateur athletes, neutrophil measurement immediately post triathlon and in the recovery period greatly increased in both athlete groups, lymphocytes increased in both elite and amateur athletes immediately post exercise and in the recovery period, and monocyte counts increased immediately post triathlon in both groups as well.

Because triathlons are such a demanding event, extreme training causes great immune system changes. When there is immune suppression, the body is more susceptible to infection, resulting less training time due to disease or infection. Neutrophils make up 50-60% of the total leukocytes and are essential in defending the immune system, and were found that exhaustive exercise increases these cells both during and after. Lymphocyte counts increased during the triathlon and then decreased to

values found before the event. Increases of the lymphocytes are due to the recruitment of all the white blood cells with exercise.

The outcomes of this study demonstrate that there was an increase in leukocytes, neutrophils, IgG, IL-6, CK, and CRP. The variables that decreased were lymphocytes, monocytes, IgA, and IgM in both elite and amateur athlete groups. Results showed that the elite group recovered faster than those in the amateur group after the triathlon event. Findings of this investigation provide good stepping stones to future investigations, and it is suggested that there be more studies conducted in this area [4].

Chapter III

METHODS

Subjects

Participants were both male between the ages of 11-44 and were recruited from high schools, college, and tennis facilities in Bowling Green, KY. Individuals had to have prior or current tennis experience at the high school, collegiate or recreational level, and had to have played in the past three months. It was determined that at least eight participants were needed for this study. Subjects completed two days of testing, with and without a bionic tennis glove. The study was approved by Human Subjects Review Board (see Appendix I) and all participants provided informed consent prior to participation in the investigation.

Protocol

Subjects were monitored throughout both testing procedures, with and without the Bionic™ Tennis Glove. Initial measurements such as age, height, weight, skinfold analysis, and heart rate were recorded for each player, as well as blood samples being obtained by a finger prick before and after the tennis sessions. Height and weight were measured using Detect-Medic Scale and attached stadiometer (Detecto Scales Inc., New York), and skinfold measurements taken at the chest, abdomen, and thigh were measured using calibrated Lange skinfold calipers. Warm up for the participants included running around two tennis courts three times, stretching, five serves to the deuce court and five serves to the ad court, ten crosscourt forehands and ten crosscourt backhands. The tennis protocol was designed to mimic an actual tennis match and started with five serves to the

deuce court and five serves to the ad court, then individuals hit twenty-four forehands and twenty-four backhands against an oscillating ball machine. Each bout of serves and ground strokes were repeated ten times, with one minute rests in between each session.

Blood Sample Protocol

Before and immediately after completing the tennis trial, subjects were pricked with a lancet on the non dominant hand to obtain at least two capillary tubes of blood (approximately 120 μl). Whole blood was then added to the antibody cocktail, which was mixed according to the antibodies that were tested, which were CD4, CD8, CD19, CD95, and CX_3CR1. Blood samples were obtained in both sessions with and without the bionic glove. There were four test tubes for each pre and post measurement which are lymphocytes, helper T cells, cytotoxic cells, B cells, delayed apoptosis, and migration. The blood protocol included adding whole blood to the antibody cocktail, which were mixed according to the antibodies tested. Incubate the samples for thirty minutes in the dark at room temperature. After incubation, I will centrifuge samples for ten minutes, and then decant the samples. I will then add 300 ml of red blood cell (RBC) lysis buffer, and Vortex the samples well. Then, I will incubate samples for fifteen minutes in the dark at room temperature. After incubation, I will add 300 ml of PBS. Then, centrifuge samples for ten minutes, and decant. Last, I will vortex the samples, and they are then ready to be read on the Flow Cytometer. This process will be completed for each pre, post, and 1-hr post samples.

1. Add 10 µ whole blood to each antibody cocktail.
2. Incubate blood samples in dark room for thirty minutes at room temperature.
3. Centrifuge blood samples for ten minutes, then decant.
4. Add 300 µ red blood cell (RBC) lysis buffer, vortex well.
5. Incubate blood samples in dark room for fifteen minutes at room temperature.
6. Add 300 µ PBS.
7. Centrifuge blood samples for ten minutes, then decant.
8. Samples read on Flow Cytometer.

The blood sample protocol will be completed for pre and post blood samples. There were eight blood samples acquired, four samples collected for the pre measurement, and four samples collected for the post measurement.

Statistical Analysis

The statistical analysis that was used for this study was a paired sample T-test, with a P value of 0.05.

Chapter IV

RESULTS

	Age	Height (cm)	Weight (lbs)	Skinfold
Average	18.9	178.1	146.7	10.9
SD	±3.3	±6.8	±17.8	±3.5

There are multiple comparisons being made in this study between lymphocyte subsets and the Bionic™ Tennis Glove across two days of testing. Results evaluated were helper T cells (CD4), cytotoxic cells (CD8), migration (CX_3CR1), B cells (CD19), delayed apoptosis (CD95), and the bionic glove in pre and post measurements.

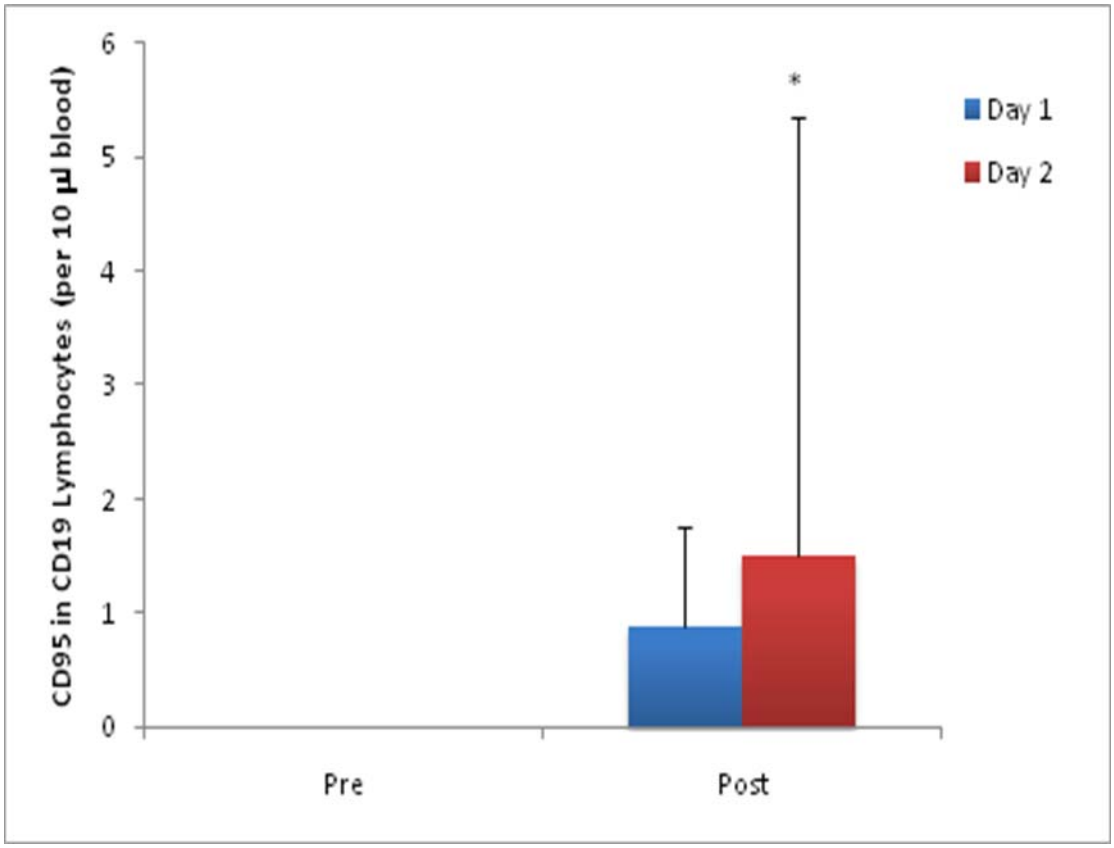

Figure 1. Apoptosis (CD95) in B lymphocytes (CD19) significantly increased with the second simulated tennis match (P = 0.007). * indicates significant difference between pre and post measurements

Figure 1 shows great increase between pre and post measurements of CD19 lymphocytes for apoptosis measurement (CD95) on day two of testing. (P= .007).

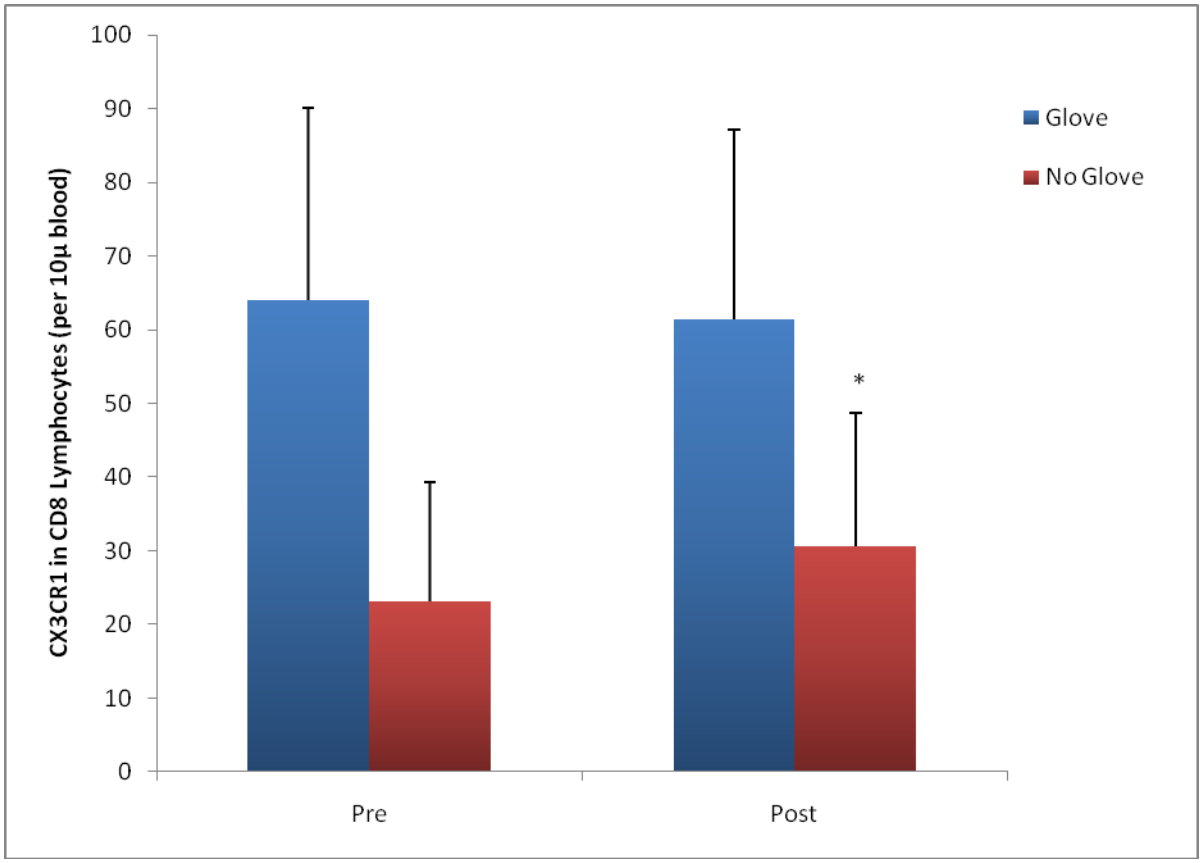

Figure 2. Cell migration (CX_3CR1) in cytotoxic lymphocytes (CD8) decreases on Day 2 of the simulated tennis match without wearing the Bionic™ Tennis Glove (P=.042). *indicates significant difference between pre and post measurements

Figure 2 shows there is an increase in CD8/CX_3CR1 in pre counts and an increase then decrease in post counts without wearing the Bionic™ Tennis Glove. There is a large

jump in the cytotoxic T cell and migration counts in the post measurement showing a rise in these lymphocyte subsets after the simulated tennis match (P= .042).

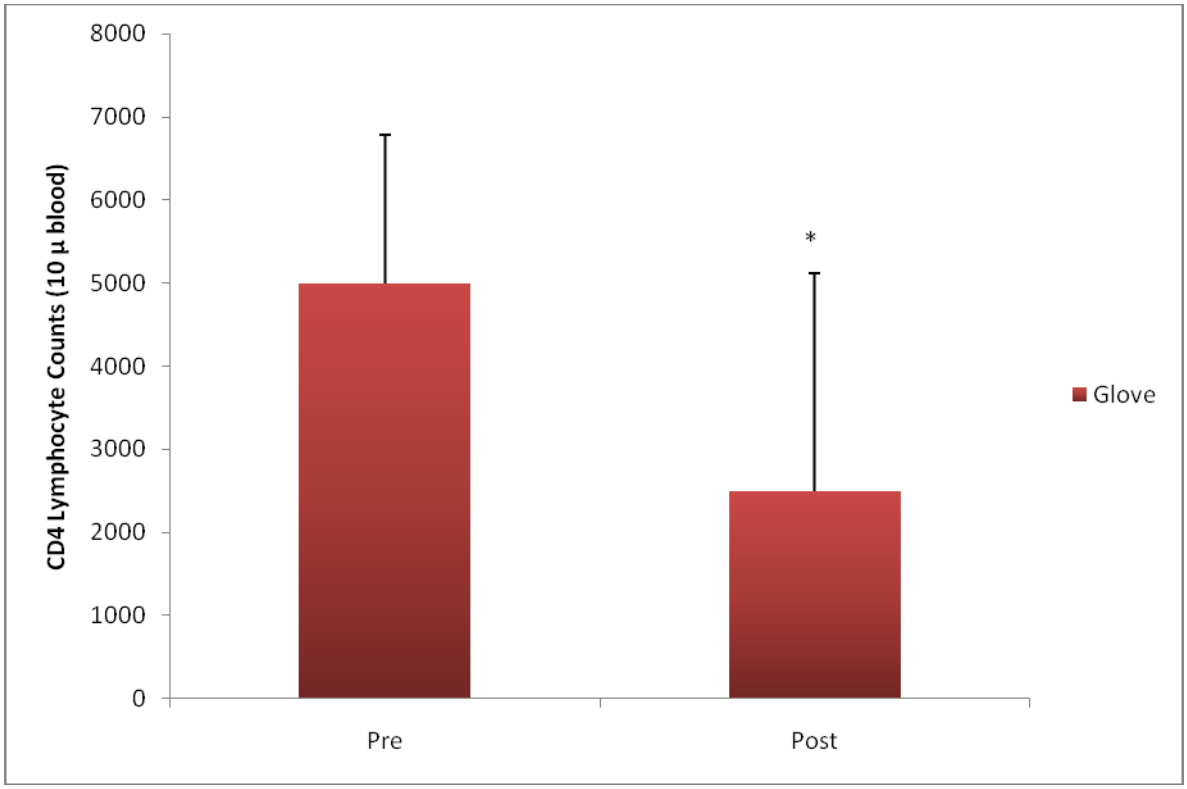

Figure 3. Helper T cell (CD4) counts decreased after the simulated tennis match with the Bionic™ Tennis Glove (P=.043). *indicates significant difference between pre and post measurements

This figure shows the difference between CD4 counts before and after the simulated tennis match. Measurements between the helper T cells decreased in the post count measurement with the Bionic™ Tennis Glove. Results of this table show that each subject reduced in CD4 cell count the second day of testing (P= .043).

Many variables were tested and showed significance in key points which were pre and post CD19/CD95 measurements (P= .007), pre and post CD8/CX_3CR1

measurements without the Bionic™ Tennis Glove (P= .042), and pre and post CD4 measurements with the Bionic™ Tennis Glove (.043).

There was no significance in the measurements between pre and post CD4/CX_3CR1, CD4/CD8, CD8, CD8/CD95, CD19, and CD19/CX_3CR1 with or without the Bionic™ Tennis Glove.

Chapter V

DISCUSSION

The purpose of this investigation was to determine lymphocyte subset counts before and after a simulated tennis match. It is established that moderate levels of exercise increase immune function while decreasing the risk of infection, and that maximal levels of exercise can increase the risk of infection [14]. Signs of immune changes which are related to exercise are inflammation and decreased T and B cell activity, and some results show that fatty acids help control inflammation, and the release of cytokines [12]. By examining the outcomes of this study, recommendations for tennis match prevention and treatment plans for players, coaches, and trainers can be made. It is obvious that the maintenance of health and exercise is extremely important to not only elite athletes but to all populations [14]. Previous studies have found that over a 12-15 week period, when compared to inactivity, brisk walking decreases sick days by half without any change in immune function at rest [16]. Multiple hypotheses were made regarding helper T cells, cytotoxic T cells, migration, and delayed apoptosis before beginning this study, all of which were thought to have no change after the simulated tennis match.

A main result of this study indicated that there was a decrease in mainly post cell counts in pre and post CD19/CD95 measurements (P= .007). Other significant outcomes showed an increase in CD8/CX_3CR1 in pre counts and an increase then decrease in post counts without wearing the Bionic™ Tennis Glove (P= .042), and a decrease in CD4 in

the post count measurement with the Bionic™ Tennis Glove (P= .043). Results of the current study were similar to Nieman's (2000) outcomes, being that Nieman found a

decrease in lymphocytes after the tennis match [3] as did this investigation. Even though the protocols had slight differences and Nieman collected data on only one day as opposed to two days, the conclusions are comparable. Previous studies have found that with exercise, there is lymphocyte apoptosis, and different stresses on the body such as heat, anxiety, and stress to the body can induce immune system changes [18]. Stress to the body during the simulated tennis match could explain the decrease in cytotoxic cells, B cells, and helper T cells.

There has not been a great deal of investigations conducted over simulated tennis matches, and lymphocyte subsets so the results that were found in this area of study are important to future findings and studies. A previous study by Neiman et al found that lymphocyte counts (primarily NK) decreased 19% 1hr post exercise [3], and even though the present study did not collect 1-hr post data, results did show that lymphocyte counts decreased immediately after the simulated tennis match. Mars et al found that lymphocyte counts decrease after exercise and with maximal exercise, lymphocyte functions are reduced [13] which is a comparable finding to this current study. Decreased cell counts after the simulated tennis match are similar results that have been found in previous studies and correspond with the current literature. These previous studies all found that lymphocyte cell count decreased in the hour following exercise. The current study showed a reduction in cell count immediately after, indicating that cell

count decreased during exercise. The reduction in lymphocyte subsets during the simulated tennis match could be due to the amount of tennis balls hit in an hour and a half time span, or the decrease could be a result of the subjects completing two hitting sessions with just one day between testing.

The findings of this study suggest that helper T cells decrease immediately following a tennis match, cytotoxic T cells migrate immediately following a tennis match, and delayed apoptosis in B cells decreases immediately after a tennis match. Mars et al found that lymphocytosis happens with exercise and T cells, B cells, and Natural Killer (NK) cells are recruited, and has been demonstrated that there is a significantly larger increase in B cells rather than T cells [13]. Even though results from this study did not see an increase in B or T cells as explained by Mars et al, the explanation of lymphocytosis and exercise helps clarify and greatly relates to the reason for the decrease in cytotoxic cells, helper T cells, and B cells. Mendez-Villanueva et al stated that it is unclear at what extent players experience fatigue during high-level tennis match play and what the physiological mechanisms are that are likely to contribute to the deterioration in performance [15], which is the reason for testing the Bionic™ Tennis Glove in this study. The Bionic™ Tennis Glove is designed to help with hand fatigue and was studied across two hitting sessions in this investigation, and the findings suggest that there is no evidence the Bionic™ Tennis Glove had any significance on the players' lymphocyte subset counts. It is unclear at what extent players experience fatigue during high-level tennis match play and what the physiological mechanisms are that are likely to contribute to the deterioration in performance [15]. Match play often lasts longer than one hour and in some cases longer than 5 hours [6], so future investigations should

explore a tennis player's fatigue and whether the Bionic™ Tennis Glove would be helpful in preventing or prolonging the decline in a player's tennis performance due to weakness and exhaustion.

By conducting this study, results can assist in shedding light on effects of the immune system after a simulated tennis match. The study's outcomes can help make recommendations for after match treatment. There have been many surveys demonstrating immune system repression resulting in upper respiratory tract infections after performing in marathons and other various running events [5]. There are multiple precautions for endurance athletes to take in order to protect the body's immune system against foreign pathogens which are limiting stress, well balanced diet, avoid overtraining, proper sleep, limit hand/eye contact, no rapid weight loss, avoid sick people, and get proper vaccinations [2]. Knowledge of prevention and treatment methods are low in the field of tennis and immune functions, so findings in this area could prevent elite athletes from contracting infections between matches.

Recommendations that could be made for pre and post match treatment are getting adequate sleep, exercising moderately, moderate training between matches, avoid overtraining, control anxiety, maintain a proper balanced diet, and avoid hand/eye contact. These treatment suggestions are especially important directly after a tennis match and within the next 1-2 weeks since lymphocytes decrease for up to 6 hours after prolonged exercise [2], and because there is an "open window" for infection 1-2 weeks following exhaustive exercise [2]. When an individual exercises intensely, up to 6 hours after exercise, NKCA (natural killer cell activity) are lowered 40-60% [2].

Because exercise immunology is an important and essential field of study, researchers are discovering new findings every day. Future investigations should examine the effects of a tennis match on the immune system more in depth. Education in this area could not only help make training and nutrition recommendation to athletes, coaches, trainers, and parents, but could help enhance the knowledge and future studies in related sports.

APPENDIX I

Human Subjects Review Board

APPLICATION FOR APPROVAL OF INVESTIGATIONS INVOLVING THE USE OF HUMAN SUBJECTS

PLEASE TYPE OR USE A WORD PROCESSOR

Submit to the Office of Sponsored Programs, 301 Potter Hall, by the first working Monday of the month for screening prior to the HSRB meeting. Please add additional space between items as needed to describe your project.

The human subjects application must stand alone. Your informed consent document(s), survey instrument, and site approval letter(s) should be attached to the application and referred to in your write up of the appropriate sections so that reviewers may read them as they read your application. Thesis proposals or other documents that are meant to substitute for completing the sections of the application will not be read and should not be attached.

1. Principal Investigator's Name: ____Mark Schafer_____

   Email Address: ___mark.schafer@wku.edu_____

   Mailing Address: __1906 College Heights Blvd # 11089__1059 Smith Stadium_

   Department: _Kinesiology, Recreation and Sport____ Phone: _270-745-5857__

   Completion of the Citi Program Training? Yes _X____ No_____

   Found at www.citiprogram.org Date ___2/3/2010_____

   Co-Investigator: _____Scott Lyons_____

   Email Address: _____scott.lyons@wku.edu_____

Mailing Address: _____1906 College Heights Blvd # 11089__1056 Smith Stadium ___

Department: ___ Kinesiology, Recreation and Sport __ Phone: __270-745-6035__

Completion of the Citi Program Training? Yes __X___ No_____

Found at www.citiprogram.org Date ___8/2010_____

2. If you are a **student**, provide the following information:

Faculty Sponsor: _____ Department: _____ Phone: _____

Faculty Mailing Address: _____

Completion of the Citi Program Training? Yes _____ No_____

Found at www.citiprogram.org Date _____

Student Permanent Address (where you can be reached 12 months from now):

_____

Is this your thesis or dissertation research? Yes _____ No_____

Policy of Research Responsibility. The Western Kentucky University Human Subjects Review Board defines the responsible party or parties of the research project as the Principal Investigator and Co- Principal Investigator. In those cases when a student holds the title of Principal Investigator, the Faculty Sponsor (Advisor, Supervisor, Administrator, or general managing Council) will conduct oversight of the research project and share in the accountability to assure the responsible conduct of research. Researchers outside of the Western Kentucky University campus system are required to provide proof of training to obtain approval for WKU Human Subjects protocols. This

proof must be presented by the Compliance Official at the researcher's institution to the WKU Compliance official. When no training requirement exists at the researcher's host institution, training must be conducted through affiliation of Western Kentucky University CITI Program.org requirements. WKU faculty, staff, and students are required to complete the CITI Program Training modules outlined by the WKU HSRB.

3. Title of project: Evaluation of hand fatigue and shot accuracy when wearing the bionic tennis glove

4. Project Period: Start __upon HSRB approval__ End __January 31, 2011_____

month, day, year

Note: *Your project period may not start until after the HSRB has given final approval.*

5. Has this project previously been considered by the HSRB? Yes ____ No__X__

If yes, give approximate date of review:

6. Do you or any other person responsible for the design, conduct, or reporting of this research have an economic interest in, or act as an officer or a director of, any outside entity whose financial interests would reasonably appear to be affected by the research?

Yes _____ No _X_____

If "yes," please include a statement below that may be considered by the Institutional Conflict of Interest Committee:

7. Is a proposal for external support being submitted? Yes____ No__X___

If yes, you must submit (as a separate attachment) one complete copy of that proposal as soon as it is available and complete the following:

a. Is notification of Human Subject approval required? Yes _____ No _____

b. Is this a renewal application? Yes _____ No _____

c. Sponsor's Name:

d. Project Period: From: To:

8. You must include copies of all pertinent information such as, a copy of the questionnaire you will be using or other survey instruments, informed consent documents, letters of approval from cooperating institutions (e.g., schools, hospitals or other medical facilities and/or clinics, human services agencies, individuals such as physicians or other specialists in different fields, etc.), copy of external support proposals, etc.

9. Does this project SOLELY involve analysis of an existing database? Yes ____ No __X_

If yes, please provide the complete URLs for all databases that are relevant to this application, then complete Section A and the signature portion of the application and forward the application to Sponsored Programs:

If the database is not available in an electronic format readily available on the internet, please provide evidence that the data were collected using procedures that were reviewed and approved by an Institutional Review Board, then complete Section A and the signature portion of the application and forward the application to Sponsored Programs.

In the space below, please provide complete answers to the following questions. Add additional space between items as needed.

I. PROPOSED RESEARCH PROJECT

A. Provide a brief summary of the proposed research. Include major hypotheses and research design.

The Bionic™ Tennis Glove was developed by an orthopedic hand surgeon and consists of an enhanced design that attempts to mitigate intrinsic hand fatigue when playing tennis. Some of the key features include: anatomical relief pads, pre-rotated finger design, and motion zones. It is proposed that each of these features will improve the hand grip on the tennis racquet and lead to improved performance when playing tennis.

The purpose of the study is to determine if recreational and competitive tennis players will experience intrinsic hand skeletal muscle fatigue during simulated tennis match. The investigation will also examine if tennis players wearing the Bionic™ Tennis Glove during a standard 2 out of 3 set simulated match protocol will experience less hand fatigue and enhanced shot accuracy compared to not wearing the glove.

Research Design and Methods

The investigation will employ a with-in subject, multiple observation experimental design consisting of two separate 60 to 90 minute sessions. (Table 1.) For both sessions, subjects will report to the Tennis Performance Institute (TPI) in Bowling Green, Kentucky to perform a simulated tennis match.

Table 1. Experimental Design

Sessions

(Day 1)	(Day 2)
Informed Consent and Assent instructions	Review Study
Physical Activity Questionnaire (PAR-Q)	Finger-stick blood sample (Pre, mid, & post)
Medical History	Tennis match
Anthropometric (Height, Weight, & Body Composition)	
Study Instructions (Tennis objectives, VAS, hand grip, RPE)	
Finger-stick blood sample (Pre, mid, & post)	
Glove fitting	
Tennis match	

Time : 0 ⟶ 48 to 72 hrs

Forty male (N=20) and female (N=20) volunteers 11-44 years of age will participate in the study. Subjects will have played varsity high school tennis, or college varsity tennis, or competed in USTA-sponsored tennis tournaments. Subjects must have played within the last 3 months. Subjects will be free of musculoskeletal limitations and of diagnosed cardiovascular or metabolic disease. At the time of recruitment and prior to the simulated tennis match protocol, each subject will complete the Physical Activity Readiness Questionnaire (PAR-Q), Medical History, and sign an informed consent prior to participation. The PAR-Q, Medical History from, and the informed consent are attached to this document. To be included in the study, subjects will have to be in a “low risk” category according to the American College of Sports Medicine (ACSM, 2010). Subjects will be excluded from the study based on the following criteria: (a) Answering yes to one

or more of the questions on the PAR-Q without obtaining clearance from their physician (b) Currently pregnant (c) Subjects with implantable devices such as Pacemaker, Automatic Implantable Cardioverter Defibrillators (AICD) (d) Orthopedic (Acute or chronic musculoskeletal injury), cardiovascular (coronary artery disease), respiratory (Chronic Obstructive Pulmonary Disease or Asthma), metabolic conditions (Diabetes), and current smoker. Current smoker is defined as someone who currently smokes or who has quit less than six months ago.

Subjects will be instructed to wear loose fitting clothing (i.e. shorts and t-shirt) and to report to TPI hydrated 2 hours after eating for both exercise trials. Subjects will be instructed not to consume caffeine, or alcohol during the day of the exercise trials. In addition, subjects will be asked to abstain from their regular exercise routine the day of the experimental trials. The first and second study sessions will be conducted at the same time of day. The experimental trials will be conducted on an indoor tennis court at the TPI where ambient temperature will range from 70^0 F to 74^0 F (21^0 to 23^0 C) and percent humidity will be less than 60%.

Prior to the simulated tennis match, subject's body weight, height, and body composition will be measured. The subjects will be given instructions for the hand fatigue Visual analogue (VAS) scale (Grant, 1999), perceived exertion (RPE) (Robertson, 2004), hand grip dynamometer (ACSM, 2010), and the protocol for the simulated tennis match. A heart rate monitor will be placed on the subject to determine heart rate throughout the tennis match. A blood sample will be obtained prior to, immediately following, and 1 hour post simulated tennis match using universal precautions. On day two, the subjects will be given a review of the study scales and tennis match protocol.

Subjects will be randomly assigned to a counterbalance order in which they wear the Bionic™ tennis glove for the first and second data collection trials. Each subject will be fitted for a Bionic tennis glove that best suits their hand size. Subjects will be using their own tennis racquet for each of the trials. The same racquet will be used for both trials.

Simulated tennis match protocol will consists of each subject hitting 480 ground strokes fed at a constant speed, spin, and placement by a ball machine. The

subject will be positioned at the baseline. Subjects will conduct 10 separate hitting sessions with 1 minute rest breaks between sessions. Each session will consist of 10 serves and 48 crosscourt ground strokes (24 forehand and 24 backhands).

Subjects will be tested pre, mid, and post simulated match play for dominant hand strength and heart rate. Subjects will also be tested for hand fatigue using the VAS and RPE scale during the middle and post data collection periods. During each dynamometer test phase, 3 separate measurements will be taken using the average values for each phase. The first hand dynamometer test phase will occur a minimum of 1 minute prior to initiating ball strike in session 1. The second phase of hand dynamometer testing will occur during the rest break just prior to the 6th session at the 230th hit-ball point. Post – hand dynamometer test will be taken 1 minute after the 10th and last session at the 460th hit-ball point.

Accuracy will be measured throughout all 10 hitting sessions, including 480 ground strokes and 100 serves. The tennis court will be divided into 2 symmetrical quadrants bisected in the middle between the center mark on the baseline and the center line. Subjects will hit ground strokes from the baseline aiming crosscourt to the bisected court. If the crosscourt target is hit within the singles line, then it is scored as +1; and if hit outside the target area, it is scored as -1. Serves are accurate if hit within the crosscourt service box and will be recorded in the same +1/-1 scoring system.

B. Describe the source(s) of subjects and the selection criteria. Specifically, how will you obtain potential subjects, and how will you contact them?

Subjects will consist of healthy trained tennis players at Western Kentucky University and in the Bowling Green community. Potential subjects will be recruited using information flyers that will be posted at selected sites in the Bowling Green community and Western Kentucky University campus. Potential subjects who respond to the flyer will have the nature, risk and potential benefits and rights as a research subject explained to them. If the subject agrees to participate in the study and meets the inclusion criteria, he/she will be scheduled for a study date.

C. Informed consent: Describe the consent process and attach all consent documents.

On day one of the study protocol, subjects will complete the PAR-Q, medical history, and informed consent. Upon completing the paperwork, it will be

determined if the subjects meet the study criteria and they are considered “low risk” according to the (ACSM, 2010).

D. Procedures: Provide a step-by-step description of each procedure, including the frequency, duration, and location of each procedure.

The subjects body weight (kg) and height (cm) will be determined using a Detect-Medic Scale and attached stadiometer (Detecto Scales Inc., New York). Subjects will be asked to remove their shoes and will be wearing a t - shirt and shorts.

The subjects body composition will be measured using calibrated Lange skinfold calipers. The objective is to measure subcutaneous fat to determine body fat. The procedure is explained below.

1. Firmly grasp a double fold of skin and the subcutaneous fat between the thumb and index finger of your left hand and lift up away from the body. The skinfold is lifted 1 cm above the site to be measured

2. Lift the fold by placing the thumb and index finger 8 cm (3 inches) apart on a line that is perpendicular to the long axis of the skinfold. The long axis is parallel to the natural cleavage lines of the skin. For individuals with extremely large skinfolds, the thumb and finger will need to be separated more than 8 cm in order to lift the fold.

3. Keep the fold elevated while the measurement is taken (do not let go of the skinfold after placing the calipers).

4. Place the jaws of the caliper perpendicular to the fold, approximately 1 cm below the thumb and index finger, and release the jaw pressure slowly

7. Take the skinfold measurement within 2 seconds after the pressure is released.

8. Open the jaws of the caliper to remove it from the site. Close the jaws slowly to prevent damage or loss of calibration. Let go of the skinfold after removing the calipers.

9. Take a minimum of two measurements at each site. If values vary from each other by more than 4 mm, take additional measurements.

10. Take skinfold measurements in a rotational order rather than consecutive readings at each site.

11. Take the skinfold measurements when the client's skin is dry and lotion- free.

12. Do not measure skinfolds immediately after physical activity because of fluid shifts to the skin

Finger-stick blood samples will be obtained using Universal Precautions at rest for a baseline measurement, immediately following the exercise bout, and 1h following cessation of the exercise bout. Approximately 100 microliters of blood will be obtained with each sampling period, with the total combined amount of blood taken each day being less than half of one teaspoon. Blood will be subjected to biochemical analysis for the determination of lymphocyte subfractions (helper T, suppressor T, and B lymphocytes) and for markers of cell death (early apoptosis, late apoptosis, and necrosis). The investigators have undergone Bloodborne Pathogen training and documentation will be kept on file at Western Kentucky University.

1. Using Universal Precautions, first sterilize the subject's fingertip.

2. Next, puncture the site using an automated lancet.

3. Collect blood into heparanized capillary tubes.

Heart rate (HR) in beats/minute will be measured using wireless Polar Monitoring System (Woodbury, NJ). A heart rate monitor will be placed around the subjects chest at just below the pectoralis major and secured in place with an elastic strap. The receiver is a wrist watch that the subject will have placed on their non-dominant arm.

A rating of perceived exertion scale (RPE) will be used to determine a subjective level exertion when playing tennis. Prior to the simulated tennis play, the subjects will receive standard instructions on RPE scaling procedures. These procedures include: definition of RPE, scale instructions, setting high and low memory anchor points. Subjects will then be oriented to the Borg Scale through the following anchoring procedures. The investigator will first read the definition of RPE: *The perception of physical exertion is defined as the subjective intensity of effort, strain, discomfort, and/or fatigue that you feel during exercise.* Finally, the investigator will read a set of standardized set of instructions to the subjects (Robertson, 2004).

The Visual Analogue Scale (VAS) will be used to determine hand fatigue. Subjects will indicate their level of fatigue by making a mark on a 100 mm horizontal line with a pen. At the far left of the scale it will indicate “none” and at the far right of the scale it will indicate “very severe”.

Hand Grip will be conducted according to ACSM guidelines (ACSM , 2010). The subject will stand with feet shoulder width apart with the hand dynamometer in the dominant hand. Arm will be to the side and the subject will be instructed to perform a maximal grip. The subject will be instructed not to hold their breath or bend their elbow greater than ninety degrees when performing the hand grip procedure.

E. How will confidentiality of the data be maintained? (Note: Data must be securely kept for a minimum of three years on campus.)

During and after study participation, research data will be locked in the principal investigator’s office at Smith Stadium 1059 in a locked file cabinet. A number to maintain confidentiality will identify subjects. The subject’s name and contact information will be kept in a separate file to avoid identification. The data will be kept for a minimum of three years after completion of the study.

F. Describe all known and anticipated risks to the subject including side effects, risks of placebo, risks of normal treatment delay, etc.

Risk associated with the use of a heart rate monitor include redness, irritation, and chafing. An ointment to relieve any discomfort will be provided if necessary.

For finger-stick blood samples can cause minor bruising around the sample area and slight discomfort during the sampling procedure.

The simulated tennis play is a submaximal bout of exercise that poses no greater risk than an actual tennis match.

The investigators hold a current certification from the American Heart Association (AHA) in CPR and AED. Each investigator has a card indicating the certification.

G. Describe the anticipated benefits to subjects, and the importance of the knowledge that may reasonably be expected to result.

The subject may have no benefit to participation. The subjects will learn about their body composition.

H. List of references (if applicable):

American College of Sports Medicine. (2010). ACSM's Guidelines for Exercise Testing and Prescription 7th ed. Philadelphia: Lippincott Williams & Wilkins.

Grant, S., Aitchison, T., Henderson., Christie, J., Zare, S., McMurray, J., Dargie, H. (1999) A comparison of the reproducibility and sensitivity to change of Visual Analogue Scales, Borg scales, and Likert scales in normal subjects during submaximal exercise. *Chest*, 116, 1208-1217.

Robertson, R. (2004). *Perceived Exertion for Practitioners*. Champaign, IL: Human Kinetics.

Additions to or changes in procedures involving human subjects, as well as any problems connected with the use of human subjects once the project has begun, must be brought to the attention of the HSRB as they occur.

II. SIGNATURES

A. I certify that to the best of my knowledge the information presented herein is an accurate reflection of the proposed research project.

_____ _____

Principal Investigator Date

_____ _____

Co-Investigator Date

B. Approval by faculty sponsor (required for all students):

I affirm the accuracy of this application, and I accept the responsibility for the conduct of this research, the supervision of human subjects, and maintenance of informed consent documentation as required by the HSRB.

_____ _____

Faculty Sponsor Date

C. Approval by Department Head is not required (Some departments require approval by the Department Head. Please verify with your department head if their signature is required). If PI is a director or department head, then the PI's immediate superior should sign.

I confirm the accuracy of the information stated in this application. I am familiar with, and approve of the procedures that involve human subjects.

_____ _____

Department Head (or immediate superior) Date

D. Advising Physician*:

I certify that I am a duly licensed physician in the State of Kentucky and that, acting as advising physician, I accept the procedures prescribed herein.

_____ _____

Physician’s Name and Signature Date

*Physician signature is needed only if the project involves medical procedures and the investigator is not a licensed physician.

Project Title: Evaluation of hand fatigue and shot accuracy when wearing the bionic tennis glove

Investigator: _____Dr. Mark Schafer (270) 745-5857 Kinesiology, Recreation and Sport

---

(This portion is for HSRB use only.)

HSRB Determination:

Exempt from Full Review ( ) Expedited Review ( ) Full HSRB Review ( )

( ) Disapproval

( ) Approval ( ) Above minimal risk ( ) Minimal risk

a. approval, subject to minor changes

b. approval in general but requiring major alterations, clarifications or assurances

c. restricted approval

Date of review: _____

Comments:

_____ _____

Human Subjects Review Board Chair Date

_____ _____

Compliance Manager Date

If you have questions regarding review procedures or completion of this HSRB application, contact the **Office of Sponsored Programs**:

Director -- Dr. Steve Haggbloom, Human Protections Administrator, (270) 745-4652

E-mail: Steven.Haggbloom@wku.edu

Compliance Coordinator -- Mr. Paul Mooney, Human Protections Administrator, (270) 745-2129

E-mail: Paul.Mooney@wku.edu

INFORMED CONSENT DOCUMENT

Project Title: Evaluation of hand fatigue and shot accuracy when wearing the bionic tennis glove

Investigator: Dr. Mark Schafer (270) 745-5857 Kinesiology, Recreation and Sport

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.

The investigator will explain to you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask him/her any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the researcher any questions you may have.

If you then decide to participate in the project, please sign on the last page of this form in the presence of the person who explained the project to you. You should be given a copy of this form to keep.

1. **Nature and Purpose of the Project:** This research study will test and evaluate hand fatigue of tennis players during a simulated two out of three set match protocol both with and without the use of the Bionic™ Tennis Glove. The study will seek to understand if hand fatigue is present when playing tennis and if the Bionic™ Tennis Glove will reduce the impact of fatigue and improve performance.

2. **Explanation of Procedures:** If you decide to take part in this research study, you will complete two separate 60 - 90 minute study sessions, each separated by a 2 -3 day period. Each session will consist of a simulated tennis match consisting of approximately 480 ground strokes. To minimize risks, you will be asked to

complete a Physical Activity Readiness Questionnaire (PAR-Q) and a medical history form which asks questions about your current health status. If you have an orthopedic (muscle or bone), cardiovascular (Heart), and/or metabolic disease (i.e. coronary artery disease (Heart Disease), prior myocardial infarction (Heart Attack), peripheral vascular disease (Blockages in legs), chronic obstructive pulmonary disease (Lung disease), and diabetes mellitus (High/low Blood sugar) you will be excluded from participation in this research study. All procedures will take place at the Tennis Performance Institute (TPI) in Bowling Green, KY.

Procedures during both sessions:

1). Height and weight will be measured using a standard physician scale.

2). Body composition will be measured using skinfold calipers. The investigator will access sites on your chest, belly, waist, upper arm and pinch each site using the calipers.

3). A heart rate monitor will be placed around your chest and secured in place with an elastic strap to determine your heart rate when playing tennis.

4). You will be asked to rate your level of fatigue and exertion when playing tennis.

5). A blood sample will be drawn from the end of your finger on your non-playing hand. A finger-prick procedure will be used at the beginning and end of the study session.

6). You will squeeze a hand grip dynamometer with your dominant playing hand to determine your hand grip strength.

7). You will participate in simulated tennis match consisting of approximately 480 ground strokes.

3. **Discomfort and Risks:** Risk associated with the use of a heart rate monitor include redness, irritation, and chafing. For finger-stick blood samples can cause minor bruising around the sample area and slight discomfort during the sampling procedure. The simulated tennis play is a submaximal bout of exercise that poses no greater risk than an actual tennis match.

4. **Benefits:** You may have no benefit from participating in this study. However, you will learn about your percent body fat.

5. **Confidentiality:** Any information about you obtained from this research will be kept confidential. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results.

6. **Refusal/Withdrawal:**

   Refusal to participate in this study will have no effect on any future services you may be entitled to from the University. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks.

_____ _____

Signature of Participant Date

_____ _____

Witness Date

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT

THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY

THE WESTERN KENTUCKY UNIVERSITY HUMAN SUBJECTS REVIEW BOARD

Paul Mooney, Compliance Coordinator

TELEPHONE: (270) 745-4652

PARENTAL INFORMED CONSENT DOCUMENT

Project Title: Evaluation of hand fatigue and shot accuracy when wearing the bionic tennis glove

Investigator: Dr. Mark Schafer (270) 745-5857 Kinesiology, Recreation and Sport

Your child is being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement for your child to participate in this project.

The investigator will explain to you and your child in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask him/her any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the researcher any questions you and son or daughter may have.

If your child then decides to participate in the project with your permission, please sign on the last page of this form in the presence of the person who explained the project to you and your child. You should be given a copy of this form to keep.

1. **Nature and Purpose of the Project:** This research study will test and evaluate hand fatigue of tennis players during a simulated two out of three set match protocol both with and without the use of the Bionic™ Tennis Glove. The study will seek to understand if hand fatigue is present when playing tennis and if the Bionic™ Tennis Glove will reduce the impact of fatigue and improve performance.

2. **Explanation of Procedures:** If your child decides to take part in this research study, they will complete two separate 60 - 90 minute study sessions, each separated by a 2 -3 day period. Each session will consist of a simulated tennis match consisting of approximately 480 ground strokes. To minimize risks, your child will be asked to complete a Physical Activity Readiness Questionnaire (PAR-Q) and a medical history form which asks questions about their current health status. If your child has an orthopedic (muscle or bone), cardiovascular (Heart), and/or metabolic disease (i.e. coronary artery disease (Heart Disease), prior myocardial infarction (Heart Attack), peripheral vascular disease (Blockages in legs), chronic obstructive pulmonary disease (Lung disease), and diabetes mellitus (High/low Blood sugar) they will be excluded from participation in this research study. All procedures will take place at the Tennis Performance Institute (TPI) in Bowling Green, KY.

Procedures during both sessions:

1). Height and weight will be measured using a standard physician scale.

2). Body composition will be measured using skinfold calipers. The investigator will access sites on your chest, belly, waist, upper arm and pinch each site using the calipers.

3). A heart rate monitor will be placed around your chest and secured in place with an elastic strap to determine your heart rate when playing tennis.

4). You will be asked to rate your level of fatigue and exertion when playing tennis.

5). A blood sample will be drawn from the end of your finger on your non-playing hand. A finger-prick procedure will be used at the beginning and end of the study session.

6). You will squeeze a hand grip dynamometer with your dominant playing hand to determine your hand grip strength.

7). You will participate in simulated tennis match consisting of approximately 480 ground strokes.

3. **Discomfort and Risks:** Risk associated with the use of a heart rate monitor include redness, irritation, and chafing. For finger-stick blood samples can cause

minor bruising around the sample area and slight discomfort during the sampling procedure. The simulated tennis play is a submaximal bout of exercise that poses no greater risk than an actual tennis match.

4. **Benefits:** Your child may have no benefit from participating in this study. However, they will learn about their percent body fat.

5. **Confidentiality:** Any information about your child obtained from this research will be kept confidential. All records related to your child's involvement in this research study will be stored in a locked file cabinet. Their identity on these records will be indicated by a case number rather than by their name, and the information linking these case numbers with their identity will be kept separate from the research records. They will not be identified by name in any publication of the research results.

6. **Refusal/Withdrawal:**

   Refusal to participate in this study will have no effect on any future services your child may be entitled to from the University. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks.

_____ _____

Signature of Parent or Legal Guardian Date

_____ _____

Witness Date

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT

THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY

THE WESTERN KENTUCKY UNIVERSITY HUMAN SUBJECTS REVIEW BOARD

Paul Mooney, Compliance Coordinator

TELEPHONE: (270) 745-4652

INFORMED ASSENT

Project Title: Evaluation of hand fatigue and shot accuracy when wearing the bionic tennis glove

Investigator: Dr. Mark Schafer (270) 745-5857 Kinesiology, Recreation and Sport

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.

The investigator will explain to you in detail the purpose of the project. You may ask him/her any questions you have to help you understand the project. Please read this explanation and discuss with the researcher any questions you may have.

If you then decide to participate in the project, please sign on the last page of this form in the presence of the person who explained the project to you. You should be given a copy of this form to keep.

1. **Nature and Purpose of the Project:** This research study will test and evaluate hand fatigue of tennis players during a simulated two out of three set match protocol both with and without the use of the Bionic™ Tennis Glove.

2. **Explanation of Procedures:** If you decide to take part in this research study, you will complete two separate 60 - 90 minute study sessions. Each session will consist of a simulated tennis match consisting of approximately 480 ground strokes. All procedures will take place at the Tennis Performance Institute (TPI) in Bowling Green, KY.

Procedures during both sessions:

1). Height and weight.

2). Body composition will be measured.

3). A heart rate monitor will be placed around your chest.

4). You will be asked to rate your level of fatigue and exertion when playing tennis.

5). A blood sample will be drawn from the end of your finger on your non-playing hand.

6). You will squeeze a hand grip device.

3. **Discomfort and Risks:** Risk associated with the use of a heart rate monitor include redness, irritation, and chafing. For finger-stick blood samples can cause minor bruising around the sample area and slight discomfort during the sampling procedure. The simulated tennis play is a submaximal bout of exercise that poses no greater risk than an actual tennis match.

4. **Benefits:** You may have no benefit from participating in this study. However, you will learn about your percent body fat.

5. **Confidentiality:** Any information about you obtained from this research will be kept confidential. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a case number rather than by your name, and the information linking these case numbers with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results.

6. **Refusal/Withdrawal:**

Refusal to participate in this study will have no effect on any future services you may be entitled to from the University. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks.

I, _____, understand that my parents (mom, dad, or guardians) have given permission (said it's okay) for me to take part in a project about _____ under the direction of _____.

I am taking part because I want to. I have been told that I can stop at any time I want to and nothing will happen to me if I want to stop.

Signature _____ Date _____

ID # _____

Exercise Physiology Laboratory

Physical Activity Readiness Questionnaire (PAR-Q)

Now I am going to ask you a few questions to determine if you are eligible to participate in the study.

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

   No ___ Yes ___ If yes, specify: _____

2. Do you feel pain in your chest when you do physical activity?

   No ___ Yes ___ If yes, specify: _____

3. In the past month, have you had chest pain when you were not doing physical activity?

   No ___ Yes ___ If yes, specify: _____

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

   No ___ Yes ___ If yes, specify: _____

5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?

   No ___ Yes ___ If yes, specify: _____

6. Is your doctor currently prescribing drugs (for example, water pills) for a blood pressure or heart condition?

   No ___ Yes ___ If yes, specify: _____

7. Do you know of any other reason why you should not do physical activity?

   No ___ Yes ___ If yes, specify: _____

ID # _____

WKU
Exercise Science
Department of Kinesiology, Recreation & Sport

Exercise Physiology Laboratory

MEDICAL HISTORY

Now I am going to ask you a few questions to determine if your health status …

	YES	NO
1. History of heart problems, chest pain, or stroke?	_____	_____
2. Increased blood pressure?		
3. Any chronic illness or condition?	_____	_____
4. Difficulty with physical exercise?		
5. Advice from a physician not to exercise?	_____	_____
6. Recent surgery? (Last 12 months)		
7. Pregnancy? (Now or within the last 3 months)	_____	_____
8. History of breathing or lung problems?		
9. Muscle, joint, back disorder, or any previous injury still affecting you?	_____	_____
10. Diabetes or thyroid conditions?	_____	_____
11. Cigarette smoking habit?		
12. Increased blood cholesterol?	_____	_____
13. History of heart problems in your immediate family?		
14. Hernia or any condition that may be aggravated by lifting weights?	_____	_____
15. Do you have any condition limiting your movement?		
16. Are you aware of being allergic to any drugs or insect bites?	_____	_____
17. Do you have asthma?		
18. Do you have epilepsy, convulsions, or seizures of any kind?	_____	_____
19. Do you follow any specific diet?		
	_____	_____
Please explain in detail any "YES" answers:	_____	_____
	_____	_____

BIBLIOGRAPHY

1. Bente Klarlund Pederson and Laurie Hoffman-Goetz. Exercise and the Immune System: Regulation, Integration, and Adaption. PHYSIOLOGICAL REVIEWS Vol. 80, No. 3, July 2000.

2. David C. Nieman. Exercise Effects on Systemic Immunity. Immunology and Cell Biology (2000) 78, 496–501.

3. David C. Nieman, Michael W. Kernodle, Dru A. Henson, Gerald Sonnenfeld, and Darla S. Morton. The Acute Response of the Immune System to Tennis Drills in Adolescent Athletes. 2000. Vol.71, No.4, pp. 403-408.

4. Dr Chan-Ho Park, Dr Tae-Gon Park, Professor Tae-Un Kim, Professor Yi-Sub Kwak. Changes of immunological markers in elite and amateur triathletes. International SportMed Journal, Vol. 9 No. 3, 2008, pp. 116-130.

5. E.M. Peters. Exercise, Immunology and Upper Respiratory Tract Infections. Int J Sports Med 1997; 18: S69-S77.

6. Fernandez J, Mendez-Villanueva A, Pluim BM. Intensity of tennis match play. Br J Sports Med. 2006 May; 40(5):387-91; discussion 391.

7. Girard O, Lattier G, Micallef JP, Millet GP. Changes in exercise characteristics, maximal voluntary contraction, and explosive strength during prolonged tennis playing. Br J Sports Med. 2006 Jun; 40(6):521-6.

8. Girard O, Millet GP. Physical determinants of tennis performance in competitive teenage players. J Strength Cond Res. 2009 Sep; 23(6):1867-72.

9. James Wilfred Navalta, Brian Keith McFarlin, Thomas Scott Lyons. Does exercise really induce lymphocyte apoptosis? Fronteirs in Bioscience E2,478-488, Jamuary 1, 2010.
10. James Wilfred Navalta, Brian Keith McFarlin, Thomas Scott Lyons, John Clifton Faircloth, Nicholas T. Bacon, and Zachary J. Callahan. Exercise-induced lymphocyte apoptosis attributable to cycle ergometer exercise in endurance-trained individuals. Appl. Physiol. Nutr. Metab. 34: 603–608 (2009).
11. James W. Navalta, Darlene A. Sedlock, Kyung-Shin Park, and Brian K. McFarlin. Neither gender nor menstrual cycle phase influences exercise-induced lymphocyte apoptosis in untrained subjects. Appl. Physiol. Nutr. Metab. 32: 481-486 (2007).
12. König D, Berg A, Weinstock C, Keul J, Northoff H. Essential fatty acids, immune function, and exercise. Exerc Immunol Rev. 1997; 3:1-31.
13. M. Mars, S. Govender, A. Weston, V. Naicker, and A. Chuturgoon. High Intensity Exercise: A Cause of Lymphocyte Apoptosis? Biochemical And Biophysical Research Communications 249, 366-370 (1998), Article No. RC989156.
14. Marlee Gleeson. Overview: Exercise Immunology. *Immunology and Cell Biology* (2000) 78, 483–484.
15. Mendez-Villanueva A, Fernandez-Fernandez J, Bishop D. Exercise-induced homeostatic perturbations provoked by singles tennis match play with reference to development of fatigue. Br J Sports Med. 2007 Nov; 41(11):717-22; discussion 722.

16. Nieman D.C., Pedersen B.K. Exercise and Immune Function: Recent Developments. Sports Medicine, Volume 27, Number 2, February 1999, pp. 73-80(8).
17. Roy J. Shepard. Overview of the Epidemiology of Exercise Immunology. Received 27 January 2000; Accepted 27 January 2000.
18. Sharon Phaneuf, Christiaan Leeuwenburgh. Apoptosis and exercise. Medicine & Science in Sports & Exercise. Accepted for publication May 2000.