Biomarkers in Athletes: A Meta-Analysis In Female Soccer and Field Hockey Players

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BIOMARKERS IN ATHLETES: A META-ANALYSIS IN FEMALE SOCCER AND FIELD HOCKEY PLAYERS

by

SOPHIE L. HOWARD

A thesis submitted in partial fulfillment of the requirements for the Honors in the Major Program in Sports and Exercise Science in the College of Education & Human Performance and in the Burnett Honors College at the University of Central Florida Orlando, Florida.

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Thesis Chair: Thomas J. Fisher, PhD
ABSTRACT

The purpose of this study was to examine the prevalence and potential abnormalities of biomarkers in female soccer and field hockey players by conducting a meta-analysis of previous studies. In this study, previous research on certain biomarkers (Creatine kinase, lactic acid, iron, hemoglobin, white blood cells, and cortisol) in collegiate, elite and national level female soccer and field hockey players was collected and evaluated. Studies on baseline measurements for these biomarkers in female soccer and field hockey athletes were collected and their group means were considered. These values were collectively put into individual forest plots, one for each biomarker, and were thereafter compared to a given normal laboratory blood value range for the general population. Whereas iron, white blood cell count and especially hemoglobin tended to lie either towards or beneath the lower limit of the reference range assigned to the general population, CK and cortisol have a tendency to be higher in athletes compared to the general population. The findings for lactic acid did not have a significant tendency in either direction. The findings made throughout this study indicate the importance of proper nutrition for the athletes. Furthermore, the findings reiterate and remind coaches and health professionals of the importance on not only the education on proper nutrition for athletes, including sufficient iron intake and possible iron and vitamin supplementation but also the importance of adequate rest and time for recovery to limit the risk of overtraining and high intensity exercise related illness and infection.
DEDICATION

For all my soccer friends out there striving to become a better athlete.

For my thesis chair Dr. Thomas Fisher and especially my ‘sub-chair’ Dr. Maren Fragala, who made the completion of this thesis possible by supporting, helping and advising me throughout the process.

And especially for my parents, who are the foundation of my ambition to go above and beyond, continuously looking for that extra challenge to become a better self. Thanks Mum and Dad, you have always guided the path to make me the woman I am today.
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Lastly, thanks to all my friends and family for listening to me every day when talking about the progress of my thesis and for all your understanding when bailing out on you because I was, once again, “really close to the end”.
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CHAPTER ONE: INTRODUCTION

Many different sports throughout the world are currently being intensively studied. However, the broad spectrum of sport variety, gender difference, and the influence of supplementation make it difficult to get a good general overview of the research that has been conducted within athletics. For the understanding of each sport, its demands and effects on body and mind, it is very important and helpful to further examine, and observe different markers within athletes that might give some clarification or identification of the physical and physiological demands and effects of each sport. A biomarker is a biochemical substance that can be measured accurately and give indications of a medical state or of a particular biological process (Strimbu, 2010). For athletics, the substances that are most important are those that change due to exercise or exercise induced or related processes within the body such as hemoglobin, cortisol and creatine kinase (CK). Compared to performance tests, which are frequently incorporated into the training program and are often performed to determine athletes’ fitness levels, biomarkers can give insights into what is happening in the athletes’ bodies. While performance tests do reveal some information about an individual’s level of fitness and response to fatigue, the scores that are achieved during fitness testing lack the ability to display possible reasons for why some athletes do not adapt and recover as well as others. With the help of biomarkers, however, coaches and the athletes themselves are able to examine their levels and can potentially identify reasons for their certain levels of performance and possibly also prevent or reverse performance impairments, such as early occurring or long-lasting fatigue and excessive muscle soreness. Therefore, this thesis examines the importance of extensive and detailed research on biomarkers in athletes to enforce and support optimal performance
conditions. Thus, the purpose of this study is to conduct a meta-analysis of select biomarkers within female soccer and field hockey players in order to possibly identify certain patterns of prevalence in regards to deficiencies, out of norm values or general trends. This study will help to draw a general conclusion regarding the importance of the examination of certain blood markers specifically for females within these two sports.

The following sections of this chapter provide a general overview of the sports of soccer and field hockey and the importance of the separation of gender when researching biomarkers in athletes.

Soccer, being played by over 250 million people across the continents, is the world’s number one sport (Statistic Brain, 2014). It is not surprising that so much effort and time is spent continuously trying to improve performance. Whereas male soccer players draw all the attention and the biochemical impact of a soccer match on male soccer players has extensively been researched (Ascenao, 2008), female soccer players have so far not been sufficiently observed and studied.

In terms of the gamut of sports, soccer is very similar to field hockey. Whereas field hockey is played on a slightly smaller field and consists of four 15-minute quarters, soccer, herewith neglecting youth team regulations, is played for 90 min with two 45-minute halves. However, both sports are team sports with a game and match being based on two teams competing with multiple field players and one goalkeeper each. Both sports are endurance based team sports in which athletes throughout a game perform many high intensity bouts. Next to this physical aspect, in both sports, the athletes need to rely on their highly developed level of skill in
order to score or defend a goal while sustaining accumulating fatigue. However, whereas in soccer the athletes use their feet to control, distribute, or strike the ball, field hockey players have field hockey sticks with which they play the game. Therefore, in order to increase the number of studies to that will be included in this meta-analysis; field hockey athletes are also examined, especially since, based on the small number of studies that can be found, female field hockey players also have not been sufficiently researched.

Even though as mentioned, male soccer players have been intensively researched, it is essential to separate both genders based on the potential for differences, one of which is hormonal and physiological influences. Women, in comparison to men, differ in regard to several aspects of athleticism. This is based on influential factors and occurrences within the female body such as menstrual cycle and hormonal imbalance. Not only is the female menstrual cycle, which can result in hormonal fluctuation, a factor negatively affecting performance, but also the ability to transport oxygen, the general stroke volume (the amount of blood pumped out of the heart through one contraction), the heart rate and consequently the cardiac output, which is the product of the two last mentioned factors, differ between men and women. When focusing on physiological demands, several different blood markers within the athletes can be very helpful to not only get an overall better understanding of the sports’ demands, but also to identify certain norms and tendencies present in these particular athletes, who unfortunately are often subject to iron and hemoglobin deficiencies (Landahl, 2005)

Therefore, this study took on the task of looking at all the current research on the evaluation and consideration of biomarkers in female soccer and field hockey players by performing a meta-analysis to determine an overall prevalence and trend in marker values. The
following chapters provide a summary of related research to support this study and to explain the importance of each marker considered for the analysis.
CHAPTER TWO: LITERATURE REVIEW

Soccer and field hockey, in contrast to many single-effort sports, such as endurance races, are complex sports that consist of many bouts of high intensity runs throughout an endurance based event relying on fast recovery to sustain increasing fatigue (Bloomfield, 2007). Therefore, the physiological understanding of a soccer and a field hockey game requires the examination and consideration of the relations between several hematological and biochemical parameters. The multiple high intensity sprints throughout a match require immediate energy production. As these bouts accumulate throughout the game, it is essential for the athlete to sustain and prevent fatigue and muscle damage occurring with increasing time (Andersson, 2010). Quickly eliminating and counteracting waste products accumulating within the hard working muscles and sufficiently supplying these with oxygen and other nutrients is a key factor in enabling the athletes to “last” for the duration of a 60 or 90-minute game. The interaction of many different biochemical parameters and the availability of these variables in a sufficient manner are extremely crucial in both the athletes’ athleticism and their ability to quickly recover from such acute stress (Cairns, 2006).

When considering high level performance, many biomarkers have different influences and a different level of impact depending on physical and physiological demands. Hemoglobin, a protein within red blood cells: carries oxygen to the different tissues within the body. Since oxygen is not very soluble in water, which is the main component of blood, an oxygen transport protein is necessary to enable oxygen to make its way through the blood. The availability of hemoglobin therefore determines the supply of oxygen to different tissues and consequently influences the tissues’ ability to function properly. Closely related to hemoglobin is Iron, an
essential mineral that serves as a critical factor for specific biochemical–physiologic processes that are important for endurance exercise performance (Landahl, 2005). The formation of hemoglobin and the activity oxidative enzymes all rely on iron for their function.

When exercise intensity rises and consequently oxygen demand exceeds the amount that can be taken up, increasing lactic acid is produced as a by-product. This occurs when pyruvic acid, supplying energy to living cells through the citric acid cycle, ferments to produce lactate when oxygen is lacking. Blood lactate concentration is a very useful marker of exercise intensity and adaptation to training. The exact role of how lactic acid affects performance and results in fatigue is however still controversial and has to be continued to be researched (Cairns, 2006).

Likewise, CK is also a biomarker that increases due to intense exercise (Andersson, 2008; Heisterberg, 2013; Heneishi, 2007). CK is a compact enzyme that forms the core of an important energy complex known as the phosphocreatine system (Baird, 2012). CK’s main responsibility is catalyzing the reaction of creatine and ATP to form ADP (Adenosine Diphosphate) and Phosphocreatine, the foremost energy source for short and intense work demands, such as sprints. The more CK is available, the more ATP (Adenosine triphosphate) can be produced for energy, if sufficient creatine is available. This consequently allows the athlete to perform more high intensity actions throughout a particular intervention.

Besides the previously stated muscle damage resulting from strenuous exercise, soccer and field hockey games greatly affect the immune system. It is known that exercise and white blood cells are closely related. With exercise, white blood cell count increases and these are sent
through the body more rapidly in order to possibly detect illnesses sooner. However, it is in this case important to differentiate between moderate, everyday exercise and long-lasting and intense events such as marathons, soccer, or field hockey games. These strenuous events could actually decrease the amount of white blood cells circulating through the body, which consequently weakens the immune system and increases the risk of infection or illness. Also, a decreased number of white blood cells can increase the presence of stress-related hormones, such as cortisol, which can be used as an indicator for a disturbance in the anabolic-catabolic balance, and may result in decreased performance (Hoogeveen, 1996).

As several blood-based biomarkers provide relevant information into how an athlete is responding positively or negatively to training and competition, their observation and tracking may provide new tools to monitor, particularly in high level female athletes. Before blood-based biomarkers can be used practically by coaches and athletes, a greater understanding of the information that they provide to the female soccer or field hockey player is necessary. For this, it is essential to collect baseline measures reported in previous studies, which in this case, are biomarkers from blood samples from athletes in a complete resting condition. These baseline values will provide a more reasonable measure of typical biomarkers in female athletes to reveal patterns of prevalence and whether or not biomarkers in these women are elevated or deficient when compared to a normal range for the general population. Hypothetically, it is expected that this population will have increased levels of cortisol and CK and decreased levels of iron, hemoglobin, and white blood cells compared to the general population. These hypotheses are based on findings that were observed in previously conducted studies on biomarkers of male soccer players (Grospiaga, 2004, Heisterberg, 2013, Silva, 2008). Such information may provide
coaches and athletes further insights into training arrangement and athlete conditions of female athletes.
CHAPTER THREE: METHODOLOGY

Study Selection Criteria

The gathering of data will include the collection of previously published studies on female soccer and field hockey players participating in higher level competition between the ages of 15 and 36. All studies used were only soccer and field hockey related studies whose subjects underwent specific practice or game sessions. A total of 21 studies were used directly for the meta-analysis, from which 17 dealt with soccer players and 4 considered field hockey players. The investigations included single-effort session, an observation of several practice and game sessions throughout a longer period of time, such as a season and sport specific fitness tests. Resistance training and any non-soccer and non-field hockey-related exercise efforts were excluded. Only studies with baseline measurements, meaning without or before any particular intervention or any sort of exercise, will be included. The types of studies that were considered in this analysis are cross-sectional research studies that only measured biomarkers from athletes and causal comparative studies that make comparisons between biomarker values of athletes and non-athletes. However, since only a limited number of studies included these comparisons, only the values from the athletes were included in this meta-analysis. The following biomarkers were considered for this analysis: CK, lactic acid, iron, hemoglobin, white blood cell count, and cortisol. For each biomarker studies were collected. Several studies evaluated multiple markers so that these could be used for multiple biomarkers’ collections. The number of studies that were found and considered for each marker individually ranged between 5 and 9. Therewith, for each biomarker a collection of studies was performed with between 5 and 9 studies each.

***See Inclusion-Exclusion Table in Appendix A***
Search Strategies

The current investigation has been prepared using systematic review and meta-analysis in an effort to evaluate a heterogeneous group of athletes. The following keywords, "biomarker", "blood lactate", “female soccer”, “female field hockey”, “oxidative stress” and “stress response” were used in a web search query in the databases of Medline/PubMed, the University of Central Florida Libraries and Google Scholar. Available congress proceedings and abstracts were also used. Publications from August 1996 to March 2015 were included.

Coding

I will be coding the included studies by subdividing these into categories based on form of publication, exercise intervention, subject size and level of play. The studies will be collected in a table and then organized based on coding. This table will also include marker, group mean, standard deviation value, and the measurement unit, which will also be coded. I will be the solely conductor and monitorer in this analysis.

***See Coding Manual in Appendix B and Coding Table in Appendix C***
CHAPTER FOUR: FINDINGS

The following chapter will include graphs that demonstrate each study’s mean value for the relevant biomarker in relation to the reference ranges from the Merck Manuals, which provide normal laboratory blood values for the general population. Since a normed reference for women only is not available, the ranges from the Merck Manuals; which includes men, women, athletes and non-athletes; had to be used. Therefore, these results compare mean resting values of female soccer and field hockey players to biomarker baseline measurements found within the general population. Several studies gave group means for multiple biomarkers, whereas some only dealt with one individual biomarker out of the six that were considered in this analysis. As mentioned, a total of 20 studies were used for the meta-analysis. A complete overview of number of studies and which study was included in which’s biomarker’s collection can be found in the coding table that is attached in the appendix (Appendix C).

Statistical Analysis

The statistical analyses were carried out using Excel 2013 (Microsoft, 2013) using data from the primary reports. If the study’s subjects’ individual marker values were given instead of a mean value, the subjects’ group mean baseline measurement was calculated using the following formula:

$$\bar{x} = \frac{\sum x}{N},$$
where X is the biomarker value and N is the sample size. Using the *GlobalRPh Unit Conversion Table* (McAuley, 1993), the mean values from each study were converted into a standardized mean and plotted in single forest plots for each biomarker to compare study means to each other and to the mean of these means. The forest plots were generated using the Exploratory Software for Confidence Intervals (ESCI Meta-Analysis, 2012) based on study group means and standard deviations. The variance of mean, margin of error of the confidence interval, and the 95% confidence intervals were also calculated by the software. The individual study group means were weighted according to their confidence intervals when estimating the average of the study means. Studies with smaller confidence intervals were weighted more heavily than studies with larger confidence intervals. Reference ranges for the general population, found in the Merck Manuals Normal Laboratory Blood Values (Merck & Co., 2009), were also included in the forest plots to compare the study means and averages of these means to the normal population.

The studies indicted with an asterisk (*) sampled female field hockey players. All other studies were on female soccer players. The square size reflects the weight of each study’s group mean, which is based on its confidence interval. Small confidence intervals resulted in heavier weighted mean values, which is consequently demonstrated through larger squares within the forest plots. The vertical lines that extend from each square indicate the study’s confidence interval. The shorter the lines, the smaller the confidence interval. The red diamond at the bottom of the plot illustrates the weighted averages from the individual studies. The vertical peaks of the diamond indicate the average of the means from the single studies, and the horizontal edges display the average of the 95% confidence intervals from the single studies. The upper horizontal line is the measurement scale for each biomarker. The two black vertical lines indicate the
normal range for the general population. This is true for all forest plots apart from the one for the findings for cortisol which includes two different reference ranges and therefore four different vertical lines. In order to collate the displayed group means to the appropriate studies, each study’s citation is given to the right of that study’s statistical parameters.

**Figure 1: Creatine Kinase in U/L**

In Figure 1, CK group means from 5 different studies are displayed and put into relation with the reference range of 30 – 170 U/L. Of these 5 studies, 80% of the group means fall within this range all tending to give values towards the upper range limit. Only Tsubakihara et al.’s mean value (210.3±128.2 U/L) exceeds the upper range limit by more than 40 U/L. When also considering the studies’ standard deviation, 3/5 of the studies cross the upper limit. Fallon et al. gives a very large standard deviation, covering almost 200 U/L.
The reference range for lactic acid was given as 0.67-1.8 mmol/l. Shown in figure 2, all studies’ group means fall within the given range with Spencer et al. and Bishop et al. demonstrating values of exact 1.8 mmol/l. Interestingly, both these studies considered female field hockey players as their population. Both Ali et al.’s “NF” population (1.1 mmol/l) and Sunderland et al. (0.95 mmol/l) fall within the lower half of the given range.
Figure 3 demonstrates the group means iron values for 8 studies. This graph shows a value tendency towards the lower limit of the given reference range (11-29 µmol/l) with 87.5% of the studies’ mean values falling within the range. There is no outstanding difference between female soccer and female field hockey (Hinrichs, 2010) players. Only Gibson et al.’s group mean lies above the given range for the general population.
Figure 4 shows the hemoglobin group means for 8 different populations in relation to the reference range of 140-170 g/L. 87.5% of the studies lie below the lower limit of the given range. With a mean of 152 g/L, only Gropper et al. gives a value that lies within the range. Interestingly, compared to all other biomarkers considered, all studies show a relatively small standard deviation. Only Kang et al. shows a noticeable standard deviation in both of his populations. No study’s group mean comes close to the upper part of the reference range.
Figure 5 demonstrates the white blood cell count in $10^9$ cells/L and whereas 66.67% of the studies clearly lie within the reference range (3.9-10.7 $10^9$ cells/L), Kang et al.’s control group mean with a value count of 4.1 $10^9$ cells/L lies extremely close to the range’s lower limit and Kang et al.’s subject group mean (3.88 $10^9$ cells/L) lies below the limit. The general tendency for this forest plot runs towards the lower end and limit of the given reference range.
Figure 6, the figure displaying the collected group means for cortisol, differs from all other forest plots through the existence of two ranges. In comparison to all other considered biomarkers, cortisol’s baseline measurements are extremely dependent on sampling time. Peaking early in the morning and gradually decreasing throughout the day, cortisol values are usually higher in the morning compared to late afternoon or evening sampling. These measurements can however be impacted and altered through exercise. With this, it is important to know when the blood sampling took place for each study. Whereas for Aizawa et al. (6:00 p.m.), Avloniti et al. (8:00 a.m.), and Edwards et al. (3:00 p.m.) the time of sampling was given, Haneshi et al. is lacking this information. Given this, Aizawa et al. and Edwards et al. should be examined in relation to
the given reference range for 5:00 p.m. (83-359 nmol/l), whereas Avloniti et al. is to be compared to the given range for 8:00 a.m. (251-552 nmol/l). Since Haneishi et al. doesn’t provide a sampling time, this study is to be examined given both ranges. Generally speaking, the nine studies’ cortisol group means that are shown in Figure 6 are spread out very broadly, with large standard deviations. Haneishi et al.’s group means tend to lie more within the given reference range for an 8:00 a.m. sampling. Whereas Aizawa et al. and Avloniti et al.’s group means each lie within their separate reference ranges, Edwards et al. greatly exceeds the upper limit of its considered reference range. It is noticeable that there are multiple studies which, based on their large confidence intervals, are weighted pretty lightly. Despite the wide value range that the considered populations give, an estimated 50% of the studies’ group means can be said to lie within the appropriate ranges. This estimation was made according to the information that is provided for the different studies’ sampling time. However, as briefly mentioned, a majority of these are modestly weighted.
CHAPTER FIVE: DISCUSSION

The purpose of this meta-analysis was to collect and identify resting values of select biomarkers within female soccer and field hockey players and to compare these to a reference range given for the general population. With this, it was intended to identify certain value tendencies or patterns of prevalence in regards to deficiencies and out of norm values. The general findings are demonstrated in Figures 1 through 6, which are going to be discussed in the following sections.

Through this analysis a general tendency towards elevated but within range baseline values for the particular athletes was found. Raised CK levels are predominantly associated with muscular cell damage and it is known that individuals, such as soccer and field hockey players who regularly undergo high-intensity physical activities, have significantly increased resting CK levels compared to their sedentary or moderate-intensity exercising counterparts (Baird, 2012). High loads of eccentric muscle work reinforce an increase in this enzyme. Further elevation of resting CK occurs when several intense exercise sessions, such as practices that are performed back-to-back without sufficient rest. This could possibly cause the increased elevation of resting CK found by Tsubakihara et al. (2013), whose subjects underwent a structured, extensive, weekly training program, which consisted of five lifting or skill sessions, prior to providing their baseline measures. CK measurements are usually a predictor for enzyme activity at the time of sampling. Therefore, athletes, who despite not having undergone any strenuous exercise for several days but yet show drastically increased resting measurements, should be closely observed. Clinically, an extreme elevation of CK can indicate heart attacks, severe muscle break down, and
muscle dystrophy. This elevation could be an early sign of muscle weakness and therewith related diseases.

The accumulation of lactic acid, which is an indirect indicator of training status, training adaptation and exercise intensity is often observed and evaluated in order to detect and treat overtraining in athletes (Le Meur, 2013). When, during exercise, oxygen demand exceeds the amount that can be supplied by the athlete, the body starts winning or producing energy through converting glycose into pyruvic acid which then ferments to produce lactic acid (Cairns, 2006). In our findings we discover an overall normal range of lactic acid group means. Both Spencer et al. and Bishop et al. evaluate female field hockey players and give mean values that lie directly on the upper limit of the considered reference range. Testing repeated sprint ability, these findings could indicate a decreased buffer capacity. Moreover, these generally higher resting lactic acid values within female field hockey players could be due to higher workloads, insufficient recovery or lower training status. Since however both studies’ subjects are members of the Australian National Squad, the latter is assumption is unlikely.

In general, high resting lactic acid values can indicate poor adaption to training or insufficient recovery and rest. For both, female soccer and field hockey players it is essential to recover from previous games or practices as soon as possible and to allow the body to get rid of the accumulated lactic acid within the muscles to enable those to function appropriately with onset of the next intervention. The achievement of the elimination of lactic acid is a sign of well adapted and well trained athletes or possibly an increased rest period to allow more time for the body to regenerate. Lactic acid accumulation is usually eliminated within 1 hour of recovery.
Prior training and therefore a higher training status can support the clearance of lactic acid (Spencer, 2004).

Since Iron and hemoglobin are two very closely related biomarkers, their findings should be discussed and analyzed in close cohesion. Iron deficiency is the most common nutritional deficiency in the world (Landahl, 2005). The results for iron, showing a value tendency towards the lower limit of the deliberated reference range, are therefore not surprising. For hemoglobin, all but one study give a group mean below the given reference range and therefore indicate a strong tendency of deficient hemoglobin existence at rest within female soccer and field hockey players. Iron, an essential compound of the hemoglobin molecule, and hemoglobin itself are essential blood components that supply the muscles and cells with sufficient oxygen. Both field hockey and soccer are endurance based sports through which these athletes require large amounts of oxygen within the different systems of the body in order for these to function. For this, high iron and hemoglobin values would be very beneficial for these athletes and their performance. Now considering the findings previously displayed, it becomes clear that the female soccer and field hockey players tend to have not only low but compared to the general population even deficient baseline measurements for iron and hemoglobin. It therefore becomes clear that it is essential to monitor female soccer and field hockey players on their iron and hemoglobin storage in order to possibly detect insufficiency or even deficiencies in this area.

If iron and hemoglobin are both responsible for making sure that the different body systems, for exercise especially the muscles, are sufficiently supplied with oxygen, it is then clear how low iron stores can affect the accumulation of lactate. Insufficient oxygen supply leads to an increased accumulation of lactic acid and prevents the muscles from working to their fullest
potential. This shows how iron and hemoglobin status are closely related to the evaluation of lactic acid within the same subjects. These findings show that for coaches and athletic trainers it is important to educate their athletes on not only the importance of iron and hemoglobin for their performance but also on possible ways to counteract these low resting values. It is essential for coaches, athletic trainers and even sports nutritionist to inform their athletes about possible ways to incorporate iron rich food in their diet. The findings definitely show that athletes should pay close attention to the amount of iron they take in on a daily basis and if needed to possibly add iron supplements to help prevent low iron stores and therefore help the athletes to maintain appropriate iron and hemoglobin resting levels. When considering the findings for iron group means, it is noticeable how Gibson et al., as the only study on iron baseline measurements, exceeds the considered reference range. This can very well be attempted to be explained when considering the demonstrated calculation and averaged iron intake based on a 4-day food record.

Gibson et al. reveals a mean DRI of 205%. With this, the elevated iron baseline measurement can be explained. Similar to this, Gropper et al. indicates a similar outlier value within the hemoglobin findings by being the only group mean within the given range. Here again, when referring to the determination of the percentage of recommended daily intake of iron, Gropper et al. provides a mean coverage of 100%. Moreover, it was known that several subjects, without knowing if these belonged to the soccer playing individuals, were taking iron supplements which also could contribute to the elevated resting levels.

Similar to the low iron and hemoglobin presence, the white blood cell count also demonstrated low resting values. The findings demonstrated in figure 5 indicate that the athletes resting white blood cell count values tend to lie towards the lower limit of the reference range.
This shows that even though regular exercise is known to increase white blood cells in one’s system (Andersson, 2008, Avloniti, 2007, Fallon, 2001, Gibson, 2011, and Kang, 2004), the daily exercise that female field hockey and soccer players undergo is strenuous enough to lastingly decrease the count of white blood cells. Through this, the athletes themselves are more susceptible to illness and infection than individuals who regularly exercise but at lower intensities and therefore don’t challenge their systems as much. The cause for Kang et al.’s markedly low mean values can be associated with a training volume for up to 7 to 9 hours of the day previously to the testing.

As mentioned earlier, a low value of white blood cells can be closely related to the increase of stress-related hormones such as cortisol. This is reflected and represented in the analysis’ findings for cortisol. The findings show an overall study mean towards the upper limit of the considered reference range. Even though we do not have a conclusive value way beyond the range, the observed tendency gives an insight into the stress component of both sports on these athletes. Exercise is seen by the body as a form of stress, and therewith, with the onset of exercise, cortisol is released to provide the body with more glucose in the blood for immediate energy. The release of cortisol in response to stress is despite its bad reputation essential and critical for our body’s health and survival. But with almost anything, too much of a good turns into a bad and elevated resting cortisol values can have crucial negative effects, such as a compromised immune system. Through prolonged and frequently repeated endurance exercise, the increased levels of cortisol catch up with our bodies and turn into elevated resting cortisol values. That a 60- or 90 minute game increases cortisol levels has been discovered and agreed upon in many studies (Aizawa, 2006, Avloniti, 2007, Edwards, 2006, and Haneishi, 2007),
however, this increase in resting values underlines the importance of sufficient recovery for the athletes to control this increase appropriately. For coaches, this again means giving the athletes sufficient time to regenerate between practice sessions and especially games in order to perform to the best of their ability throughout the season.

It is further important to mention the relation between some of these biomarkers. Since iron and hemoglobin are, as previously described, closely related, low iron stores are often accompanied by low hemoglobin values. Similar is to be observed with white blood cell count and cortisol. However, in this case, high cortisol values are related to low white blood cell count, as mentioned in chapter two. Furthermore, if iron and hemoglobin are both responsible for making sure that the different body systems, for exercise especially the muscles, are sufficiently supplied with oxygen, it is then clear how low iron stores can affect the accumulation of lactate. Insufficient oxygen supply leads to an increased accumulation of lactic acid and prevents the muscles from working to their fullest potential. This shows how iron and hemoglobin status are closely related to the evaluation of lactic acid within the same subjects.

Limitations

When conducting this meta-analysis, it became clear very soon that female athletes have not been extensively and by no means sufficiently researched. Based on an insufficient number of studies relevant for this purpose, the target population for this study, initially limited to female soccer players, had to be expanded. Since the two sports of field hockey and soccer have as described a lot of communalities, it can be expected that also physical demand and physiological effects are very similar, if not equal. Despite the similarity of the two sports, expanding the
study’s target population to two sports continues to prevent making strong conclusions specifically for the one sport only. Also, since reference values for the general population were only to be found in form of references ranges and not values as anticipated, it was not possible to calculate effect sizes for each study and to demonstrate these in the forest plots. Instead, the group means had to be put in relation to the given reference range. Even computing a z-test was not possible, since it is not known what percent of individuals fall in the range that is given. For this, the assumption that the distribution of the population dependent variable is normal and the midpoint of the range is the mean could have been made. However, for this study’s purpose, the statistical analysis is appropriate and provides a valuable contribution to this field.

Lastly, as can be seen above, the group means for cortisol are compared to two existing reference ranges for measurement at different times. Cortisol is one of the blood markers that are very susceptible to the timing of sampling. It is therefore essential to differentiate between morning and evening samplings. Whereas we are given some sampling time frames in certain studies (Aizawa, 2006, Avloniti, 2007, and Edwards, 2006), this information is lacking in others (Haneishi, 2007), which makes it difficult to make strong conclusions or premises. Moreover, the presence of high error variations within the considered studies make it difficult to draw conclusions from the group means. These errors are measured by the confidence intervals, which consequently provide the weight. Even though two different reference ranges are given and it is therefore possible to compare the group means to the appropriate range given for each time of sampling, it certainly becomes more difficult to get an overall value tendency for this biomarker.
CHAPTER SIX: CONCLUSION

Overall, this study gives athletes, coaches and athletic trainers a good analysis of several markers (CK, lactic acid, iron, hemoglobin, white blood cells, and cortisol) that are essential for their athletes’ performance. The revealed tendency for low baseline iron, hemoglobin and white blood cell measurements and generally increased high CK and cortisol resting values in female soccer and field hockey players, indicate the importance of proper nutrition, adequate rest and possible supplementation consideration. This analysis does not only give a good overview of baseline mean values (possible future reference values) for female soccer and field hockey players, but it also provides information and comparable values to indicate values unusual or uncommon in female soccer and field hockey players. This could be important in order to possibly detect early signs of over-training or marker deficiencies, which is crucial in allowing and supporting the athletes to maintain their performance throughout a season without regression due to a too high exercise-to-rest ratio. This study collects findings form previous and current research on biomarkers in female soccer and field hockey players and makes conclusions about an overall baseline measurement tendency for this population.

Future Research

The foremost importance that this study reveals is the need for more research on female soccer and field hockey players in order to have more studies to include in such a meta-analysis and to consequently make stronger conclusions about biomarkers in female soccer and field hockey players. With these stronger conclusions, there would be the possibility to establish a
female soccer and field hockey player reference value or even range that then can be used to make comparisons between evaluated baseline values and the concluded “normal” values for this population. Generally, the new established reference range for these female athletes could not only be compared to the general population, including non-athletes, but it would provide a female soccer and field hockey specific range which can be used for further monitoring. This range would allow and support the detection of out-of-norm baseline measurements and therewith related illness, overtraining or muscle weakness. Based on the lack of studies in this area of research, only a very small amount of biomarkers could be considered for evaluation. To make such a meta-analysis helpful and supportive regards the advancement in research for the physiological component of both sports, many more biomarkers have to be looked at. Because of insufficient research on electrolytes and minerals for example, both had to be neglected for this study. Despite the limited research with which our findings can be compared, the results from this investigation can be used to inform future research in this population and possibly for an establishment of specific sport nutrition guidelines.

Implications for athletes, coaches, and sport fitness professionals

This study detects several tendencies of selected biomarker group means for female field hockey and soccer players. Whereas iron, white blood cell count and especially hemoglobin tend to lie either towards or beneath the lower limit of the reference range assigned to the general population, CK and cortisol have a tendency to be higher in athletes compared to the general population. The meta-analysis’ lactic acid mean value seems to be very similar and within the general population range, however still tending towards the upper limit. These prevailed mean
values reinforce the importance of close observation of athletes throughout the season. The high cortisol and low white blood cells values indicate the importance of sufficient rest for the athletes. Whereas intense and frequent practice is essential to bring the athletes to the next level, it is especially for the long run even more important to provide them with sufficient rest and time for recovery in order to be able to maintain and improve performance. Since, as mentioned, these athletes, based on their lower white blood cell count, are more susceptible to illness, it is essential for them to let their body’s recover from previous practice sessions and games. Also, the aspect of illness and infection emphasizes the importance of appropriate nutrition for the athletes.

For coaches, sports nutritionists and athletic trainers it is essential to make sure female athletes get enough of all vitamins to help support the immune system in order to counteract the high cortisol and low white blood cell values. With this said, it is also essential to educate the athletes about the importance of iron in their diet for their performance and to make sure they incorporate iron rich foods or if needed iron supplements in their diet to help prevent too low iron stores and hemoglobin presence. Similar to cortisol, generally elevated CK and lactic acid values are natural occurrences in elite athletes. These “natural occurrences” however have to be closely observed since they could quickly, if not treated carefully with adequate rest, inhibit the athletes’ performance. It is essential to have an eye on these values in order to detect possible enzyme activity disturbances or initial stages of overtraining.

Overall, this study’s findings call for close observation and if possible frequent monitoring of the athletes in order to early on detect iron deficiencies, possible muscle weakness and initial signs of inadequate recovery. Moreover, it calls for education and consideration of vitamin and iron supplementation to allow the athletes to perform to their fullest potential. Even
though it is recommended that nutritional needs are generally met through a well-balanced diet, this can be very difficult and in some cases almost impossible for athletes, through which supplementation becomes a great way to meet the for athletes altered nutritional needs.
APPENDIX A:
INCLUSION – EXCLUSION TABLE

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Example 1</td>
<td>Example 2</td>
</tr>
<tr>
<td>Example 3</td>
<td>Example 4</td>
</tr>
<tr>
<td>Example 5</td>
<td>Example 6</td>
</tr>
<tr>
<td><strong>Inclusion</strong></td>
<td><strong>Exclusion</strong></td>
</tr>
<tr>
<td>--------------</td>
<td>---------------</td>
</tr>
</tbody>
</table>
| **Participants** | - Subjects between 18 and 35 years  
- female | - Subjects older than 35 or younger than 18  
- male |
| **Sport** | Soccer and field hockey | Any other sport |
| **Intervention** | Soccer and field hockey specific practices or games (one-time or several throughout season), sport specific fitness tests | Strength training, any other non-soccer and non-field hockey related practice or exercise events |
| **Testing** | Resting values, baseline measurements | Values affected by exercise |
| **Types of Studies** | Comparison: Athletes vs Non-athletes; cross-sectional research | Case studies |
| **Biological markers** | Exercise related substances (Creatine kinase, lactic acid, iron, hemoglobin, white blood cell count and cortisol) |  |
APPENDIX B:
CODING MANUAL
Unit: (1) Conventional Unit (2) SI Unit (3) Other

Level of play: (1) Collegiate (2) Elite/Professional (3) Junior elite (4) International competition (5) Other (6) Unknown

Exercise Intervention: (1) Single Practice (2) Single Game (3) Multiple Practices (4) Multiple Games (5) Prolonged Intervention (6) Baseline Measurements (7) Other (8) Unknown

Form of Publication: (1) Journal (2) Book or Book Chapter (3) Dissertation or Master’s Thesis (4) Convention Paper or other speech (5) Unpublished Manuscript (6) Other (7) Unknown

Subject size, biomarker group mean, and standard deviations were fill-in values.
APPENDIX C:
CODING TABLE
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<th>Marker</th>
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<th>SD</th>
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<th>Intervention (C.)</th>
<th>Form of Publication (C.)</th>
<th>Author, Year</th>
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<td>win</td>
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<td>24*</td>
<td>3*</td>
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<td>19*</td>
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* values are estimated from graphs given in study

*** shaded studies considered female field hockey players
REFERENCES


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