The psycho-hormonal influence of fatigue on amateur female soccer players

A Broodryk

orcid.org / 0000-0001-7396-1775

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Promoter: Dr C Pienaar

Co-Promoter: Dr M Sparks

Assistant Promoter: Dr DJ Edwards

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Student number: 21673144

DECLARATION

The co-authors of the three articles, which form part of this thesis, Doctor Cindy Pienaar (Promoter), Doctor Martinique Sparks (Co-promoter and project leader) and Doctor David Edwards (Assistant promoter) hereby give permission to the candidate Ms. Adéle Broodryk to include the three articles as part of her PhD thesis. The contribution (advisory and supportive) of the co-authors was kept within reasonable limits, thereby enabling the candidate to submit this thesis for examination purposes. This thesis, therefore, serves as a fulfillment of the requirements for the degree doctor of philosophae in human movement science within the school of Biokinetics, Recreation and Sport Science in the Faculty of Health Sciences at the North-West University (Potchefstroom campus), South-Africa.

Dr. Cindy Pienaar
Promoter and co-author

Dr. Martinique Sparks
Co-promoter and co-author

David John Edwards

Dr. David John Edwards
Assistant promoter and co-author

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SUMMARY

Soccer is rapidly becoming the most popular team sport played globally for both genders. However, literature describing the physical, physiological and psychological facets of female players is scarce. Various components such as fatigue, a stressful competitive atmosphere, perceived anxiety and negative mood states can increase cortisol secretion and consequently alter (optimum) performance. A typical match is comprised 95% of aerobic activities and a mere 5% of anaerobic activities, though literature suggest that it is the anaerobic actions that cause fatigue, heighten the perceived anxiety and increase cortisol secretion.

Therefore, the main objectives of this study were to determine the effect of an aerobic and anaerobic fatiguing test (AFT) and a tournament on salivary cortisol and the psychological states of amateur female soccer players, and if a relationship existed between cortisol, anxiety, mood and/or the AFTs and match outcomes.

The participants were 50 amateur female soccer players (age: 22.0 years; stature: 158.9 cm; mass: 55.5 kg) from two tertiary institutions, who completed the aerobic (Yo-Yo intermittent recovery test) and anaerobic fatiguing test (5-metre shuttle run test) over two consecutive days, two weeks prior to a tournament. Cortisol levels (saliva), anxiety (Spielberger state-trait anxiety inventory) and mood (incredibly short profile of mood states [ISP]) were measured immediately before (pre) and 15 minutes after (post) each fatiguing test. The same measurements were conducted an hour prior to (pre), and 15 minutes after (post) every match during a six-match tournament over five consecutive days while the players were fitted with GPS systems to monitor their external and internal loads. Immediately post-AFT, blood lactate (BLa^-), maximal heart rate (HR_{max}) and rate of perceived exertion (RPE) were recorded.

Statistical analysis included a linear mixed model in order to investigate time point differences. Afterwards, Pearson's rank correlation was used to examine the possible relationships between cortisol, anxiety, mood and/or the AFT's and match outcomes.

An anaerobic fatiguing test led to significant cortisol and total mood disturbance (TMD) increases, with no relationship between cortisol or any psychological measurement. Cortisol correlated with BLa^-, and TMD with RPE at post-AFT. Mood and anxiety scores correlated strongly at all times.

An aerobic fatiguing test led to significantly increased cortisol and various mood subscales. Cortisol correlated positively with the absence of anxiety before, and with ISP-fatigue post-AFT. HR_{max} correlated with TMD before, and ISP-fatigue after the AFT. Furthermore, cortisol and BLa^- and ISP-vigour and RPE correlated at post-AFT.

The tournament led to significant cortisol and TMD increases. No direct relationship was observed between cortisol and/or mood and/or anxiety, though prior to a victory, cortisol correlated positively with TMD, ISP-tension, ISP-fatigue and ISP-depression. Prior to a defeat, cortisol and ISP-anger and ISP-confusion correlated positively and afterwards it correlated negatively with ISP-vigour. A defeat resulted in significantly higher TMD responses compared to a win.

In conclusion, this is the first study to investigate the effect of two fatiguing tests and a tournament on the psycho-hormonal states of amateur female soccer players. Emphasis should be placed on maximising anaerobic-related training, as anaerobic activities affect the cortisol and mood fluctuations seen during a match. Implementing a mood questionnaire is useful to indicate underlying physiological and psychological stress. Lastly, ensuring a positive mood state (either concerning training or match facets) can be beneficial in altering the psycho-physiological stress reaction.

Key words: Soccer; women; cortisol; mood; anxiety; competition; training

OPSOMMING

Sokker is vinnig besig om wêreldwyd die gewildste spansport vir beide geslagte te word. Daar is nietemin baie min literatuur wat die fisiese, fisiologiese en psigologiese fasette van vrouespelers beskryf. Verskeie faktore (onder meer uitputting, stresvolle kompeterende atmosfeer, angstigheid en negatiewe gemoedstoestande) kan kortisol-afskeiding vermeerder, wat gevolglik (optimale) prestasie kan beïnvloed. ʼn Tipiese sokkerwedstryd beslaan 95% uit aërobiese aktiwiteite terwyl slegs 5% uit anaërobiese aktiwiteite bestaan, alhoewel navorsing voorstel dat dit die anaerobiese aktiwiteite is wat lei tot uitputting, verhoogde angstigheid en kortisol sekresie.

Die hoofoogmerke van hierdie studie is dus om die effek van ʼn aërobiese en anaërobiese uitputtingstoets en ʼn toernooi op speekselkortisol en die psigologiese toestand van amateur- vrouesokkerspelers te bepaal, en vas te stel of daar ʼn verwantskap is tussen kortisol, angstigheid, gemoedstoestand en/of die uitputtingstoetse en wedstryduitslae.

Die deelnemers was 50 amateur- vrouesokkerspelers (ouderdom: 22.0 jaar; lengte: 158.9 cm; massa: 55.5 kg) van twee tersiêre inrigtings. Hulle het die aërobiese (klimtol- onderbroke hersteltoets) en anaërobiese uitputtende toets (5-meter- heen-en-weerhardlooptoets) oor twee opeenvolgende dae voltooi, twee weke voor ʼn toernooi. Kortisol vlakke (speeksel), angstigheid (Spielberger se toestand-eienskap-angstigheidopname) en gemoedstoestand (ongelooflike kort profiel van gemoedstoestande [ISP]) is gemeet op dag een onmiddellik voor (pre) en 15 minute na (post) elke uitputtingstoets. Dieselfde metings is weer uitgevoer ʼn uur voor (pre) en 15 minute na (post) elke wedstryd gedurende ʼn seswedstrydtoernooi wat plaasgevind het oor 5 agtereenvolgende dae, terwyl die spelers met globale posisioneringsisteme toegerus is om hulle eksterne en interne ladings te monitor. Onmiddellik na die uitputtingsstoetse is bloedlaktaat (BLa^-), maksimale harttempo (HR_{max}) en die tempo van waargenome inspanning (RPE) aangeteken.

Statistiese analise het ʼn lineêre gemengde model ingesluit om tydstipverskille te ondersoek. Daarna is Pearson se rangkorrelasie gebruik om die moontlike verwantskappe tussen kortisol, angstigheid, gemoedstoestand en/of die uitputtingsstoetse en wedstryduitslae te ondersoek.

ʼn Anaërobiese uitputting toets het tot ʼn beduidende toename in kortisol en totale gemoedstoestandversteurings (TMD) gelei, maar geen verwantskap tussen kortisol en enige psigologiese metings aangedui nie. Kortisol het positief gekorreleer met BLa^- en TMD met die RPE na die uitputtingstoets. Daar was deurgaans ʼn sterk korrelasie tussen die tellings vir gemoedstoestand en angstigheid.

’n Aërobiese uitputtingstoets het tot beduidend verhoogde kortisol en verskeie gemoedstoestandsubskale gelei. Kortisol het positief gekorreleer met die afwesigheid van angstigheid voor, en met ISP-uitputting na die aërobiese uitputtingstoets. HR_{max} het gekorreleer met TMD voor en ISP-vermoeienis na die anaërobiese uitputtingstoets. Verder het kortisol, BLa^- en ISP-energiek en RPE gekorreleer na die aërobiese uitputtingstoets.

Die toernooi het gelei tot ’n beduidende toename in kortisol en TMD. Geen direkte verwantskap tussen kortisol en/of gemoedstoestand en/of angstigheid is waargeneem nie, alhoewel kortisol met TMD, ISP-spanning, ISP-vermoeienis en ISP-depressie positief gekorreleer het na ’n oorwinning. Voor ’n neerlaag het kortisol positief gekorreleer met ISP-woede en ISP-verwarring en negatief daarna met ISP-energiek. ’n Neerlaag het tot beduidend hoër TMD-reaksies aanleiding gegee as ’n oorwinning.

Ter afsluiting, hierdie studie is die eerste wat die effek van twee uitputtende toetse en ’n toernooi op die psigo-hormonale toestande van amateur- vrouesokkerspelers ondersoek. Die klem behoort te val op die maksimering van anaërobies-verwante oefeninge, aangesien anaërobiese aktiwiteite die kortisol- en gemoedsfluktuasies wat gedurende ’n wedstryd waargeneem word, beïnvloed. Die gebruik van ’n gemoedstoestandvraelys is nuttig om onderliggende fisiologiese en psigologiese stres aan te dui. Laastens is die versekering van ’n positiewe gemoedstoestand (hetsy in verband met oefening of fasette van wedstryde) bevorderlik vir die verandering van die psigo-fisiologiese stresreaksie.

Sleutel woorde: Sokker, vroue, kortisol, angs, gemoedstoestand, kompetisie; oefening

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LIST OF ABBREVIATIONS

ABBREVIATION	MEANING
A	Anger
ACT	Attentional Control Theory
AEE	Aerobic Endurance Exercise
AFT	Aerobic / Anaerobic Fatiguing Test
ANS	Autonomic Nervous System
ASI	Anxiety Sensitivity Index
ATP	Adenosine triphosphate
BLa^-	Blood lactate
Bpm	Beats per minute
BRUMS	Brunel Mood Scale
BSQ	Body Sensations Questionnaire
C	Cortisol / Confusion
CAR	Cortisol Awakening Response
CI	Confidence Interval
cm	Centimetres
CSAI	Competitive state anxiety inventory
D	Depression
DE	Disordered Eating
DHEA	Dehydroepiandrosterone
DHEA-s	Dehydroepiandrosterone-sulphate
e.g.	Example
ES / d	Effect Size
F	Variance between groups / Fatigue
FIFA	International Federation of Association Football
FSH	Follicle-stimulating hormone
G (1–3)	Group (1–3)
GPS	Global Positioning System
h	Hour(s)
H^+	Hydrogen ions
HI	High Intensity
HIIT	High Intensity Interval Training
HIRT	High Intensity Resistance Training
HIT	High Intensity Training
HPA	hypothalamus-pituitary-adrenal
HR	Heart rate
ISP	Incredibly Short POMS

kg	Kilograms
km.h^{-1}	Kilometres per hour
LH	luteinizing hormone
LI	Low Intensity
LIT	Low Intensity Training
m	Meter(s)
MAT	Multidimensional Anxiety Theory
m.s^{-1}	Meters per second
min	Minute(s)
ml	Millilitre(s)
mmol/L	Millimoll per litre
MRF	Mental Readiness Form
n	Number of subjects reported on
N/R	Not reported
NWU	North-West University
OC	Oral Contraceptive
OMSAT	Ottawa Mental Skill Assessment Tool-3
p	Statistical significant value
PCr	Phosphocreatine
PET	Processing Efficiency Theory
POMS	Profile of Mood States
r	Correlation coefficient
RAST	Repeated Anaerobic Sprint Test
REM	Rapid Eye Movement
RM	Repetition Maximum
RPE	Rate of Perceived Exertion
SAFA	South African Football Association
SAI	State-Anxiety Inventory
SCAT	Sport Competitive Anxiety test
SD	Standard Deviation
Sec	Second(s)
STAI	State-Trait Anxiety Inventory
STAXI	The Spielberger State-Trait Anger Expression Inventory
T	Testosterone / Tension
T(1–3)	Time point (1–3)
TMD	Total Mood Disturbances
TUT	Tshwane University of Technology
USA	United States of America
USSA	University Sports South Africa
V	Cramer's *V* value of significance
V	Vigour
VO_{2max}	Maximal Oxygen consumption
Vs.	Versus
W	Power expressed in Watts
W/sec	Watts per second
y	Years
YYIE	Yo-Yo intermittent endurance
YYIR	Yo-Yo intermittent recovery test
5-m MST	5 Meter Multiple Shuttle Run Test
ºC	Degrees Celsius

1 INTRODUCTION

1. INTRODUCTION

Soccer is the most popular team sport played globally and participation has increased remarkably among females, with over 29 million participants recorded (Datson *et al.*, 2014:1225; Ndimande-Hlongwa, 2016:2). Playing at the highest level (*e.g.* the Fédération Internationale de Football Association (FIFA) Women's World Cup) is significant within the soccer culture, making it an important sporting life event (Holt & Hogg, 2002:255). Participating at this level can be extremely stressful (Holt & Hogg, 2002:251), requiring players to have various technical, physical, physiological and psychological skills (Stølen *et al.*, 2005:503) in order to cope with the increased demands of the match and training volumes (Datson *et al.*, 2014:1225). Extensive research has been done in the past on male soccer players over a wide range of facets (Alexandre *et al.*, 2012:2891; Mohr *et al.*, 2005:593); however, there is a lack of data on female soccer players (Andersson *et al.*, 2008:372; Bradley & Vescovi, 2015:112).

2. PROBLEM STATEMENT

More opportunities are arising for females to engage in soccer around the world (Pelak, 2005:53). This is clear from the increase in competitive soccer matches over the last 10 years, as well as a 50% increase in participating nations during the 2015 Women's Soccer World Cup compared to the previous one (Andersson *et al.*, 2008:372; Datson *et al.*, 2014:1225; Ndimande-Hlongwa, 2016:2). However, the increase in competitive fixtures limits the time for adequate physical, mental, tactical and technical preparations (Morgans *et al.*, 2014:251). This intensifies the apparent stress experienced, as training itself can be stressful as each session has its own specific physiological and psychological demands (Cardinale & Varley, 2017:56). Soccer in South Africa is seen as a masculine sport, which limits the development of women's soccer (Pelak, 2005:57,58). This constraint was emphasised in a complaint submitted to the Commission of Gender Equality, stating that the South African Football Association (SAFA) does not take the interests of female soccer players into consideration to the same extent as those of their male counterparts (Ndimande-Hlongwa, 2016:2). As stated by Ndimande-Hlongwa (2016:6), *"Banyana Banyana (South African senior female soccer team), cannot compete successfully at an international level if SAFA does not implement the plan to create women's professional league football."* This observation is indeed true, as is evident from only the under-17 female team qualifying for the FIFA World Cup (Ndimande-Hlongwa, 2016:6).

The game of soccer is understood as intermittent in nature (Carling *et al.*, 2008:853), requiring numerous changes in the speed (jogging, walking, sprinting and high-intensity running) and direction of movements (Morgans *et al.*, 2014:251). Approximately 1387–1401 activity changes takes places during a female soccer match, resulting in a frequency change every 3–4 seconds (Andersen *et al.*, 2012:1630; Krustrup *et al.*, 2005:1244). Of these activity changes, 125–142 (or 4.8% of the total match-time) can be classified as high-intensity runs and 26 as sprints, indicating a high turnover rate for the anaerobic energy system (Bangsbo *et al.*, 2007:112). In addition, players have to perform various decisive actions during a match, which include jumps, short sprints, tackles and duel play (Stølen *et al.*, 2005:509). The aerobic system also has a large input during a female soccer match (94%), with low-intensity activities contributing 16% (standing), 44% (walking) and 34% (low-intensity running) respectively of the total match time (Krustrup *et al.*, 2005:1244). According to Reilly (1997:258) and Carling *et al.* (2008:853), low-intensity activities dominate the work rate of soccer players, as seen from the time-based ratio of low- to high-intensity exercise, being approximately 7:1. Although aerobic metabolism tends to dominate the energy delivery during a soccer match, most of the decisive actions completed are by means of the anaerobic energy system (Stølen *et al.*, 2005:509), such as high-intensity running (Alexandre *et al.*, 2012:2890). As the standard of competitions rises, so does the amount of high-speed running and sprinting (Datson *et al.*, 2014:1266), taxing both the aerobic and anaerobic energy systems (Haneishi *et al.*, 2007:586; Morgans *et al.*, 2014:251), resulting in fatigue.

Fatigue, which is defined as a decline in performance during match play (Reilly, 1997:258), usually occurs during the second half of the match and is evident in a decrease in the number of high-intensity runs (Krustrup *et al.*, 2005:1246) and an increase in the number of low-intensity activities and rest periods (Reilly, 1997:258). Andersson and colleagues (2008:376) reported the following neuromuscular and biochemical changes after a fatiguing soccer match in 22 elite female players (age: 22.1 ± 3.4 y): significantly lower ($p < 0.05$) sprint, countermovement jump performance and isokinetic strength, as well as significant increases in blood creatine kinase, uric acid, urea and perceived muscle soreness. Fatigue can be categorised either as peripheral fatigue, which occurs outside the central nervous system, or central fatigue, of which the origin is somewhere within the central nervous system (Ament & Verkerke, 2009:400). Peripheral fatigue usually leads to an increase in lactate, hydrogen ions, ammonia, sweat secretion, inorganic phosphate and magnesium in the sarcoplasm, inhibition of calcium release in the sarcoplasmic reticulum and a decline in glycogen stores (Ament & Verkerke, 2009:392). Central fatigue, on the other hand, causes the motor neural drive and excitability of the cerebral motor cortex cells to decline, a decrease in the stimulation of type III and IV nerves, a blockage in the axonal action potential's conduction and enhancement of the synaptic effects of serotoninergic neurons and cytokines release (Ament & Verkerke, 2009:392). Other physiological factors linked to temporary fatigue experienced during a match are low muscle creatine phosphate, electrical disturbances in muscle cells and the accumulation of extracellular potassium (Mohr *et al.*, 2005:594;595). Fatigue is also related to physiological and behavioural changes that may be detrimental to match performance (Bangsbo *et al.*, 2007:112; Reilly, 1997:259).

The prevalence of fatigue during and after match play shows that players face a range of technical, tactical, physical and psychological demands (Gaudreau & Blondin, 2004:1866) that could result in increased stress (Holt & Hogg, 2002:251) on their physiological and psychological systems (Haneishi *et al.*, 2007:583). Psychologically, these demands may result in unpleasant emotional feelings of tension and apprehensive thoughts, also known as state anxiety (Caci *et al.*, 2003:394), which may have a major influence on a player's performance (Varzaneh *et al.*, 2011:19). In an intrinsic case study during interviews with 21 female soccer players (age: 23.7 y) participating in a previous World Cup final, four main categories of perceived stressors were identified, the demands of international soccer, player-coach communication, distractions and competitive stressors being some of the primary stressors (Holt & Hogg, 2002:258). Competitive stressors included pre-match and match anxiety as well as the opposition, with one of the distraction stressors identified as fatigue (Holt & Hogg, 2002:261,263). In addition, another study reported a significant positive relationship ($r = 0.32$; $p < 0.001$) between training load and anxiety or perceived fatigue (Millet *et al.*, 2005:495).

Because of the stressful nature of a soccer match (Holt & Hogg, 2002:251), it could be expected to have a direct effect on a players' hormonal state, as a reciprocal relationship exists between androgens and behaviour (Oliveira *et al.*, 2009:1056). There is evidence of the dynamic relationship between androgens, competition and sometimes victory and defeat (Edwards *et al.*, 2006:135), with cortisol being the main hormone responsible for allostatic stress responses (Kirschbaum & Hellhammer, 2000:379). In this regard, salivary cortisol and testosterone, which are seen as two important hormones for the biochemical assessment of a player (Michailidis, 2014:279), have proven to increase by as much as 126% and 58% respectively in team competitions (Edwards & Casto, 2015:49). This increase might be due to the psychological effect of the competition, the physical training or a combination of the two (Edwards & Casto, 2013:158).

A similar, parallel increase in cortisol and testosterone takes place prior to a competition (Edwards *et al.*, 2006:140; Edwards & Casto, 2013:159) in both males and females, although females may experience an additional rise during a competition (Bateup *et al.*, 2002:187). This may be due to females adopting a different response pattern when faced with a challenge or stressors due to inherent parental investments (Bateup *et al.*, 2002:184). However, according to Edwards *et al.* (2006:135), research on the effect of females participating in competitions on androgens is scarce. In a study of 17 female rugby players (U/23), an increase of 30% in players' cortisol levels from baseline to just prior to the match was noticed, with a further increase of 51% during the course of the match (Bateup *et al.*, 2002:184). Bateup *et al.* (2002:188) stated that two factors were associated with a cortisol rise: the extent to which the opposing team was more challenging than expected, and whether the team won or lost. Not only is cortisol involved in the metabolism and mobilising of energy resources to provide sufficient fuel (Dickerson & Kemeny, 2004:356); it is also involved with behaviour associated with aggression, arousal and mobilisation of physiological resources to deal with threats or challenges (Bateup *et al.*, 2002:183). Testosterone, on the other hand, retains dual-sided, fear-reducing and aggression-increasing motivational properties (van Honk *et al.*, 2005:219). This emphasises the relationship that exists between testosterone and cortisol with regard to the psychological state of a player (Edwards *et al.*, 2006:135).

Unfortunately, the biochemical analysis of testosterone is an expensive and area-bound procedure, emphasising the importance of analysing cortisol to assess the stress a player is confronted with during training or a match. Furthermore, research involving females, and more specifically soccer players, in South Africa is scarce. This leaves coaches, sport scientists or trainers with limited answers on either optimising training to reduce the influence of stress and its role on the onset of fatigue or implementing strategies to minimise the negative effects thereof.

It is against this background that the following research questions are posed: Firstly, what is the effect of an anaerobic fatiguing test on salivary cortisol and the psychological states of amateur female soccer players, and does a relationship exist between these variables and/or the fatiguing exercise? Secondly, what is the effect of an aerobic fatiguing test on salivary cortisol and the psychological states of amateur female soccer players, and does a relationship exist between these variables and/or the fatiguing exercise? Thirdly, what is the effect of a tournament and the match-outcomes (win/lose) on salivary cortisol and the psychological states of amateur female soccer players, and does a relationship exist between these variables and/or the match outcome? Answers to these questions will provide soccer coaches with useful information regarding their players' psychological and hormonal state following various fatiguing tests, as well as after a match that was either won or lost. This will aid them in maximising their performance by improving the players' psychological approach to various forms of fatigue.

3. OBJECTIVES

The objectives of this study are:

- to determine the effect of an anaerobic fatiguing test on the salivary cortisol and psychological states of amateur female soccer players, and whether a relationship exist between these variables and/or the fatiguing exercise;
- to determine the effect of an aerobic fatiguing test on salivary cortisol and the psychological states of amateur female soccer players, and whether a relationship exists between these variables and/or the fatiguing exercise;
- to determine the effect of a tournament and the match outcome (win/lose) on salivary cortisol and the psychological states of amateur female soccer players, and whether a relationship exists between these variables and/or the match outcome.

4. HYPOTHESES

- The anaerobic fatiguing test will result in a statistical significant increase ($p < 0.05$) in cortisol, anxiety and mood states of amateur female soccer players. Also, a positive correlation between cortisol and total mood disturbances, and between anxiety and total mood disturbances are hypothesised. Furthermore, a positive relationship is expected between the anaerobic fatiguing exercise variables (maximal heart rate and blood lactate levels) and cortisol, as well as the negative mood states.
- The aerobic fatiguing test will result in a statistical increase ($p < 0.05$) in salivary cortisol and anxiety states, as well as a significantly negative effect ($d \geq 0.8$) on the mood states of amateur female soccer players. A positive relationship is expected between anxiety and cortisol or mood states and a negative

relationship is expected between cortisol and mood states. Furthermore, a positive relationship is expected between the aerobic fatiguing exercise and positive mood states, with no relationship expected for cortisol and anxiety.

- A progressive increase ($d > 0.8$, $p < 0.05$) in cortisol and anxiety, together with a decrease in positive mood states, will take place over the course of the tournament. Moreover, a positive relationship is expected between anxiety and cortisol or mood states and a negative relationship between cortisol and mood states. A victory will result in statistically better mood states and lower cortisol responses, whereas a losing outcome will result in higher anxiety, negative mood states and cortisol.

Provided below is a conceptualised framework of how the current study will form part of a larger project entitled: "Investigating performance indicators and injury risk factors for the development and performance of female soccer players."

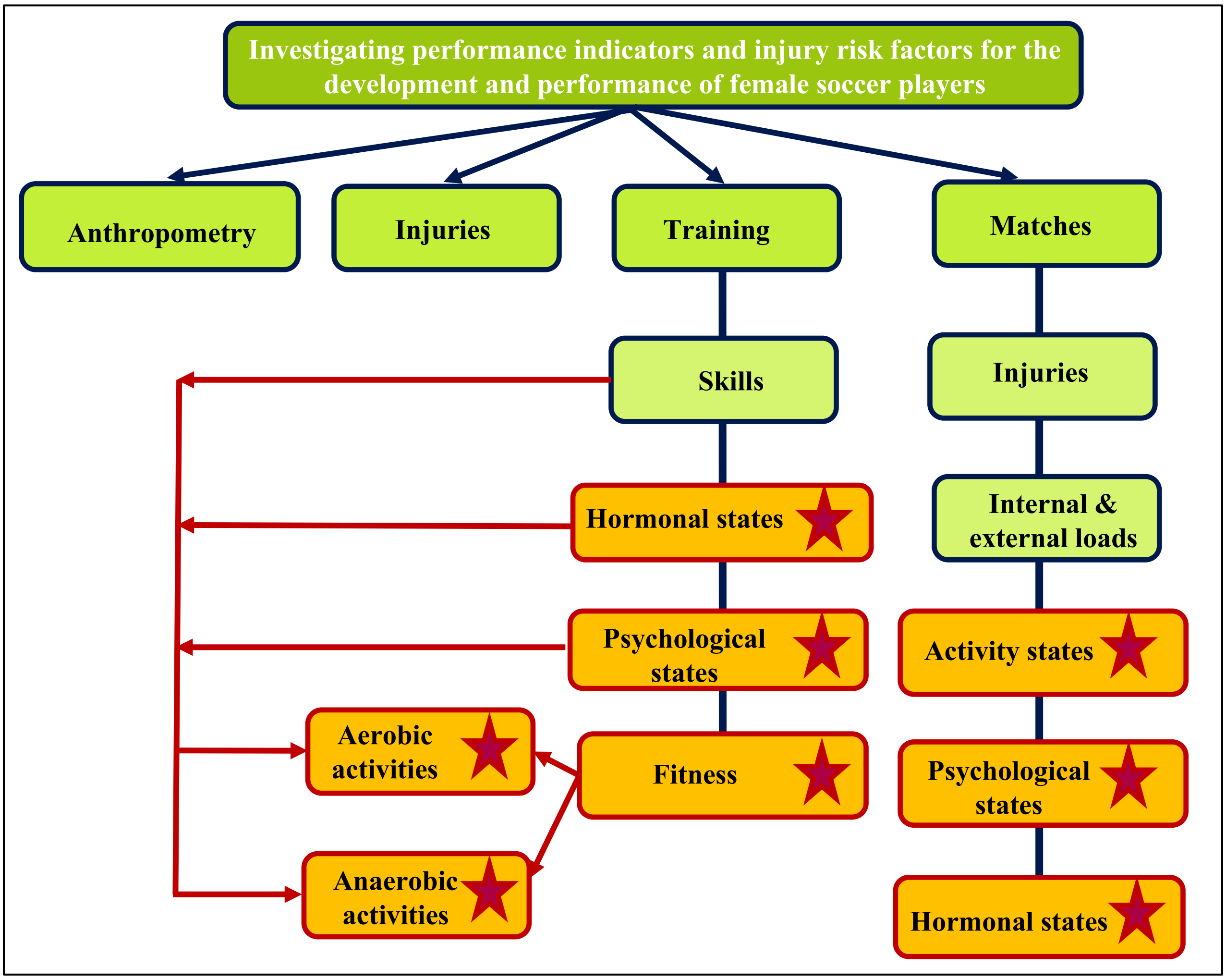

Figure 1. Conceptualised model of how the current study will form part of a larger project entitled: **"Investigating performance indicators and injury risk factors for the development and performance of female soccer players"**

 Facets of interest for the current study.

A complete methodology outline of the study is provided in Appendix A.

5. STRUCTURE OF THE THESIS

The thesis will be submitted in article format as approved by the senate of the North-West University and will be structured as follows:

Chapter 1: Introduction. A bibliography will be provided at the end of the chapter in accordance with the guidelines of the North-West University.

Chapter 2: Literature review: The influence of fatigue on the hormonal and psychological aspects of female athletes.

Chapter 3: Article 1: The psycho-hormonal influence of anaerobic fatigue on semi-professional female soccer players. This article was published in *Physiology & Behavior*.

Chapter 4: Article 2: The effects of aerobic fatigue on the psycho-hormonal state of amateur female soccer players. This article will be presented for possible publication in the *Journal of Sports Sciences.*

Chapter 5: Article 3: The effects of a soccer tournament on the psycho-hormonal states of collegiate female players. This article will be presented for possible publication in the *Journal of Strength and Conditioning Research.*

Chapter 6: Summary, conclusions, limitations and recommendations.

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2 LITERATURE REVIEW: THE INFLUENCE OF FATIGUE ON THE HORMONAL AND PSYCHOLOGICAL ASPECTS OF FEMALE ATHLETES

1. INTRODUCTION

Soccer, also known as football, is the most popular team sport played worldwide and what was once considered a male-type sport has passed over to the female realm. This development is clearly seen in the 29 million females playing it globally and the 50% increase in participating teams during the 2015 FIFA Women's World Cup (Ndimande-Hlongwa, 2016:77). Though an increase was seen worldwide, this was not the case in South Africa, as the South African Football Association (SAFA) was not taking female soccer into consideration to the same extent as male soccer (Ndimande-Hlongwa, 2016:76). Fortunately, as the popularity of female soccer increased over the years, South African females challenged the gender boundaries and formed their own teams in the late 1960s (Pelak, 2005:64). Since then, South African women's soccer participation has increased remarkably; however, the senior South African team has never qualified for the FIFA Women's World Cup (Ndimande-Hlongwa, 2016:82; Pelak, 2005:73), which might be attributed to a number of reasons. Playing at the required professional level requires players to be fast, fit, technically gifted (Holt & Hogg, 2002:260) and able to cope with the demands of the match (Alexandre *et al.*, 2012:2890), thereby exposing the players to a high competition load at a domestic and international level (Thorpe *et al.*, 2017:27). This then spirals to training, with every form of training having specific psychological and physiological demands, varying by dose and type (Cardinale & Varley, 2017:56).

All of the components listed above, either in combination or because they are absent, can lead to matches being extremely stressful (Holt & Hogg, 2002:251), which can heighten perceived anxiety (Casto & Edwards, 2016a:25) and consequently lower performance if this anxiety exceeds the normal range (Raglin & Morris, 1994:47). To fully understand the stress experienced during a soccer match, the physiological as well as the psychological factors associated with the match should be taken into account (Haneishi *et al.*, 2007:583). Physiological influences of stress and mood states may intervene and have rebound effects on performance (Chennaoui *et al.*, 2016:2). A major stressor in soccer is fatigue, which is more evident during the second half of the match, as seen in a decrease in physical performance (reduced work-rate) of the players on the field (Mohr *et al.*, 2005:593; Reilly, 1997:258; Reilly *et al.*, 2008:358). A distinction should be made between a competitive match *vs*. training, as a competition produces a greater degree of anxiety, mental and physical stress (Aizawa *et al.*, 2006:322), as well as the drive to win or outperform the opponent (Casto & Edwards, 2016b:1). In addition, a different response to both the physiological and biochemical parameters within the body occurs during training *vs*. competition (Aubets & Segura, 1995:149). Therefore training sessions should try to simulate the physiological demands of soccer, as these are contributors to the fatigue observed (Svensson & Drust, 2005:601). Managing the players' fatigue subsequently, will aid in monitoring whether players adapt positively or negatively to the collective stresses of competitions and training (Thorpe *et al.*, 2017:27). Unfortunately, insufficient research has been done to determine whether training might delay the onset of fatigue or reduce the detected performance decline (Reilly *et al.*, 2008:359). It is imperative to manage an athlete's fatigue, predominantly in monitoring their loads, to indicate whether they adapt positively or negatively to the collective stresses of competition and training (Thorpe *et al*., 2017:27).

Fatigue can be linked to a range of contributing factors (Bangsbo *et al.*, 2007:112; Thorpe *et al*., 2017:27) affecting the physical, physiological and psychological aspects of a match (Reilly, 1997:259). Although a competition is regarded as a social interaction, teams participate to gain access to something valuable, whether it be the feeling of victory or the victory itself (Casto & Edwards, 2016a:22). The drive to win has a neuroendocrine basis (Casto & Edwards, 2016b:1), with cortisol being the main hormone responsible for the stress response experienced during a soccer match (Kirschbaum & Hellhammer, 2000:379). Not only is an increase in cortisol observed following physical exertion (Chennaoui *et al.*, 2016:2), but it also has a direct influence on the psychological state of an individual (Bateup *et al.*, 2002:183). These psychological characteristics involved with cortisol changes include aggression, arousal, addressing challenges (Bateup *et al.*, 2002:183), depression, cognitive anxiety (Chennaoui *et al.*, 2016:2; Haneishi *et al.*, 2007:587) and fatigue, among others (Chida & Steptoe, 2009:272).

According to Alexandre *et al.* (2012:2891) and Mohr *et al.* (2005:593), numerous investigations have been done in the past on male soccer players over a wide range of facets, though limited research could be found assessing female soccer players. These publications discussed the psychological, physical (Andersson *et al.*, 2008:372; Krustrup *et al.*, 2005:1242) and physiological (Edwards *et al.*, 2006:135; Haneishi *et al.*, 2007:583) aspects of female players. A typical soccer match comprises various high- and low-intensity activities (Svensson & Drust, 2005:601), with varying intensity levels having different responses on the body. Neither the acute effects of anaerobic and aerobic exercise nor the influence of competition on the neuro-hormonal system in elite sportswomen has been fully investigated (Aizawa *et al.*, 2006:323; Karacabey *et al.*, 2005:362). Though ample research is obtainable regarding the effect of fatigue on various factors, limited research is available regarding the effect of high- and low-intensity training or competition on both the psychological and hormonal state of female soccer players. Moreover, only a few research studies could be found that evaluated the effect of fatigue on either the hormonal or psychological state of female athletes, with no studies on South African and African soccer players (See Table 1, 2 and 3).

In figure 1, a conceptualised model is presented regarding the shortcomings in the South African female soccer domain, indicating how the current literature review will aim to overcome and address these limitations and close the gap in literature available on the psycho-hormonal states of these players.

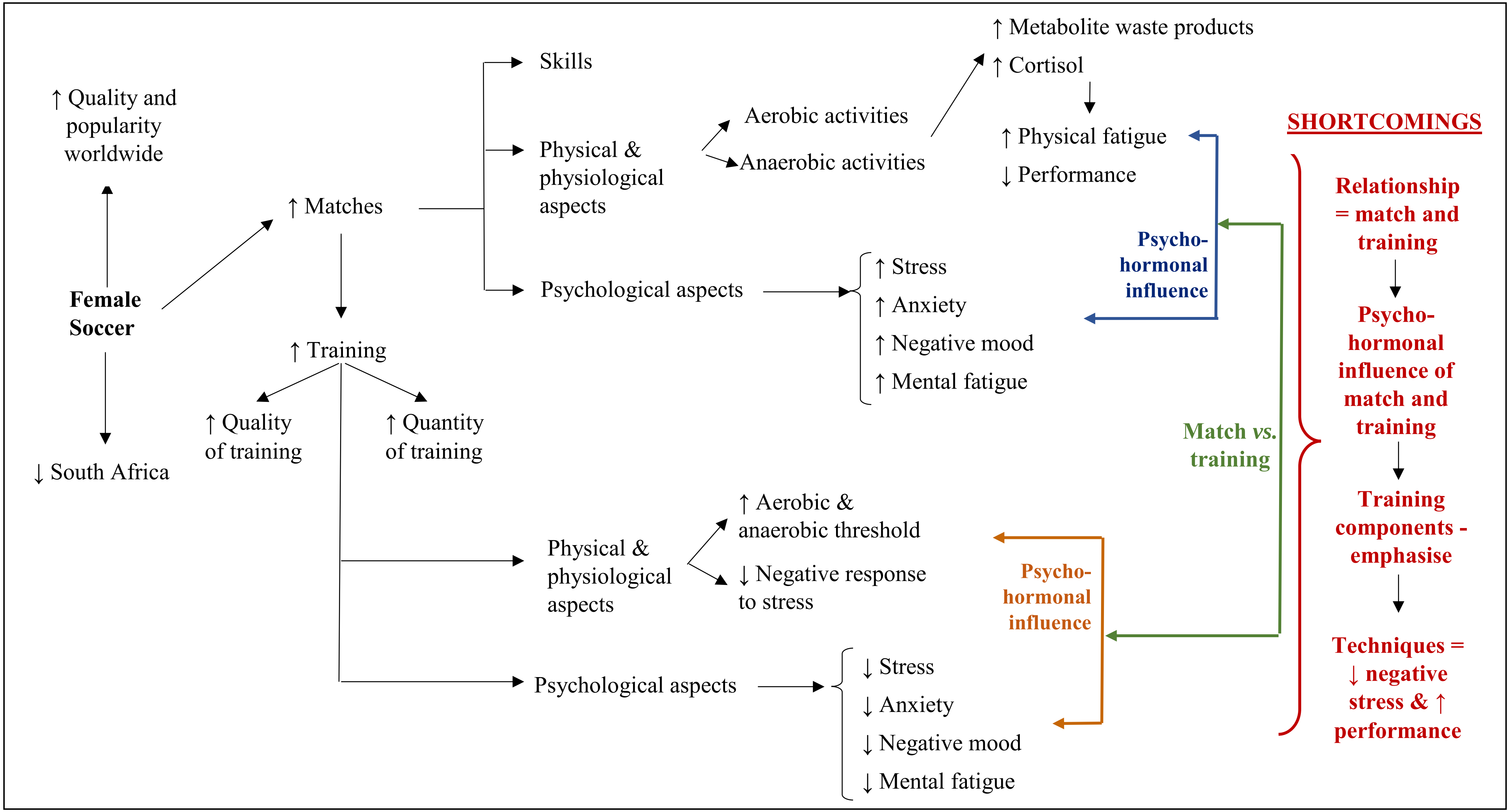

Figure 1. Conceptualised model of the shortcomings in the South-African female soccer domain and how the literature review will overcome the limitations.

This literature review was compiled in the light of the above-mentioned shortcomings. The first aim of this literature review is to provide the reader with a thorough overview of fatigue as a stressor and the effect thereof on the human body and performance. Secondly, female athletes will be discussed according to their hormonal and psychological response to stress. This will be followed by summative tables and summaries of the literature found on various aspects. Fourthly, the relationship between the hormonal and psychological state of female athletes will be addressed, followed by the additional factors that should be considered during the interpretation of the literature. Finally, a summary in the form of a collective scheme of the effect of various stressors on the hormonal and psychological components of female athletes will be provided.

Searches were narrowed down to include only articles from the past 12 years (commencement of literature research, 2005–2017) on female athletes in the summative tables, but older research studies were included in the discussion section in the context of this review if needed. In addition, studies mentioned in the tables should have included at least one of the following influences: cortisol, mood or anxiety, for comparison reasons. Furthermore, only studies that made use of adult female populations (age: ≥ 18 years) as test subjects and where testing took place in a training regime were included. Key words used during the searches included, but were not limited to, the following: fatigue, exhaustion, cortisol, hormones, soccer, football, anxiety, mood, psychological state. Computer searches were performed using the Medline, Masterfile, SportsDiscus, Academic Research and Academic Search Premier Databases. The MetaCrawler, Google Scholar and Scirus internet search engines were also used to gather all available research.

In the following section a brief overview of the effect of fatigue as a stressor will be provided. This will be discussed first according to the effect of stress and its pathway in the human body, followed by its effects on the physical, physiological and psychological systems. This will be followed by an overview of actively competing females and the influence of training or competition on their hormonal and psychological states. A linkage between the hormonal and psychological state and supplementary factors that should be taken into consideration will then be discussed. This will provide the reader with the background information necessary to interpret the findings of the various research articles that will follow in subsequent chapters.

2. FATIGUE AS A STRESSOR

The cause of fatigue can be categorized into four areas, namely, physical illnesses, demographic factors, lifestyle factors and social factors, with the latter two playing a role among young adults (Lee *et al*., 2007:565). In a study on the prevalence of fatigue among university students, 45.8% of males and 48.9% of females reported feeling fatigued (Lee *et al*., 2007:567). Among the causes for this experience were illness, deprived sleep, irregular exercise, irregular diet, inferior status ranking, smoking and drinking (Lee *et al*., 2007:567). Physical training, and more specific, the activity intensity, was inversely related to fatigue, with significant dose-response relationships observed between intensity and fatigue rates ($p < 0.001$).

Evaluating fatigue within the soccer domain, either during training or competition, a wide array of outcomes have been reported on. The length of a soccer match in which a high level of performance must be sustained over more or less 90 minutes (min), will have players experiencing the onset of fatigue (Reilly *et al.*, 2008:357). In soccer the onset of fatigue is seen as a decline in physical performance (decreased work rate profiles [Reilly *et al.*, 2008:358] and high-intensity activities [Mohr *et al.*, 2005:597]) with continued play time (Reilly, 1997:258) owing to inability to perform physical work at a previously demonstrated level (Rampinini *et al.*, 2009:231). Physical fatigue is generally defined as *"an acute impairment of exercise performance that includes both an increase in the perceived effort necessary to exert a desired force or power output and the eventual inability to produce that force or power output"* (Davis & Bailey, 1997:46). During the 1999 Female Soccer World Cup, four main stressor categories were identified by Holt and Hogg (2002:258). Among these stressors were distractions (fatigue and the opponents), coaches' communication (interaction in training and games); the demands of an international soccer match (pace) and competitive stressors (anxiety, mistakes, fear and performance) (Holt & Hogg, 2002:258). To understand the effect of fatigue as a stressor fully, the word stress should first be defined.

The term stress dates back as far as the 13th century and is generally used to describe pressure or distress (a negative response) related to a specific source (Ursin & Eriksen, 2004:569). In contrast to distress, eustress (the positive stress response) is characterised by meaningfulness, hope and manageability (Nelson & Cooper, 2005:73,74). When faced with a psychological stressor, various affective and cognitive processes are activated to influence the physiological response of the human body (Dickerson & Kemeny, 2004:356), the main outcome being a secretion of cortisol (Kirschbaum & Hellhammer, 2000:379). This physiological-psychological response will be discussed *via* the top-down and bottom-up theories in section 3.2.1.2. and 4.1.1. According to the cognitive activation stress theory, the response as mentioned above is dependent on the outcome expectations of the stressor as well as the available responses to it (Ursin & Eriksen, 2004:569). Below is a representation of the four main aspects of stress as proposed by Ursin and Eriksen (2004:570).

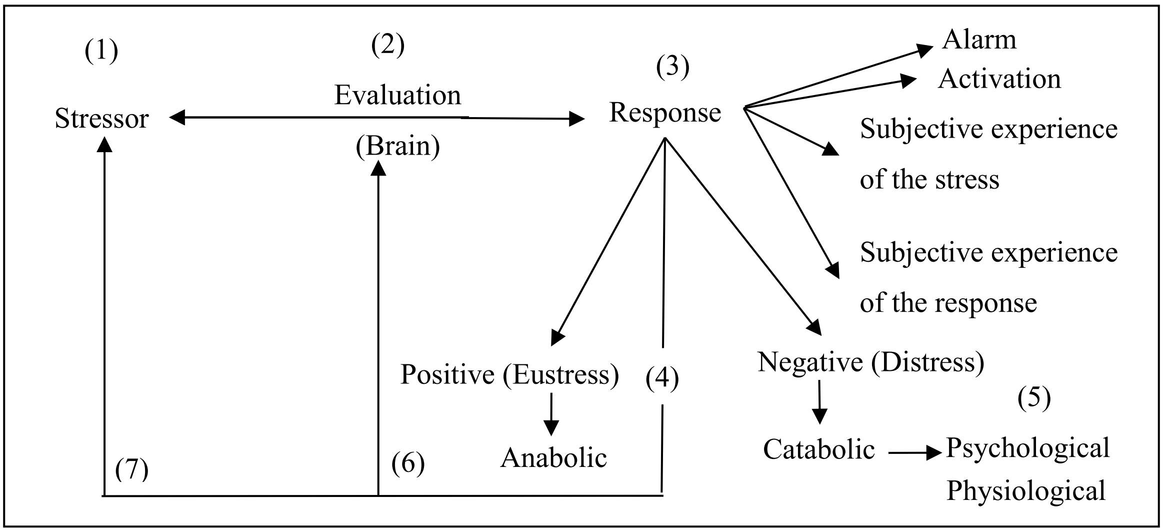

Figure 2. The pathway of the four main aspects of stress (Adapted from Ursin & Eriksen, 2004:570, 581).

The stress/stressor (1) is evaluated in the brain (2) and may then result in a specific response (3). If an alarm is triggered (3), it may be fed back to the brain (6). The physiological and psychological stress response may then be either positive or negative (4), i.e. either anabolic or catabolic. A catabolic response can lead to anxiety if high arousal occurs, which leads to uncertainty (5). The brain may alter the stimulus or the perception thereof (7), either by acts or expectancies.

An adapted version (Figure 9) is presented at the culmination of section 6 to illustrate the effect of various factors (as discussed throughout this section) on both the psychological and hormonal state. Holt and Hogg (2002:260, 263) concurred that fatigue (either as a result of the fast pace of a soccer match, or seen as a distraction), can be perceived as a stressor in female soccer players. A range of factors can contribute to fatigue (Bangsbo *et al.*, 2007:112; Russell *et al.*, 2011:231), including physiological (neural, muscular and metabolic [Green, 1997:248,250]), psychological (behaviour) [Reilly, 1997:259] and motivational factors, as well as the perception of the players [Davis & Bailey, 1997:46]) and the match characteristics, to name a few.

2.1. Facets of fatigue

2.1.1. *Neuromuscular aspects of fatigue*

Fatigue can occur for a number of reasons, whether physiological, neuromuscular, metabolic or psychological reasons. Two types of fatigue of the neuromuscular system can take place: peripheral fatigue (fatigue arising outside the central nervous system) and central fatigue (the origin of the fatigue is within the central nervous system) (Ament & Verkerke, 2009:400; Robineau *et al.*, 2012:560). Peripheral fatigue is characterised by a reduction in the force production due to exercise-induced processes away from the neuromuscular junction (Taylor & Gandevia, 2008:542). A decline in muscular contraction and subsequent performance may be due to a failure at a specific site in the neuromuscular system (Green, 1997:250). It usually leads to the accumulation of lactate, hydrogen ions (H^+), ammonia, sweat secretion, inorganic phosphate and magnesium in the sarcoplasm, as well as the inhibition of calcium release in the sarcoplasmic reticulum and a decline in glycogen stores (in extreme cases decline in blood glucose levels) (Ament & Verkerke, 2009:392).

Central fatigue, on the other hand, is defined as a force generated by voluntary muscular effort that is less than that produced by electrical stimulation (Davis & Bailey, 1997:46). Both maximal and submaximal muscle activation can result in central fatigue (Taylor & Gandevia, 2008:545). This fatigue will result in the following: influencing the motor neural drive and excitability of the cerebral motor cortex cells, a decline in the corticospinal impulses that reach the motor neurons (Davis & Bailey, 1997:47), a decrease in the stimulation of type III and IV nerves, blockage of the axonal action potentials' conduction and an enhancement of the synaptic effects of serotoninergic neurons and cytokines release (Ament & Verkerke, 2009:392), a disruption in the cerebral neurotransmitter level, especially in the serotonergic activity

(Cheung & Sleivert, 2004:102) and a decrease in muscle pH levels (Billaut & Bishop, 2009:264).

2.1.2. *Physical aspects of fatigue related to matches*

Soccer is intermittent in nature, involving high-intensity activities interspersed with lower-intensity activities (Svensson & Drust, 2005:601). Playing a soccer match at an international level can be marked by an increase in pace (Holt & Hogg, 2002:260), which subsequently quantifies the intensity level of a movement activity (Carling *et al.*, 2008:852). Therefore, practising these high-intensity activities may lead to enhanced performance (Carling *et al.*, 2008:852). In this regard, Andersen *et al.* (2012:1630) noted a total of 1 387–1 401 activity changes taking place during a match, with changes occurring every 3 seconds (sec). They further conveyed 139–142 of these instances as high-intensity runs (Andersen *et al.*, 2012:1630). Unfortunately, these activity patterns change significantly throughout the match, which might be a result of fatigue setting in (Reilly, 1997:258). Measuring sprint alterations during and at the end of a soccer match is useful for quantifying fatigue (Robineau *et al.*, 2012:555), as a sprint exercise may result in ionic distresses, which might contribute to fatigue (Billaut & Bishop, 2009:264).

Fatigue usually occurs during the second half of the match, as seen in a decrease in the number of high-intensity activities observed, as well as an increase in the number of low-intensity activities and rest periods (Reilly, 1997:258). In addition, a decline in performance (Mohr *et al.*, 2005:593), less ball involvement and a decrease in the number of short passes attempted, as well as fewer successful short passes, are noticeable when fatigue transpires (Rampinini *et al.*, 2009:230). Extensive research has been done in the past on males regarding the physical aspects of soccer (Mohr *et al.*, 2005:593), with a recent study indicating that no methodological standardisation of velocity thresholds exists to quantify locomotor activities from a match for female soccer players (Bradley & Vescovi, 2015:112). This limits the available literature regarding the occurrence and causes of physical fatigue during a female soccer match.

Regarding studies on female soccer players, research has indicated fatigue to influence not only amateur but also elite players. In this regard, Russell and colleagues (2011:226) reported slower sprinting speeds ($F_{(5,70)}$= 7.469, $p < 0.01$) following a simulation soccer match to induce fatigue. Furthermore, the particular study (Russell *et al.*, 2011:227) also reported that the players' shooting precision was influenced ($F_{(3,42)}$ = 3.134, $p = 0.035$), with shots being 25.5 ± 4.0% less accurate than before the session, as well as a decrease in the speed of the shots during the second half of the match ($p = 0.012$). Robineau and associates (2012:559–560) reported a wide range of results after a 90 min match in eight amateur soccer players (age: 20.4 ± 1.3 y). Results indicated a significant decrease ($p < 0.05$) in the quadriceps isometric and concentric maximal voluntary torque, hamstring isometric maximal voluntary torque, sprint speed for the 20 to 30 m interval, stride frequency and static jump height, which were observed at half time as well as at the end of the soccer match (Robineau *et al.*, 2012:559–560). Andersson *et al.* (2008:376) reported both neuromuscular and biochemical changes following a soccer match in 22 elite female players (age: 22.1 ±

3.4 y). They observed significantly lower ($p < 0.05$) sprint, countermovement jump and isokinetic strength performance, as well as a significant increase in blood creatine kinase, uric acid, urea and perceived muscle soreness (Andersson *et al.*, 2008:376).

2.1.3. *Physiological aspects of fatigue related to matches*

A range of physiological factors are correlated with soccer performance (Turner *et al.*, 2011:29), though a range of physiological factors are linked to the temporary fatigue experienced during a match (Bangsbo *et al.*, 2007:112). These include high muscle lactate and acidosis, muscle glycogen depletion (Bangsbo *et al.*, 2007:117, 120), low muscle creatine phosphate, electrical disturbances in the muscle cell and the accumulation of extracellular potassium (Mohr *et al.*, 2005:594, 595), as well as the accumulation of metabolites and neural adjustments (Billaut & Bishop, 2009:267,268).

As stated by Purvis and colleagues (2010:444), physiological fatigue can be a consequence of a metabolic end point that is demonstrated by depleted energy stores, excessive cytokine release, tissue injury and/or oxidative stress. As a result of a repetitive high-intensity activity period, a major metabolic disruption can take place within the muscles and muscle fibres, which could fast-track the onset of fatigue (Green, 1997:252). A large increase in creatine, free adenosine tri-phosphate (ATP), free adenosine monophosphate, inorganic phosphate and lactic acid can occur after high-intensity activity (Green, 1997:252). Another factor that contributes to fatigue is a loss of muscle glycogen, as intense training primarily utilises carbohydrates and the glycogen reserves within the muscles (Green, 1997:252,253).

The accumulation of H^+ may result in a decline in performance because of its role in glycolytic inhibition during muscular contraction (Billaut & Bishop, 2009:267). In addition, because of the length of a soccer match, a rise in core body temperature beyond an optimum value can occur and may be an added cause of fatigue (Reilly *et al.*, 2008:360). Two groups of homeostatic disturbances as a result of increased core body temperature (due to training or exercise) could contribute to decreased performance (Cheung & Sleivert, 2004:100). The first is an impairment in voluntary muscle activation, which results in fatigue, and the second an increase in strain experienced by the cardiovascular system, impairing blood pressure and blood flow to the brain, which might also accelerate fatigue (Cheung & Sleivert, 2004:100). In a study regarding the physiological demands of a female soccer match, a positive correlation was found between maximal oxygen consumption (VO_{2max}) and high-intensity running in 14 elite female soccer players (Krustrup *et al.*, 2005:1247).

2.1.3.1. **Literature on the effect of fatigue on the physiological aspects of soccer: High-intensity (anaerobic) activities**

As mentioned earlier, soccer is characterised by phases of high-intensity activities combined with low-intensity activities (Svensson & Drust, 2005:601). The capacity to perform these high-intensity activities continually is an indicator of a player's ability to withstand fatigue (Krustrup *et al.*, 2005:1247). ATP provision during anaerobic activities (which are characterised as brief periods of maximal work) is usually retained through the integration of various metabolic processes (Billaut & Bishop, 2009:267). Phosphorcreatine (PCr) depletion, reduced muscle glycogen content and intramuscular acidosis are seen as reasonable factors for the appearance of muscular fatigue (Lambert & Flynn, 2002:515). Both PCr and lactate formation are important processes for short bouts of high-intensity exercises (Sahlin *et al.*, 1998:262).

From the start of a high-intensity activity, the PCr system will take approximately 10 sec to reach complete depletion, although the breakdown thereof can provide energy for up to 20 sec (Sahlin *et al.*, 1998:263). According to Sahlin and colleagues (1998:263), a decrease in the PCr system is associated with the initial symptoms of fatigue. A large increase in the rate of ATP hydrolysis takes place during the transition from rest to near maximal or maximal exercise intensities (Green, 1997:249) and the rate of ATP turnover is limited by the availability of the ATP hydrolysing enzymes (Sahlin *et al.*, 1998:262). To prevent fatigue from occurring, the glycolysis, high-energy phosphate transfer and oxidative phosphorylation energy systems should be trained to restore ATP before complete depletion takes place, as ATP is only available in small amounts within the muscle (Green, 1997:249).

A rise in work bouts will affect the metabolic response of subsequent sessions (Billaut & Bishop, 2009:267). According to Mohr *et al.* (2005:594, 595), the anaerobic energy system might be overloaded during an intense soccer match, resulting in a fourfold rise in muscle lactate and pH levels, as well as the accumulation of interstitial potassium. Several methods have been used to observe the physiological demand of a match on a players' physiology, with blood lactate (BLa^-) being the most widely used measurement (Alexandre *et al.*, 2012:2891) to indicate anaerobic glycolysis (Reilly, 1997:259). Bangsbo and colleagues (2007:113) stated that the high prevalence of BLa^- concentrations following a soccer match might indicate a high demand from the anaerobic energy system (Mohr *et al.*, 2005:594). In this regard, various field test are generally implemented to examine the players' anaerobic energy system. The multiple sprint test is widely used to examine players' repeated sprint performance (Svensson & Drust, 2005:612). Following this test, the fatigue index can be calculated, which, if high, may indicate the inability to remove BLa^- and replenish phosphocreatine (Svensson & Drust, 2005:612). Unfortunately a large limitation for using this and similar tests, is the chance for players' to pace themselves throughout the test. This led researchers in developing another similar test, namely a high-intensity intermittent sprint protocol, as the time for each sprint is controlled, sprint distances vary, and total test duration is longer (Svensson & Drust, 2005:612).

An increase in tension on the metabolic, neural and muscular systems take place during repetitive intensive training activities (Green, 1997:250). As the duration of a high-intensity activity increases, an increase takes place at the non-metabolic component, which may consequently be seen as a huge factor in the occurrence of fatigue (Green, 1997:251). At this site, a disturbance at the energetic potential of the muscle fibre site takes place (Green, 1997:251). Though anaerobic activities can make a big contribution to the fatigue experienced, aerobic activities dominate a soccer match and should therefore also be considered.

2.1.3.2. **Literature on the effect of fatigue on the physiological aspects of soccer: low- and moderate-intensity (aerobic) activities**

Both the aerobic and anaerobic energy systems contribute to energy delivery during a soccer match (Stølen *et al.*, 2005:509). Soccer players require a high aerobic capacity in order to maintain an extraordinary performance level throughout a match (Robineau *et al.*, 2012:555). This is seen in 95% of the total match-time correlating with low-intensity activities such as walking, slow and moderate running (Robineau *et al.*, 2012:555).

Unfortunately, it is not always possible to test a whole team in a laboratory setting, as these tests are expensive and time-consuming (Turner *et al.*, 2011:31). Various field tests have been developed to test soccer players, requiring players either to cover a maximal distance in a fixed time or a set distance in the fastest time possible (Turner *et al.*, 2011:31). Two tests frequently used on the soccer fraternity to assess the ability to train intensively and intermittently over a long period are the Yo-Yo intermittent endurance (YYIE) test and the Yo-Yo intermittent recovery (YYIR) test (Turner *et al.*, 2011:31). Because of the intermittent nature of soccer and the simulation thereof during the Yo-Yo test, it is seen as a valid test to assess the soccer population (Svensson & Drust, 2005:610). Additionally, studies have reported correlations between the YYIE test and maximal heart rate (HR_{max}) (r = -0.74, p < 0.01) (Bradley *et al.*, 2014:4), total distance (r = 0.55, p < 0.05) (Bradley *et al.*, 2014:6) and high-intensity running (r = 0.7; 0.83, p < 0.01) (Bradley *et al*., 2014:6; Krustrup *et al.*, 2005:1245) obtained during a match. Compared to sprint tests (as previously mentioned), a correlation was found between the YYIE test and fatigue index from a repeated sprint test (r = -0.66–0.76, p < 0.05) (Krustrup *et al.*, 2010:439) and various high-intensity speed threshold values ($r \geq 0.58$, p < 0.05) (Bradley *et al.*, 2014:6).

During a lengthy training session at 60–80% of the VO_{2max}, fatigue at the muscular level usually occurs owing to the depletion of glycogen stores (Sahlin *et al.*, 1998:264). In addition, when 60% of the VO_{2max} is exceeded, cortisol and epinephrine concentrations increase (Karacabey *et al.*, 2005:363). The fatigue occurring during prolonged training seems to be more complex and related to factors of the central nervous system (such as the central drive and motivation) (Sahlin *et al.*, 1998:264). Similar to a maximal effort executed, central fatigue can also occur as a result of submaximal muscle activity (Taylor & Gandevia, 2008:545). The experience of fatigue is not solely caused by physiological factors, but may be due to psychological factors as well (Reilly, 1997:259) though aerobic training is often used as a modality to improve mood states, which might be due to cumulative physiological consequences (Byrne & Byrne, 1993:566).

2.1.4. *Psychological aspects of fatigue*

Although training is known to have a positive outcome on an individual's psychological state (Byrne & Byrne, 1993:565), the effect of fatigue may not be positive. Fatigue is an objective and subjective state, with the subjective symptoms including poor concentration, languor, lethargy, tiredness and lassitude (Purvis *et al.*, 2010:444). The intensity of an activity influences primarily somatic anxiety (increased heart rate, pain, muscle tone, nausea etc.) (Guszkowska, 2009:6). The pathway of fatigue from the brain to the body is presented in figure 3.

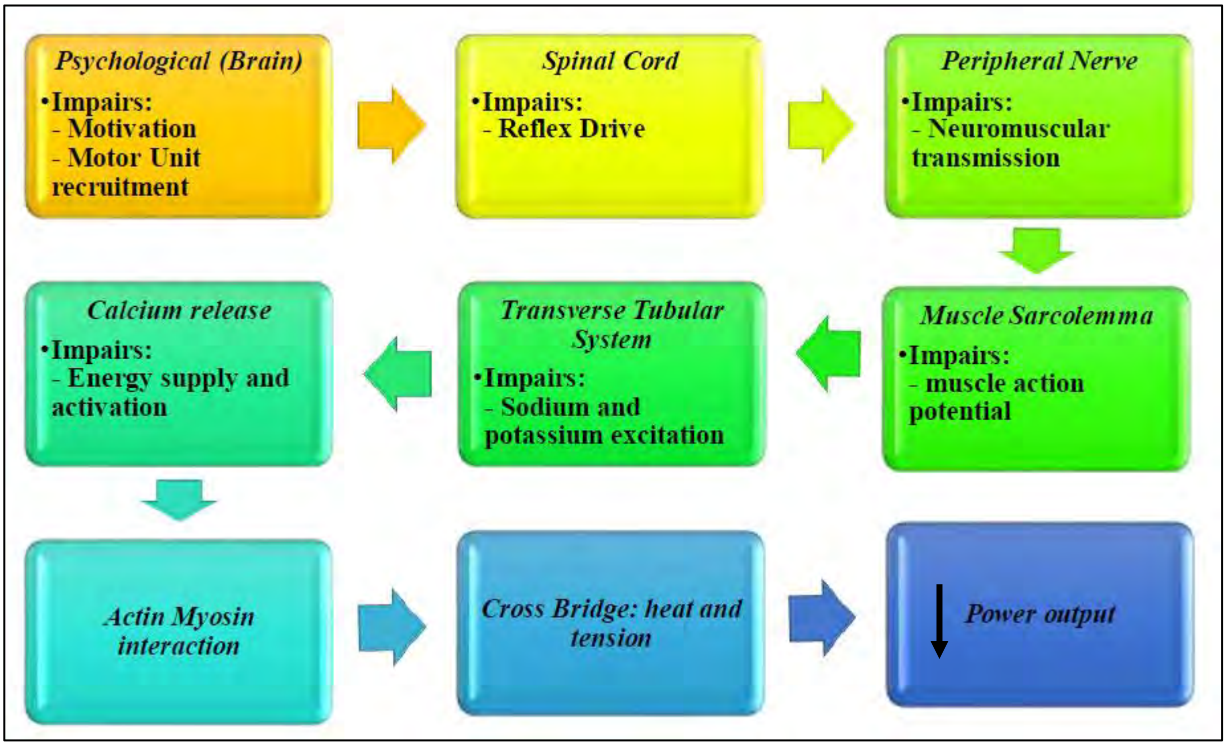

Figure 3. Possible sites of fatigue (Adapted from Gibson & Edwards, 1985:121).

2.1.4.1. **Literature on the effect of fatigue on the psychological state**

A study involving four professional triathletes (3 females, 1 male, age: 32.3 ± 3.8 y) over a 40-week season revealed a significant positive relationship (r = 0.30; p < 0.01) between training load and anxiety or perceived fatigue (Millet *et al.*, 2005:495). In addition, a significant positive relationship (r = 0.32; p < 0.01) was reported between the training load and anxiety in these triathletes (Millet *et al.*, 2005:495). During the study, the triathletes had to complete a modified State-Trait Anxiety Inventory (STAI A State questionnaire), as well as a perceived fatigue questionnaire, twice a week. In conclusion, they reported that a 15-day period following the competitive season was needed for perceived fatigue to return to baseline (pre-season) levels (Millet *et al.*, 2005:497). Rietjens *et al.* (2005:21) reported a non-significant increase (p = 0.06) in average mood score, with the worst mood state reflected at baseline following a two-week intensified training period to induce fatigue in seven well-trained cyclists (age: 25.3 ± 4.7 y). Although this study did not report any significant difference, five of the subjects demonstrated an increased mood and fatigue score during the training period (Rietjens *et al.*, 2005:21).

Furthermore, during week one of the intensified training session, the participants' rate of perceived exertion (RPE) increased significantly ($p < 0.05$), decreasing slightly during the second week but remaining significantly higher ($p < 0.05$) than the baseline values. With regard to the physiological variables, they reported a non-significant lower value of the resting and maximal values of the thyroid-stimulating hormone and cortisol, whereas the growth hormone and adrenocorticotropic hormone showed a small increase following the fatiguing session (Rietjens *et al.*, 2005:22).

This leads to the next section regarding the psychological and hormonal aspects of females in the sporting arena. Collectively, an overload in various bodily systems and the effect thereof can be seen in the following figure, as proposed by Lehmann *et al.* (1998).

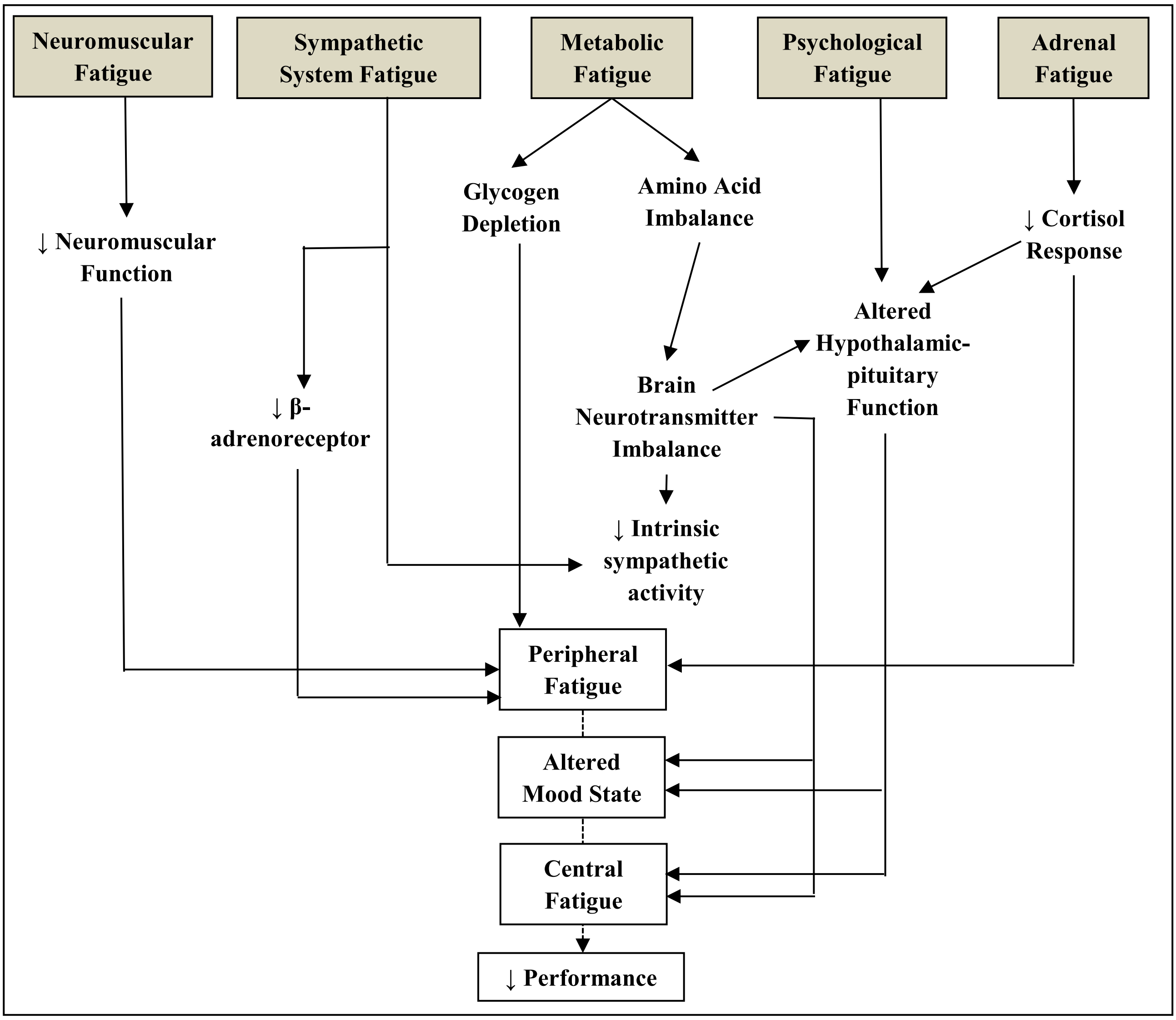

Figure 4. Schematic representation of how fatigue in various bodily systems can alter performance (Adapted from Lehmann *et al.*, 1998:1141).

3. FEMALES AND THE COMPETING DOMAIN

3.1. Hormonal aspects of female athletes

Although the female body only produces androgens in small amounts (Enea *et al.*, 2011:1), these might have an important effect on the physiological as well as psychological state. Thus, understanding the pathway and synthesis of androgens in the female body is considered imperative for optimising performance. Albumin or the sex hormone binding globulin is the primary binder for steroid hormones (Enea *et al.*, 2011:2). In this regard, androstenedione (secreted by both the adrenals and ovaries) and dehydroepiandrosterone (DHEA and its sulfo-conjugate) (secreted by the adrenals), are important in females as they can undergo peripheral conversion to more powerful androgens (such as 5α-dihydrotestosterone and testosterone) (Enea *et al.*, 2011:2). In the biosynthesis of all steroid hormones, cholesterol must firstly be converted to pregnenolone by P450scc in the theca cellular section (Enea *et al.*, 2011:2–3). From there, it can be further converted into aldosterone, testosterone or cortisol, which are all important for performance. As seen from figure 4, fatigue usually leads to an adrenal overload, decreasing cortisol secretion, which can have a catabolic effect (Chennaoui *et al.*, 2016:2). This emphasises the need to evaluate the extent to which cortisol can affect physical and psychological performance.

3.1.1. *Cortisol*

The adrenal glands synthesise and secrete aldosterone, testosterone and cortisol. In both males and females, the reproductive organs and adrenals are the primary production source for the androgens testosterone (Enea *et al.*, 2011:2) and cortisol (Kirschbaum & Hellhammer, 2000:379). Though testosterone is perceived as the most important androgen secreted (Enea *et al.*, 2011:2), only a small amount of testosterone (as well as weaker androgens) is produced by the adrenal glands and the ovaries in females (Enea *et al.*, 2011:2). Therefore cortisol is seen as a vital glucocorticoid in the female body (Kudielka & Kirschbaum, 2003:36) and is involved in physiological and psychological processes (Chennaoui *et al.*, 2016:2; Edwards & Casto, 2013:153). The hypothalamus-pituitary-adrenal (HPA) axis stimulates the secretion of cortisol (Kudielka & Kirschbaum, 2003:36) and can be activated by both physical and psychological stressors (Dickerson & Kemeny, 2004:355). The activities of the HPA axis are regulated by different brain regions (Fries *et al.*, 2009:68), making it imperative for supporting normal physiological functioning and regulating physiological systems, dealing with stressors and the functioning of important affective and cognitive processes (Dickerson & Kemeny, 2004:356).

Following the release of the HPA hormone adrenocorticotropin, cortisol is secreted in the bloodstream where it binds to specific carriers (Kirschbaum & Hellhammer, 2000:379). Cortisol is the main hormone responsible for the stress response in the body (Kirschbaum & Hellhammer, 2000:379) and immediately after a stressor, an increase can be seen in serum cortisol, with a corresponding increase observed in salivary cortisol levels 10 min later (Edwards & Casto, 2015:48). Unfortunately, a blood sample is expensive, labour-intensive and stressful, making it counterproductive for stress research (Cardinale & Varley, 2017:56; Kirschbaum & Hellhammer, 2000:379). Two percent to 15% of the cortisol released remains unbound or free, with both the unbound and free fraction being visible in blood, while only the free cortisol appears in saliva (Kirschbaum & Hellhammer, 2000:379). A strong positive correlation ($r \geq 0.90$) exists between salivary and unbound blood cortisol levels (Kirschbaum & Hellhammer, 2000:380). Therefore, analysing saliva to measure cortisol can be useful in view of the biologically active fraction of this steroid hormone (Kirschbaum & Hellhammer, 2000:379). Fortunately, saliva analysis is a popular assessment method, as no medical, personal or laboratory facilities are required for the collection or storing of the samples (Kirschbaum & Hellhammer, 2000:379).

Not only is cortisol involved during physical and psychological stressors; it also plays a vital role in providing fuel to the body by increasing the blood glucose levels, which results in the release of energy (Dickerson & Kemeny, 2004:356). Cortisol is also related to high-intensity training and is the main hormone responsible for the catabolic processes taking place (Chennaoui *et al.*, 2016:2). It inhibits the inflammatory process and immunity by reducing protein synthesis and increasing protein degradation (Chennaoui *et al.*, 2016:2). In contrast, a certain level thereof is necessary for the effects (such as increased heart rate and vasoconstriction) of catecholamine and sympathetic products to take place on the cardiovascular system (Dickerson & Kemeny, 2004:356). An important aspect to consider regarding cortisol is its robust circadian rhythm (Kirschbaum & Hellhammer, 2000:380). Various factors (cortisol awakening response, menarche, contraceptive usage and diet, among others) may influence the secretion of cortisol and will be discussed in section 4.2.1. A similar hormone that demonstrated a connection with aggression is testosterone (Archer, 2006:320).

3.1.2. *Testosterone vs. cortisol*

Females participating on an intercollegiate level have shown a substantial increase in salivary cortisol and testosterone levels (Edwards & Casto, 2013:158). Competing in team competitions has been associated with a 126% and 58% increase in females' levels of cortisol and testosterone respectively (Edwards & Casto, 2015:49). In another study of female rugby players, a 30% increase in cortisol levels from baseline to just prior to the match, with a further increase of 51% during the course of the match, was noted (Bateup *et al.*, 2002:184). In addition, the players' testosterone levels increased by 24% from baseline, increasing by a further 49% during the course of the match. According to Edwards and Casto (2015:48), it is important to determine the extent to which cortisol can regulate testosterone reactivity, as stress can vary in numerous ways. During a competition, a parallel increase can be expected in salivary cortisol and testosterone levels, demonstrating that an increase in one hormone can be significantly related to increases in the other (Edwards & Casto, 2013:159). In this regard, a significant positive correlation ($r = 0.46$, $p = 0.01$) was found between cortisol and testosterone secretion, which might be due to either the adrenal glands being the primary source of both hormones in females (Bateup *et al.*, 2002:187–188), or the adrenal glands and ovaries responding similarly to psychological and/or physical elements during competition (Edwards & Casto, 2013:159).

In Edwards and Casto's (2015) study, it was found that the lower the basal cortisol levels, the larger the testosterone response to the social stress, as well as the player rating score of the teammates (Edwards & Casto, 2013:159). Thus, if a higher cortisol baseline level can decrease the testosterone response to stress, negative testosterone-driven achievement motivation during stressful situations can occur (Edwards & Casto, 2015:48). In addition, the higher the baseline cortisol levels, the less the testosterone will increase during a competition (Edwards & Casto, 2015:50). In players exhibiting low cortisol levels, testosterone is positively related to the behavioural state, dominance (referring to power and influence over others); however, with players demonstrating a high cortisol level, this relationship is either reversed or blocked (Edwards & Casto, 2013:153,154). Because of this relationship, it is important to evaluate to what extent testosterone influences the psychological state, more specifically aggression, as aggression is linked to both cortisol and testosterone (Archer, 2006:320; Bateup *et al.*, 2002:183). This demonstrates the importance of evaluating the relationship between cortisol and psychological behaviour.

3.1.3. *Cortisol and psychological state*

A human being is comprised of three interacting components: the input/sensory system, integrators such as the central nervous system and the output/effector system (Nelson, 2017). Hormonal fluctuations do not have a direct influence on behaviour, but rather influence these three systems so that a certain response takes place in the appropriate behavioural context (Nelson, 2017). The psychological characteristics involved with cortisol changes include aggression, arousal, addressing challenges (Bateup *et al.*, 2002:183), depression, cognitive anxiety (Chennaoui *et al.*, 2016:2; Haneishi *et al.*, 2007:587) and fatigue, among others (Chida & Steptoe, 2009:272). According to Smyth and colleagues (1998:363), cortisol has a direct relationship with affect (mood), as positive affect demonstrates a relationship with lower cortisol levels ($F_{(1,901)} = 5.86$, $p < 0.05$) and negative affect with higher cortisol levels ($F_{(1,901)} = 6.91$, $p < 0.01$). Thus it is plausible that affect (mood) can mediate the relationship between cortisol and stress (Smyth *et al.*, 1998:363). Ziomkiewicz *et al.* (2015:101) reported that females expressing themselves as dominant were more prone to experiencing anxiety compared to females describing themselves as submissive. This may be due to unstable social situations forcing dominant individuals to reinforce their social position in an aggressive situation (Ziomkiewicz *et al.*, 2015:101). Furthermore, high trait anxiety may be due to negative early life experiences, which decreased social interaction development (Ziomkiewicz *et al.*, 2015:101). A female's relationship status and contraceptive usage may also have an effect on her level of dominance (Cobey *et al.*, 2015:455). Although dominance is important for achieving success, it may fluctuate according to social conditions (Cobey *et al.*, 2015:455). This emphasises the need to evaluate the literature on the effect of training and/or competition on hormones, more specifically cortisol, in the female athlete. Literature studies on the effect of training or competition on cortisol in female athletes are listed in table 1.

Table 1: Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention	Results	
Aizawa *et al.* (2006) Changes in pituitary, adrenal and gonadal hormones during competition among female soccer players.	9 elite soccer players (20.0 ± 0.4 y) Japan	Samples were collected: Before the 3-day tournament entailing six games, during (2 h after the second game of the day) and after (3 day after) the tournament, to measure: DHEA-s, cortisol and prolactin	Cortisol: Prolactin: DHEA-s: DHEA-s/C ratio: T:C ratio:	↑ During the competition compared to pre-values.** ↑ During the competition compared to pre-values.* ↓ During competition compared to pre-values.* ↓ During competition compared to pre-values.** ↓ During competition compared to pre-values.**
Azarbajani *et al.* (2010) The effect of sport competition on salivary steroids in amateur female karate athletes.	20 elite karate players (21.1 ± 3.0 y) Iran	Samples were collected: 30 min before and 5 min after the first and last competition, to measure: DHEA-s and cortisol.	Cortisol: DHEA-s: Winners:	↑ During the competition compared to pre-values. Highest before the final competition.* ↔ During competition ($p > 0.05$). ↔ Difference from losers ($p > 0.05$).
Bateup *et al.* (2002) Testosterone, cortisol and women's competition.	17 rugby players (18 – 22 y) New York, USA	Samples were collected: 24 hours (h) before, 15 min before and immediately after five rugby games, to measure: Cortisol and testosterone	Testosterone: Cortisol:	↑ From 24 h to 15 min before ($p = 0.06$) and during the game **. ↑ From 24 h to 15 min before the game** and during the game.** High correlation between C and T ($r = 0.46$).**
Bouget *et al.* (2006) Relationships among training stress, mood and dehydroepiandrosterone sulphate/cortisol ratio in female cyclists.	12 elite cyclists (21.7 ± 5.5 y) France	Samples were collected: Immediately after waking up (saliva and urine), before breakfast (saliva) and at T1 and T2 (saliva and questionnaires), to measure: Cortisol, DHEA-s and mood.	Cortisol: DHEA-s: DHEA-s/C ratio:	↑ From T1 to T2 (E*S* = 0.5).* ↔ Between T1 and T2 (*ES* = 0.1).* ↓ During the training camp (*ES* = 0.1).*

C = Cortisol; DHEA-s = Dehydroepiandrosterone sulphates; *ES* = Effect Size; T1/2 = Time point; ↑ = Increased; ↓ -= Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention	Results	
Caruso *et al.* (2014) Lactate, cortisol and testosterone values from high-speed resistive exercise workouts done by women.	11 competitive 18 novice athletes Age: N/R USA	Samples were collected: Before and 12 min after a training session (as many high-speed supramaximal impulses in 2 x 60 sec periods) to measure: Cortisol and testosterone.	Competitive group:	↑ C and mean force *vs.* novice athletes before and after the workout.* ↑ T *vs.* novice athletes after the workout.*
Casto & Edwards (2016b) Before, during, and after: how phases of competition differentially affect testosterone, cortisol, and estradiol levels in women athletes.	25 soccer players (age: 18 – 22 y) OC users: 11 Atlanta, USA	Samples were collected: At baseline 3 days prior to the first match, and four samples in association with two matches, 1 week apart, as well as 10 – 15 min before warm-up, immediately after warm-up and after the match to measure: Cortisol, estradiol and testosterone.	OC users:	↓ T *vs.* non-OC users at baseline.* ↑ C *vs.* non-OC users (home-loss match).* ↔ In C and estradiol compared to non-OC users.
			Testosterone:	Main effect for time in both matches (Home-loss & away-win).** ↑ From before to after warm-up and after warm-up to after-match.** ↓ Within 30 min after the match in both matches
			Cortisol & Estradiol:	Main effect for time in both matches (Home-loss & away-win).** ↑ From baseline to warm-up for home-match** and away-match ($p > 0.05$) and to after both match.**
Casto *et al.* (2017) Competition-related testosterone, cortisol, and perceived personal success in recreational women athletes.	35 Football players (18.0 ± 2.4 y) Atlanta, USA	Samples were collected: Ten minutes before, immediately after and 10 min after the competition, to measure: Cortisol, testosterone and perceived personal success.	Testosterone:	↑ From before to immediately and 10 min after competition.** Competition related ↑. ↔ For winning *vs.* losing outcome.
			Cortisol:	↑ From before to immediately after competition.** Competition related ↑. ↔ For winning *vs.* losing outcome or contraceptive usage. Positively related post-competition with perceived personal success. Interaction between T and C pre-competition did not predict perceived personal success score.

C = Cortisol; N/R = Not Reported; OC = Oral Contraceptives; T = Testosterone; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * - $p < 0.05$; ** - $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention		Results
Chatard *et al.* (2002) Cortisol, DHEA, Performance and training in elite swimmers.	4 Competitive swimmers (18.0 ± 2.4 y) Australia	Samples were collected: At baseline, on the morning of competition, on week days and on weekday afternoons before training, to measure: Cortisol and DHEA	Competition: Individual competition: Group train:	↑ Ratio of DHEA/C related to performance ($r = 0.34$).* DHEA related to C ($r = 0.43$).** ↔ Relationship between C and performance. 4 out of 9 = Relationship between C and performance ($r = 0.76–0.91$).* C correlated negative with number of training ($r = -0.31$).**
Chennaoui *et al.* (2016) Stress biomarkers, mood states and sleep during a major competition: “success” and “failure” athletes’ profile of high-level swimmers.	3 elite swimmers (22.0 ± 4 y) France	Samples were collected: Before and after each race over a series to measure: Cortisol, α-amylase and chromogranin-A.	Cortisol:	↑ From baseline to post-series, semi-finals and finals.* ↑ From before to after the semi-finals. ↑ For “success” group after finals *vs.* “failure” group ($Z = 1.83$, $p = 0.06$). As C ↑, fatigue also ↑ before semi- and finals ($r = 0.89$).* Correlated with depression ($r = 0.9$) from pre- to post-series and with confusion ($r = -0.95$) from pre- to post-finals.* Failure group: C correlated with vigour ($r = 0.9$).*
Cook *et al.* (2012) Comparison of baseline free testosterone and cortisol concentrations between elite and non-elite female athletes.	18 females from various sport codes (track and field, netball, cycling, swimming and bob skeleton) (25.3 ± 2.1 y) United Kingdom	Samples were collected: Over a 12 week period during a normal training and competition schedule, on the Wednesday of every week at 10:00 and before training, to measure: Testosterone and cortisol.	Elite: Cortisol: Testosterone:	Significantly higher C and T values *vs.* the non-elite athletes.* Significantly higher in weeks 4 and 12 *vs.* weeks 1 and/or 3.* Significantly higher in weeks 3, 6, 7 and/or 11 *vs.* weeks 1, 4, 8, 9 and/or 12.*

C = Cortisol; T = Testosterone; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention		Results
Crewther *et al.* (2015) Effects of oral contraceptive use on the salivary testosterone and cortisol responses during training sessions and competitions in elite women athletes.	29 elite hockey players (25.3 ± 2.1 y) OC users = 10 United Kingdom	Samples were collected: Prior to warmup before training (light session in morning and heavy session in afternoon) or competition and within 5 min after the activity to measure: Cortisol and testosterone.	Light training: Heavy training: Competition:	↑ T values for non-OC users *vs.* OC users.** ↓ C values from pre- to post-test for OC and non-OC users.** ↓ T response *vs.* heavy training and competition.** ↓ C response *vs.* heavy training and competition.** ↑ T values for non-OC users *vs.* OC users.** ↑ T and C values from pre- to post-test for OC and non-OC users.** ↑ T and C from pre- to post-competition.** ↑ T (31%) for non-OC users.**
Di Corrado *et al.* (2010) Mood states and salivary cortisol in a group of elite female water polo players: before and during the A1 National championship.	15 polo swimmers (22.9 ± 7.3 y) Italy	Mood was determined and saliva was collected: Three times per day for three consecutive days per month to measure: Cortisol.	Cortisol:	↔ In whole and experienced group ($p = 0.64$). Negative correlation between tension-anxiety and cortisol ($r = - 0.67$).**
Edwards *et al.* (2006) Intercollegiate soccer: Saliva cortisol and testosterone are elevated during competition, and testosterone is related to status and social connectedness with teammates.	18 soccer players (18 – 22 y) Atlanta, USA	Samples were collected: Prior to warm-up, 1 h before the match and 15 min after the match to measure: Cortisol and testosterone.	Post-match: Pre-match: (won/lost):	↑ T and C.** T correlated with overall teammate ratings ($r = 0.59$)* and social connectedness with teammates ($r = 0.51$).* ↔ C levels whether won or lost. Before-match T levels were correlated for both won/lost matches ($r = 0.68$).*

C = Cortisol; N/R = Not Reported; OC = Oral Contraceptives; T = Testosterone; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention		Results
Edwards & Casto (2013) Women's intercollegiate athletic competition: cortisol, testosterone, and the dual-hormone hypothesis as it relates to status among teammates.	Soccer (n = 13), volleyball (n = 46), softball (n = 16) and tennis (n = 13) (Age: N/R) OC users = 31 Atlanta, USA	Samples were collected: Before warm-up (1 h before play) and within 15 min after play, to measure: Cortisol and testosterone.	OC users: Competition: Warm-up: T and C:	↓ T levels before competition. ↑ C and T from baseline to post-competition.** ↑ C and T from baseline to post-warm-up. ↑ In C and T were correlated for the second competition ($r = 0.32$).**
Edwards & Casto (2015) Baseline cortisol moderates testosterone reactivity to women's intercollegiate athletic competition.	Volleyball (n = 28); cross-country (n = 26); softball (n = 9); tennis (n = 9) and soccer (n = 28) . (Age = NR) OC users = 46 Atlanta, USA	Samples were collected: On the day of competition prior to warm-up and within 15 min post-competition, to measure: Cortisol and testosterone.	 OC users:	↑ C and T levels in team sports.** Significant main effect of pre-competition C on magnitude of T increase.** ↓ T level** *vs.* non-users, however, ↑ in T associated with competition. ↔ C levels *vs.* non-users.
Edwards & Kurlander (2010) Women's intercollegiate volleyball and tennis: effects of warm-up, competition, and practice on saliva levels of cortisol and testosterone.	15 intercollegiate volleyball players 13 intercollegiate tennis players (Age: N/R) Atlanta, USA	<u>Volleyball:</u> Saliva was collected at baseline (30 – 40 min before warm-up), mid-warm-up and after the match. <u>Tennis:</u> Saliva was collected at baseline (prior to warm-up), at the end of warm-up and after the match. <u>Volleyball & Tennis:</u> Before and after a practice session, 1 week later to measure: Cortisol and testosterone.	Tennis: Practice *vs.* game: T and C:	↔ C levels from pre-warm-up *vs.* mid warm-up. ↑ C levels from baseline *vs.* after match.** ↑ T levels from warm-up *vs.* end of match.** ↑ C levels from baseline t *vs.* after match.** ↑ T levels from baseline *vs.* warm-up* and end of match.** ↑ C levels on competition *vs.* practice.* Correlation for mid-warm-up ($r = 0.93$).* Positive correlation after-practice ($r = 0.09$).* ↔ At any time point in the tennis players.

C = Cortisol; N/R = Not Reported; OC = Oral Contraceptives; T = Testosterone; ↑ - Increased; ↓ - Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention	Results	
Filaire *et al.* (2009) Psychophysiological stress in tennis players during the first single match of a tournament.	8 tennis players 20.2 ± 1.0 y) France	Samples were collected: Two weeks prior to a competition and during the competition (30 min upon awakening; 1 h and 10 min prior to a match; 10 min, 1 h and the evening after the match); questionnaires were collected: 15 min prior to a match to measure: Cortisol, CSAI-2.	Matches: Losers: Winners:	↑ C *vs.* resting values.** Highest C values 10 min post-match.** Evening post values – higher *vs.* evening resting values.* ↑ C on competition day on all time points.* ↑Area under concentration time curve *vs.* winners.* Positive correlation between C and CSAI-2 (cognitive state) 10 min before the match (r = 0.78).* Higher C increases *vs.* the losers.
Filaire *et al.* (2015) Dietary intake, eating behaviors, and diurnal patterns of salivary cortisol and alpha-amylase secretion among professional young adult female tennis players.	26 tennis players (19.6 ± 2.1 y) Disordered eating group (DE) = 12 Non-DE = 14 France	Saliva and questionnaires were collected: Immediately upon awakening, 30 min, 60 min and 12 h post-awakening over two days to measure: Cortisol and Alpha-amylase and trait anxiety using the trait scale of the STAI.	DE group: Non-DE group:	↑ State anxiety *vs.* non-DE group.* ↑ Cortisol levels *vs.* non-DE group at all time points except at night.** ↑ Cortisol levels after awakening.* ↑ Cortisol levels after awakening*, but ↓ rise *vs.* DE-group.** ↓ Overall day cortisol value *vs.* DE group.**
Haneishi *et al.* (2007) Cortisol and stress responses during a game and practice in female collegiate soccer players.	18 soccer players (10 starters, 20.2 ± 2.0 y) (8 non-starters, 20.5 ± 1.7 y) Tennessee, USA	Saliva and questionnaires were collected: 30 min before the game (pre-game); 10 min after the game (post-game); immediately before the regular practice (pre-practice) and immediately after the practice (post-practice), to measure: Cortisol and CSAI-2.	Starters & non-starters: Starters:	↑ C at post-game (*ES* = 1.54).* Larger ↑ in C after game than after practice (*ES* = 1.52)*. Greater ↑ in C than non-starters.* C was positively and significantly related to cognitive anxiety pre-practice (r = 0.7).*

C = Cortisol; CSAI-2 = Competitive State Anxiety Inventory; DE = Disordered Eating; *ES* = Effect Size; STAI= State-trait Anxiety Inventory; T = Testosterone;
↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention	Results	
Jiménez *et al.* (2012) Effects of victory and defeat on testosterone and cortisol response to competition: evidence for same response patterns in men and women.	50 badminton players (23.6 ± 3.8 y) Spain	Samples were collected: 40 min before and after competition to measure: Cortisol and testosterone.	Victor women: Defeated men and women:	↓ C than before competition and higher than victor men. ↑ C than before competition.**
Karacabey *et al.* (2005) The effects of exercise on the immune system and stress hormones in sportswomen.	40 sportswomen (G1 = 21.6 ± 1.4 y; G2 = 20.8 ± 1.1 y) 20 sedentary women (G3 = 20.1 ± 1.0 y) Turkey	Samples were collected: Before, immediately after and 4 h, 2 days and 5 days after a treadmill test of 30 min (G1 and G3) or a Wingate test (G2) to measure: IgA, IgG, IgM, ACTH, cortisol.	Aerobic: Anaerobic:	36% ↑ Cortisol and ACTH immediately post-exercise.* 20–30% ↓ in Cortisol and ACTH ($p > 0.05$) at 4 h post-exercise. ↔ Cortisol and ACTH ($p > 0.05$) after 2 and 4 days. 3% ↓ Cortisol and ACTH ($p > 0.05$) immediately post-exercise. ↔ Cortisol and ACTH ($p > 0.05$) at 4 h, 2 and 4 days post-exercise.
Kivlighan *et al.* (2005) Gender differences in testosterone and cortisol response to competition.	23 rowers (17 – 31 y) OC users = 6 USA	Samples were collected: Before warm-up, 20 and 40 min post-competition, to measure: Cortisol and testosterone.	Men: Females: Group:	↑ T levels *vs.* females.* ↑ C levels *vs.* males.* ↓ Pre-competition T levels *vs.* baseline for novice.** ↑ C & T levels at post-match *vs.* pre-match.*
Manshouri & Ghanbari-Niaki (2011) Six consecutive days of anaerobic interval exercise training and its hormonal effects in young females.	25 college subjects Control: 19.5 ± 0.3 y) RAST: 20.0 ± 0.4 y) Iran	Samples were collected: Before and at specific intervals, and after six consecutive days of RAST training, to measure: Obestatin, growth hormone, insulin, DHEA-s, testosterone, cortisol and glucose.	6 days of RAST:	Significant ↓ in growth hormone* and testosterone.* ↔ in Cortisol, DHEA-S or insulin within or between groups ($p > 0.05$).

ACTH = Adrenocorticotropic; C = Cortisol; G = Group; N/R = Not Reported; RAST = Running Anaerobic Sprint Test; T = Testosterone;
↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention		Results
Maya *et al.* (2016) Salivary biomarker responses to two final matches in women's professional football.	16 football players (22.5 ± 2.1 y) Chile	Samples were collected: 30 min before- and 5–10 min after two final football matches, played 3 days apart, to measure: Cortisol, testosterone, IgA and RPE.	RPE: Match 1: Match 2:	↔ Differences between match 1 or 2 ($p = 0.58$). Correlation with C for matches 1 and 2 ($r = 0.4–0.6$).* ↑ C & T from pre- to post.** ↓ T/C ratio from pre- to post.** ↑ T from pre- to post.**
McGuinan *et al.* (2004) Salivary cortisol responses and perceived exertion during high-intensity and low-intensity bouts of resistance exercise.	9 females (20.0 ± 0.9 y) USA	Samples were collected: At the beginning of each testing session and immediately after completion of the resistance exercise and 30 min after the last exercise, to measure: Cortisol and RPE.	High-intensity: High *vs.* low:	↑ C immediately after.* Significant difference in C and RPE between the two immediately post-exercise.* No correlation between C and RPE as well as C and training load immediately or 30 min post-exercise.
Obmiński (2008) Blood cortisol responses to pre-competition stress in athletes: sex-related differences.	37 females from different sport codes (volleyball, judo and taekwondo) (23 – 27 y) Poland	Samples were collected: Over two consecutive days, a control [morning and during non-exhaustion exertion (volleyball: complete rest; taekwondo: 156 maximal blows against a punching bag, 5 x 2 min with 1 min intervals; judo: zig-zag run on 100 m with maximal velocity] and a competition day to measure: Cortisol.	Volleyball: Teakwondo & Judo: Group: Menstrual cycle & outcome:	Significant differences in C in the mornings between the control day and all the tournament days.* Significant differences in C between the competition day and the morning and during the exercise test.* Lower C values during neutral conditions (morning and during non-exhausting exercise) compared to a stressful event. ↑ C values at pre-competition *vs.* baseline values.* No effect on C values (neutral or competition day).

C = Cortisol; G = Group; RPE = Rate of Perceived Exertion; T = Testosterone; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention	Results	
Obmiński *et al.* (2010) Blood indices and psychomotor skills demonstrated by elite male and female taekwondo performers during laboratory tasks of various intensity.	6 taekwondo players G1(LIT) = 6 G2 (HIT) = 1 (20 – 26 y) Poland	Samples were collected: In the morning, 3 min prior to the task [G1 = 3 x 25 single blows (75 blows); G2 = 3 x 25 of 3 various blows (225 blows)], and at 3 min and 30 min post-exertion recovery, to measure: Cortisol and testosterone.	LIT: HIT: Testosterone:	Pre-exercise: significantly ↑ C values *vs.* morning and 30 min post-exertion.* ↔ In C values at any time point ($p > 0.05$). ↔ At any time point for either G1 or G2 ($p > 0.05$).
Obmiński *et al.* (2014) Training-induced changes in aerobic and anaerobic capacity and resting hormonal status in blood in elite male and female speed skaters.	4 elite speed skaters (22 – 24 y) Poland	Samples were collected: In the morning (08:00) and after a training session (Wingate 30 sec test (anaerobic), and incremental graded test on a cycle ergometer), to measure: Testosterone and cortisol.	Cortisol:	Non-significant increase (23.9%) from pre- to post-training.
Oliveira *et al.* (2009) Testosterone responsiveness to winning and losing experiences in female soccer players.	33 soccer players (24.2 ± 4.8 y) Portugal	Psychological questionnaires and saliva were collected: 30 min before warm-up as well as before and after the match on the game-day, to measure: Cortisol, testosterone, anxiety and mood (POMS).	Losers: Winners: Hormones *vs.* psychological:	↑ T, threat perception and anxiety, ↓ in mood** and ↔ in C ($p = 0.10$). ↑ Mood and satisfaction with outcome, ↓ anxiety**, higher ↑ in T than losers after game.** ↔ Difference in C between winners and losers. Mood correlated with T ($r = 0.48$)* but not with C. Negative correlation between anxiety and T ($r = -0.36$)*, not with C. Mood change over the match had a negative correlation with T levels after the match ($r = -0.57$).**

C = Cortisol; HIT = High-intensity Train; G = Group; LIT = Low-intensity Train; POMS = Profile of Mood States; T = Testosterone; ↑ = Increased; ↓ -= Decreased; ↔ = No changes;
* = $p < 0.05$; ** = $p < 0.01$

Table 1 (cont.): Research studies on the effect of training or competition on various hormones in female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Intervention		Results
Sedgehroohi *et al.* (2011) The effect of win or loss on serum testosterone and cortisol hormones in female basketball players.	22 basketball players (20.0 y) Iran	Samples were collected: Two hours before and 15 min after the game, to measure: Cortisol and testosterone.	Fixed players: Winners *vs.* losers:	↑ T and C before and after game.** ↔ Change in T and C before and after the game ($p = 0.16–0.66$).
Wingfield *et al.* (2015) The acute effect of exercise modality and nutrition manipulations on post-exercise resting energy expenditure and respiratory exchange ratio in women: a randomised trial.	20 recreationally active females (24.6 ± 3.9 y) USA	Samples were collected: At baseline, immediately, 30 min and 60 min post-exercise to measure: Cortisol and estradiol. The exercise comprised either an aerobic endurance exercise (AEE) (30 min jogging at 45 – 45% of HR_{max}), high-intensity interval running (HIIT) (10 rounds of 60 sec running at 85 – 95% of HR_{max}) or high-intensity resistance training (HIRT) (three sets of 6 – 8 RM, 20 – 30sec rest between each set).	Estradiol: Cortisol:	↔ Between baseline or any other time point ($p = 0.636$, $ES = 0.035$). ↔ Between various exercise modalities ($p = 0.168$, $ES = 0.015$). ↔ Between pre- and post AEE ($p > 0.05$). ↑ 25% From pre- to post HIIT ($p > 0.05$). ↑ 12.5% From pre- to post HIRT ($p > 0.05$).
Yadegari *et al.* (2015) The effect of one bout resistance exercise in warm environment on serume IL-6 and cortisol in trained females.	14 subjects (26.0 ± 1.2 y) Iran	Samples were collected: 1 h before, immediately and 1 h after exercise, to measure: Cortisol and IL-6 The exercise comprised two sessions of resistance exercise in two environments.	Cortisol:	↑ Immediately post-exercise and ↓ 1 h post-exercise in warm conditions.*

AEE = Aerobic Endurance Exercise; C = Cortisol; *ES* = Effect Size; HIIT = High-intensity Interval Running; HIRT = High-intensity Resistance Training; HR = Heart Rate; RM = Repetition Maximum; T = Testosterone; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

3.1.4. *Summary of literature studies on the effect of training/competition on cortisol responses in female athletes*

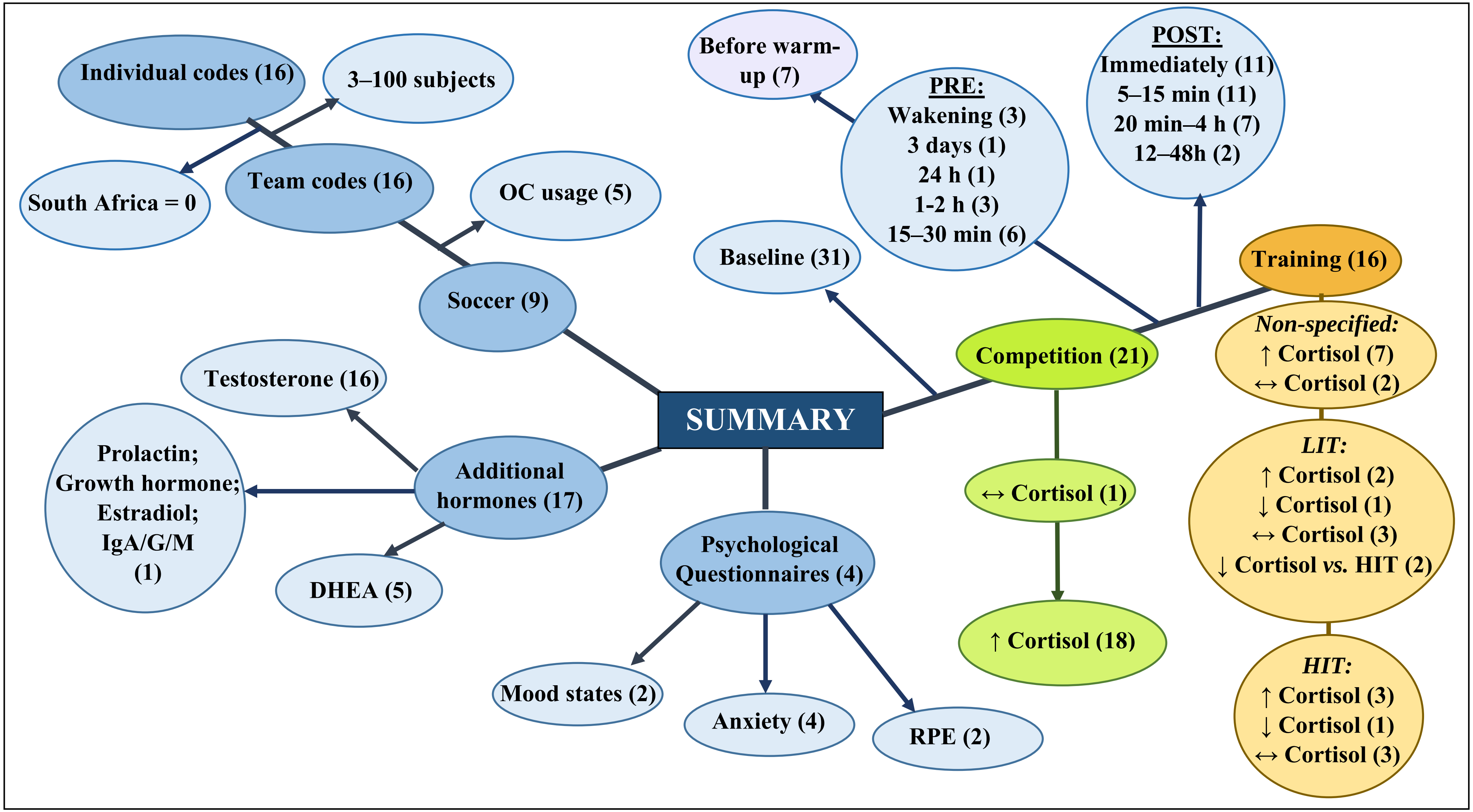

Figure 5. Summary of literature studies on the effect of training/competition on cortisol responses in female athletes.

Taken collectively, soccer players are the most commonly used study population with regard to cortisol measurements, with individual and team sports being considered equally, though not one single study was conducted on a South African team. The sample sizes used are predominantly determined by the sport code, as seen in smaller sample sizes for individual sports and larger samples for team sports, with no significant differences noted between these populations.

A major limitation in the current literature is the absence of information on whether the subjects took oral contraceptives (OC) or not. Whether the subjects took OC or not did not have an influence on their hormonal levels as reported by Edwards and Casto (2015), Edwards and Kurlander (2010), Casto and Edwards (2016) and Casto *et al.,* (2017), though Casto and Edwards (2016) found that OC users demonstrated a higher increase in cortisol compared to non-OC users when a home match was lost ($p = 0.054$). Future studies should take this into consideration, as it may have an effect on performance (refer to section 4.2.1.4).

Most studies (21) evaluated the effect of cortisol during a competition, with 16 evaluating the effect of training. All the cited studies took a sample before commencement of the test to serve as a baseline. Though protocols vary in the time period before inauguration, some studies took a sample from as long before the event as 3 days (1), 24 h (1), upon awakening (3), 1 to 2 h (3) or 15–30 min (6) prior to the match/training. Seven studies took a sample before warm-up. Following the competition or training, most studies took a sample immediately (11) or within 5–15 min (11) or even 20 min to 4 h after finishing (7). This is important, as cortisol has a robust circadian rhythm with peak values observed 30 min after awakening as well as 10–30 min after a stressor (Kirschbaum & Hellhammer, 2000:380–381). A few studies could be found that took a sample from 12–48 h (2) after the test/competition, though no significant differences were observed compared to the earlier samples. A rise in cortisol was predominantly observed during a competition, with two studies (Filaire *et al.*, 2009; Jiménez *et al.*, 2012) reporting a higher cortisol increase ($p = 0.005$) in defeated female badminton or tennis players, whereas another study (Chennaoui *et al.*, 2016) found a higher increase ($Z = 1.83$, $p = 0.06$) in cortisol for the successful group compared to the failure group following a swimming competition. Sedgehroohi *et al.* (2011) reported a cortisol increase ($p = 0.00$) in the fixed (completing 70% of the game) basketball players with no difference ($p = 0.66$) whether the outcome was winning or losing (Edwards *et al.*, 2006; Sedgehroohi *et al.*, 2011). In addition, Haneishi *et al.* (2007) found a significant increase in cortisol in female soccer players, with the starters demonstrating a larger increase compared to the non-starters ($p < 0.05$). In addition, they reported a larger increase ($ES = 1.52$) in cortisol values following a soccer match compared to training (Haneishi *et al.*, 2007). Cook and colleagues (2012) reported elite female athletes to demonstrate significantly greater cortisol values when compared to non-elite athletes ($p < 0.05$) during a normal training and competition schedule. Only one study (Chatard *et al.*, 2002), reported no significant differences in cortisol during a swimming competition or during weekdays and on weekday afternoons prior to training, though a positive relationship was seen between individual swimming competitions and performance ($r = 0.76$–0.91, $p < 0.05$) and a negative relationship with the whole group's cortisol values and the amount of training ($r = -0.31$, $p < 0.01$). When comparing training *vs.* a competition setting, cortisol tends to yield a higher response during a competition compared to training.

With regard to training sessions, an increase in cortisol was noted after high-intensity, low-intensity or non-specified training regimes. Comparing high- and low-intensity activities, a significant difference in cortisol values was reported by McGuinan *et al.* (2004). In addition to this, Crewther and colleagues (2015) found a lower cortisol value following low-intensity training for both OC and non-OC users ($p < 0.001$). In contrast, one study (Karacabey *et al.*, 2005) reported a decrease ($p < 0.05$) in cortisol levels immediately after a high-intensity training session, as well as 4 h after a low-intensity training session. Unfortunately, a few studies did not find any significant differences from training (whether high- or low-intensity training) on cortisol levels. This variance in results might be due to different hormonal response patterns depending on either the intensity level of the activity or the training status of the individuals (McGuinan *et al.*, 2004:12). A limitation noticed from the cited literature, was the absence of specifying the training protocol/intervention administered. In addition, limited studies were found comparing either aerobic or anaerobic training with an actual competition, making comparisons and practicality difficult. Future studies could provide more detailed training strategies for the reader to determine the extent and whether high- or low-intensity training has an effect on cortisol.

Cortisol is most commonly measured together with testosterone, though limited results are available regarding the relationship between cortisol and other hormones. Only a few studies evaluated the ratio or relationship between cortisol and other hormones. In this regard, Aizawa *et al.* (2006) reported a decrease ($p < 0.01$) in the DHEA-s:cortisol ratio as well as in the testosterone:cortisol ratio, which is similar to that reported by Maya *et al.* (2016) following a soccer match. Chatard *et al.* (2002) found that an increase in the DHEA-s:cortisol ratio was related to performance ($r = 0.34$, $p < 0.04$). Regarding training, Bouget *et al.* (2006:1300) reported a decrease in the DHEA-s:cortisol relationship during a training camp ($p < 0.05$), suggesting that a dose-response relationship exists between the resting DHEA-s:cortisol ratio, the training load and the assessment of stress and recovery. In addition to this, a few studies reported a positive correlation ($r > 0.3$, $p < 0.05$) between cortisol and testosterone following training (Edwards & Casto, 2013; Edwards & Casto, 2015; Edwards & Kurlander, 2010), which is to be expected, as stated earlier. Future studies can take this into consideration, as research has shown that cortisol may alter other hormonal levels, which might have an effect on either the psychological or physical performance state (section 3.1.3.).

From the current studies, a positive correlation between cortisol and cognitive anxiety before a competition or training ($r = 0.7$, $p < 0.05$) was seen, though another study found a negative correlation between tension-anxiety and cortisol ($r = -0.67$, $p < 0.01$). In addition to this, Chennaoui *et al.* (2016) reported that as cortisol increased during a swimming tournament, so did the feeling of fatigue ($r = 0.89$, $p < 0.05$) prior to the semi-finals and the finals. They furthermore found a high positive correlation between cortisol and depression ($r = 0.9$, $p < 0.05$) from pre- to post-series, as well as in confusion ($r = -0.95$, $p < 0.05$) from pre- to post-finals

(Chennaoui *et al.*, 2016). The study completed by Maya and colleagues (2016) reported a positive relationship between cortisol and the RPE following a soccer match, whereas no relationship was reported between these variables or between cortisol and training load following training (McGuinan *et al.*, 2004) or between cortisol and mood or anxiety in other research (Oliveira *et al.*, 2009).

The next section will elaborate on the importance of the psychological states of female athletes in a training or competition environment.

3.2. Psychological aspects of female athletes

Training is an effective method for enhancing one's psychological well-being (Byrne & Byrne, 1993:565). In a review dating back to 1993, up to 90% of research studies testified to lower depression and anxiety levels as a result of training (Byrne & Byrne, 1993:566). If a low possibility of an unattractive event is perceived, arousal will therefore be low; however, if the perceived probability is high, a high level of arousal occurs, which in turn leads to uncertainty (Ursin & Eriksen, 2004:581). The various stressors experienced during a competitive match may result in the appearance of another stimulus, namely anxiety (Aubets & Segura, 1995:149), which has the ability to influence performance either negatively or positively (Raglin & Morris, 1994:47; Varzaneh *et al.*, 2011:19).

3.2.1. *Anxiety*

Anxiety is a motivational and emotional state that arises in threatening circumstances (Eysenck *et al.*, 2007:336). Unpleasant feelings such as tension, stress and somatic signs and symptoms are all associated with anxiety (Patel *et al.*, 2010:325). Anxiety or the expression of anger may determine the way soccer is played, emphasising the importance of analysing the players' attitudes and readiness to adhere to the rules of the game (Junge *et al.*, 2000:23). An interesting finding reported by Man and colleagues (1995:212) was that players who performed exceptionally well during training and minor games performed poorly in important competitions, which might be due to the influence of anxiety on performance (Varzaneh *et al.*, 2011:19). This observation can be due to two possible theories, the Multidimensional Anxiety Theory (MAT) or the cusp catastrophe model of anxiety. According to the MAT proposed by Martens *et al*. (1990), cognitive anxiety — the players' concerns on performing exceptional — demonstrates a negative linear relationship with performance, whilst somatic anxiety — the players' perception over their physiological response to psychological stress — has an inverted U-shape relationship with performance (Hardy, 1999:227–228). The third component of MAT is self-confidence — seen as the players' belief that they are adequate in meeting the task at hand — which has a positive linear relationship with performance (Hardy, 1999:228). Furthermore, according to the inverted U-hypothesis, if anxiety lies within a reasonable range, performance will be maximised, whereas if it falls below or higher than the moderate levels, performance will decline (Raglin & Morris, 1994:47). With an increase in anxiety, individuals tend to worry about a threat to the goal and as a result they try to develop an effective anxiety-reducing strategy (Eysenck *et al.*, 2007:336). The second plausible model (cusp catastrophe model of anxiety) takes into account the effect of cognitive anxiety, physiological arousal (instead of somatic anxiety) and performance (Hardy, 1999:228, 229). According to Hardy (1999:229), the effects of physiological arousal on performance is determined by cognitive anxiety, i.e. if the anxiety is low, performance will increase and *vice versa.* According to this model, when cognitive anxiety is high together with a rise in physiological arousal, a sudden drop in performance might occur (Hardy, 1999:229). A possible explanation of the cusp catastrophe model is the Processing efficiency theory (PET), which suggests how players respond in two ways to cognitive anxiety (Hardy, 1999:230).

3.2.1.1. **Processing efficiency theory**

The PET is built on two assumptions, a.) worry and b.) the mechanism of the working memory (Eysenck *et al.*, 2007:337). Worry is an element of state anxiety that has an effect on the effectiveness (quality of the task performance as measured by the accuracy of the response) and efficiency (the relationship between the performance effectiveness and the effort spent in the task performance) of performance (Eysenck *et al.*, 2007:336–337). Two effects can take place as a result of worry: either cognitive interference affecting the temporary storage capacity of the working memory, or an increase in motivation to minimise the anxiety state (Eysenck *et al.*, 2007:337). The latter is applicable if the individual makes use of auxiliary processing strategies and recourses that limit the impairment of performance effectiveness (Eysenck *et al.*, 2007:337). (More about this in section 4.1.1.)

The working memory model comprises a.) a central executive system, which is involved in self-regulatory functions and information processing; b.) a visuospatial sketchpad for processing any visual and spatial information and c.) a phonological loop for rehearsing and storing verbal information (Eysenck *et al.*, 2007:337). The main effects of worry and anxiety are assumed to take place on the central executive system, as worrisome thoughts interfere with the processing-and-storage function, leading to an increased burden on the self-regulatory system to inhibit negative thoughts and produce auxiliary processing actions (Eysenck *et al.*, 2007:337). However, a limitation of the PET is the imprecise indication of which central executive functions are affected by anxiety, as well as the effects of distracting stimuli on individuals. The attentional control theory (ACT) is built on the strengths and limitations of the PET (Eysenck *et al.*, 2007:337).

3.2.1.2. **Attentional control theory**

The ACT is based on the key assumption that a distinction between performance effectiveness and processing efficiency exists with regard to central executive functioning (Eysenck *et al.*, 2007:337). According to the ACT, the effects of anxiety on attentional processing are deemed important for understanding the anxiety mechanism in relation to performance (Eysenck *et al.*, 2007:338). Attentional control is a key function for the central executive system, which can be directly influenced by anxiety (Eysenck *et al.*, 2007:338). This influence is seen in two main tasks of the central executive, namely inhibition and shifting (Eysenck *et al.*, 2007:340). Firstly, with an increase in the time spent to achieve a performance level, a decrease in processing efficiency takes places – inhibition (Eysenck *et al.*, 2007:340). Secondly, anxiety can cause individuals to exert higher effort in order to maintain consistent performances – shifting (Eysenck *et al.*, 2007:340).

When individuals are faced with a threat, their attentional recourses are directed to stimuli related to the threat, whether external (threatening task-irrelevant distractors), or internal (worrisome thoughts) (Eysenck *et al.*, 2007:338). An assumption exists that anxiety can still impair attentional control even though no internal or external stimuli are present. This can be seen when individuals perceive themselves under threat, leading to increased anxiety and decreased attentional control of a specific task (Eysenck *et al.*, 2007:338). The postulation that anxiety impairs attentional control can be seen in the light of the two attentional systems distinguished by Corbetta and Shulman (2002).

The first system is referred to as a goal-directed attentional system, which is affected by knowledge, current goals and expectations, whereas on the other hand, the stimulus-driven attentional system responds to prominent stimuli (Corbetta & Shulman, 2002:209; Eysenck *et al.*, 2007:338). The goal-directed attentional system is convoluted in the top-down attention control system and the stimulus-driven attentional system in the bottom-up attention to control (Corbetta & Shulman, 2002:209; Eysenck *et al.*, 2007:338).

Top-down processing can be defined as the flow of information from higher to lower centres, usually from previous experiences rather than sensory stimuli, whereas bottom-up processing is information processing from sensory input, through perceptual analysis towards motor output, without involving feedback flowing backwards from "higher" to "lower" centres (Corbetta & Shulman, 2002:201). According to the ACT, a disruption in the balance between these two attentional systems takes place in response to anxiety (Eysenck *et al.*, 2007:338). This is seen in an increase in the stimulus-driven (external stimuli) decrease in the goal-directed attentional system (internal stimuli), making an individual more susceptible to irrelevant stimuli (Eysenck *et al.*, 2007:338). (Will be discussed more thoroughly in section 4.1.1.)

Cognitive symptoms include a sense of confusion, poor concentration, lack of confidence, fear, forgetfulness, feelings of weakness, negative thoughts and self-talk, indecisiveness, thoughts to avoid participation, irritability and inability to follow instructions (Patel *et al.*, 2010:327). Somatic symptoms include an increase in heart rate, blood pressure, breathing and sweating, a need to urinate, muscular tension, neck and shoulder tightness, trembling, distorted vision, blushing, yawning, twitching, vomiting, nausea, diarrhoea, sweaty hands and feet and loss of sleep and appetite (Patel *et al.*, 2010:327). In addition, behavioural signs and symptoms include repetitive movements, aggressive outbursts, fidgeting, biting of fingernails, acting defensively, covering the face with the hands or avoiding eye contact (Patel *et al.*, 2010:327). Anxiety can be divided into various categories as measured by a variety of questionnaires.

3.2.1.3. **Types of anxiety**

Anxiety is often seen as a result of pressure for success and performance in sport (Horikawa & Yagi, 2012:1), with competitive anxiety distributed into both somatic and cognitive components (Varzaneh *et al.*, 2011:19). Cognitive anxiety is the mental component of anxiety caused by negative expectations, while somatic anxiety refers to the affective and physiological elements developed directly from autonomic arousal (Martens *et al.*, 1990:9). According to Martens and colleagues (1990:171), a positive linear relationship exists between self-confidence and performance, whereas a negative linear relationship exists between performance and cognitive anxiety. Regarding somatic anxiety, a curvilinear relationship exists where both higher and lower values can negatively affect performance (Martens *et al.*, 1990:171). A study involving 303 athletes (233 males and 70 females; age: 24.2 ± 5.1 y) of different sporting codes reported a positive correlation between somatic and cognitive anxiety ($r = 0.44$, $p < 0.01$), while a negative correlation ($r = -0.43$, $p < 0.01$) was observed between cognitive anxiety and self-confidence (Fernandes *et al.*, 2013:709). Thus it can be assumed that athletes that report lower levels of self-confidence tend to experience higher levels of somatic and cognitive anxiety, and *vice versa* (Fernandes *et al.*, 2013:711).

A distinction should be made between state and trait anxiety. Trait anxiety refers to the predetermined perception and response to environmental stimuli that are either threatening or non-threatening, while state anxiety is an emotional response that involves feelings of tension and apprehensive thoughts, as well as heightened autonomic nervous system activity (Horikawa & Yagi, 2012:1; Martens *et al.*, 1990:9). According to Horikawa and Yagi (2012:4), the interaction between stress (a situation interpreted negatively) and trait anxiety may determine state anxiety. An individual with low trait anxiety experiences fewer threats in many situations and tends to have lower state anxiety scores compared to a player with high trait anxiety (Horikawa & Yagi, 2012:1). However, if individuals' exhibit higher trait anxiety levels, their cognitive interpretation of anxiety is less positive, which could impair performance (Horikawa & Yagi, 2012:2). In addition, females who experience a high level of trait anxiety tend to experience a high level of state anxiety, which might indicate a higher stress reaction to competitive situations (Ziomkiewicz *et al.*, 2015:101). Various factors (such as gender, type of sport and level of ability), tend to influence the dimensions of competitive anxiety and will be discussed subsequently (Martens *et al.*, 1990:75).

3.2.1.3.1. *Type of sport*

Regarding the type of sport, athletes in individual sports face different stressors compared to athletes from team sports (Holt & Hogg, 2002:253). Fernandes *et al.* (2013:710) stated that athletes from individual sport codes tend to report lower cognitive anxiety scores ($p < 0.05$) compared to team sport athletes. Furthermore, they reported no significant differences between self-confidence and somatic anxiety for either team or individual sport codes (Fernandes *et al.*, 2013:710), which is in contrast to the findings reported by Martens *et al.* (1990:147). They indicated that their results might have been due to the athletes' perception that they had higher levels of control over what they could do during a competition, compared to those taking part in a team sport (Fernandes *et al.*, 2013:712).

3.2.1.3.2. *Level of competence and competition*

Players' level of competitive experience seems to have an influence on their interpretation of the competitive situation (Fernandes *et al.*, 2013:706). According to the research of Fernandes and colleagues (2013:710), if an athlete demonstrates a broad level of competitive experience, a subsequent increase ($p < 0.05$) in self-confidence can be seen with no significant differences in somatic and cognitive anxiety observed ($p > 0.05$). Unfortunately this is not always seen in the results and outcome of a competitive match. This notion was acknowledged by Man and colleagues (1995:212) who reported poor execution from players in important competitions, at national and international level, although they usually performed exceptionally well during training and minor games. In their study, they assessed the effect of low-stress conditions (such as a less-important training game) and high-stress conditions (highly competitive games) on trait and state anxiety and self-confidence.

Their results indicated that during low-stress conditions, state anxiety is significantly lower compared to high-stress conditions ($F_{(1,43)} = 29.35$, $p = 0.00$), though this was not the case for trait anxiety ($F_{(1,43)} = 0.38$, $p = 0.54$) (Man *et al.*, 1995:217). Moreover, a significant main effect was only seen in trait anxiety for cognitive anxiety ($F_{(1,43)} = 0.05$ $p = 0.033$); however, the players' stress levels influenced both somatic ($F_{(1,43)} = 11.14$, $p = 0.00$) and cognitive anxiety ($F_{(1,43)} = 23.54$, $p = 0.00$) (Man *et al.*, 1995:217). They concluded that high-stress games resulted in significantly higher levels of both somatic and cognitive anxiety; whereas during low-stress games state anxiety tended to be lower (Man *et al.*, 1995:218). This might be due to the players' predictions of the forthcoming competition and the outcome thereof. In this regard, Raglin and Morris (1994:49) found a significant positive correlation ($r = 0.69$, $p < 0.05$) between the anxiety experienced prior to competition and the predictions made two days earlier in nine female volleyball players when using the State-Trait Anxiety Inventory (STAI) questionnaire.

In addition to this, another study (Horikawa & Yagi, 2012) investigated the effect of a controlled and a stressful condition on 10 soccer penalty shootout trials and reported a significantly lower ($p < 0.01$) number of successful goals under stressful conditions. Under the control conditions the players were free to shoot at the start of the whistle, whereas in the stressful condition (characterised by manipulating the antecedent condition by providing them with pressured instructions, as well as a higher level of verbal instructional pressure to shoot successfully) they were strictly instructed to increase their successful goal score from the control condition (Horikawa & Yagi, 2012:2). The STAI questionnaire was completed before and after shooting and the players were divided into high- and low-trait anxiety groups according to their baseline STAI scores. The results indicated a significantly higher ($p < 0.05$) mean state anxiety score of the high-trait anxiety group compared to the low-trait anxiety group, as well as higher mean state anxiety scores under the stressful condition ($p < 0.01$) compared to the control condition (Horikawa & Yagi, 2012:3).

3.2.1.3.3. *Gender*

Gender is another factor that has an effect on the experience of competitive state anxiety (Borrego *et al.*, 2012:120). Studies reported females of different sporting codes experiencing significantly higher cognitive ($p < 0.01$–0.05) and somatic ($p = 0.07$) anxiety prior to competition, as well as lower self-confidence levels ($p < 0.01$), compared to males (Borrego *et al.*, 2012:122; Esfahani & Gheze Soflu, 2010:239; Fernandes *et al.*, 2013:709). Borrego and colleagues (2012:125) stated that the above-mentioned results concerning the somatic and cognitive anxiety of 44 female soccer players might be due to them feeling less self-confident, though the female volleyball players used in Esfahani and Gheze Soflu's study (2010:239), reported feelings of higher self-confidence than their male counterparts. Furthermore, a significant difference ($p < 0.01$) for both males and females was reported between somatic and cognitive anxiety and the player's attraction to the group (Borrego *et al.*, 2012:122) and a low to moderate significantly negative correlation ($p < 0.01$) was reported between group task integration and somatic and cognitive anxiety, respectively (Borrego *et al.*, 2012:123).

3.2.1.3.4. *Anxiety and intellectual styles*

The level of anxiety that individuals demonstrate is part of their perception of the specific situation (Zhang, 2009:348). The preferred channel of processing this situation can be regarded as the individual's intellectual style (Zhang, 2009:348). Individuals' intellectual style refers to their hemispheric preferences as well as their cognitive, thinking and learning styles (Zhang, 2009:347). A distinction should be made between three types (hierarchical, conservative and external) of intellectual styles. Individuals who demonstrate a type 1 (hierarchical) intellectual style tend to be more right-hemispheric, reflective, creative, field-independent, adaptive and open-minded (Zhang, 2009:348). They are more self-assured as they demonstrate a welcoming attitude to challenging tasks and higher resistance to anxiety-related situations (Zhang, 2009:350). Type 2 (conservative) style individuals are considered to be left-hemispheric, impulsive, pessimistic and less adaptive, with a lower level of cognitive complexity (Zhang, 2009:348). Anxiety can be positively associated with this style, with the individuals having the tendency to be more anxious about things due to a lack in self-confidence, thus experiencing higher anxiety compared to type 1 (Zhang, 2009:350). Individuals demonstrating a type 3 (external) intellectual style may demonstrate either type 1 or 2 characteristics, depending on the situation's demands (Zhang, 2009:348).

3.2.1.3.5. *Interpretation of anxiety*

When a stressful or anxiety-related situation occurs, an individual can interpret the indicators thereof as either facilitative (positive) or debilitative (negative) (Thomas *et al.*, 2007:380). In Thomas *et al.*'s

(2007:382) study, the participants were classified as "facilitative" (positive interpretation) if they interpreted the state and trait level for both somatic and cognitive symptoms as positive, whereas a classification of a "debilitator" (negative interpretation) was made if the interpretation was negative. They determined the following differences between these two types:

a.) "Facilitators" replace negative cognitive performance symptoms and images with positive ones, whereas "debilitators" replay these negative performances and destructive symptoms cognitively (Thomas *et al.*, 2007:386);
b.) "Facilitators" make use of various psychological skills to overcome a negative experience, whereas "debilitators" attempt to use these skills but are unable to maintain control over their use and function (Thomas *et al.*, 2007:389);
c.) "Facilitators" demonstrate internal control over negative pre-performance cognition, whereas "debilitators" have little internal control over negative somatic and cognitive symptoms (Thomas *et al.*, 2007:389);
d.) "Facilitators" make use of both internal and external strategies to control and replace symptoms experienced, whereas "debilitators" use external strategies to stabilise the negative symptoms experienced (Thomas *et al.*, 2007:391–392).

In conclusion, this emphasises the need to evaluate the effect of various training or competition stressors on the psychological state of female athletes. Literature studies regarding various anxiety questionnaires in female athletes are listed in table 2.

Table 2: Research studies on the effect of training or competition on various anxiety questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age, Country)	Questionnaires	Intervention		Results
Borrego *et al.* (2012) Relationship between group cohesion and anxiety in soccer.	44 soccer players (17.1 ± 1.6 y) Portugal	- Group Environment Questionnaire (task cohesion) - Competitive State Anxiety Inventory 2 (CSAI-2)	Questionnaires were completed: 60 min before the game.	Females:	↑ Cognitive anxiety before competition *vs.* males.** ↑ Somatic anxiety ($p = 0.07$) *vs.* males. Task cohesion ≠ pre-competitive anxiety ($p > 0.05$), except for group task and self-confidence.**
				Task integration:	Correlated significantly with self-confidence (low-positive), somatic (low-negative) and cognitive (moderate negative) anxiety.**
				Individual attraction:	Significantly related to cognitive anxiety (low-negative).**
Bozkus *et al.* (2013) The effects of age, sports experience and physical self-perception on competition anxiety levels of female football players.	83 football players (18.3 ± 1.8 y) Turkey	- Physical Self-perception Inventory - Sport Competitive Anxiety test (SCAT-C)	Questionnaires were completed: Before the start of the match (Time: N/R)	Self-perception:	Highest subscale = sport ability, lowest subscale = strength. Anxiety = moderate.
				Competitive anxiety:	Correlated negatively with sport experience ($r = -0.22$)** and competence ($r = -0.36$).**
				Sport experience:	Correlated with sport ability ($r = 0.3$)*, physical condition ($r = 0.52$)** body attractiveness ($r = 0.28$)**, strength ($r = 0.3$)* and general physical competence ($r = 0.42$).**
Broman-Fulks *et al.* (2004) Effects of aerobic exercise on anxiety sensitivity.	41 recreational active subjects (21.2 ± 5.1 y) USA	- Anxiety Sensitivity Index - Body Sensations Questionnaire (BSQ) - STAI	Questionnaires were completed: Immediately before, after 5 min cooldown and 1 week follow-up. 20 min aerobic exercise (high or low intensity), 2 – 4 x a week, 6 sessions over 2 weeks.	ASI:	Main effect for session ($F_{(2,104)} = 49.98$).** ↑ At pre-treatment *vs.* post-treatment and follow-up. ↓ From pre- to post-treatment and to follow-up.* Effect for high- ($F_{(2, 56)} = 42.5$)** and low-intensity session ($F_{(2,48)} = 13.72$).**
				BSQ:	Related with ASI ($r = 0.33$)*, STAI-state ($r = 0.31$)* and STAI-trait ($r = 0.32$).* ↓ Mean scores for high-intensity group *vs.* low-intensity group.

ASI = Anxiety Sensitivity Index; BSQ = Body Sensations Questionnaire; CSAI-2 = Competitive State Anxiety Inventory; SCAT = Sport Competitive Anxiety Test; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 2 (cont.): Research studies on the effect of training or competition on various anxiety questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age, Country)	Questionnaires	Intervention		Results
Butt *et al.* (2003) The intensity and directional interpretation of anxiety: fluctuations throughout competition and relationship to performance.	62 hockey players (22.0 ± 4.5 y) Miami, USA	- Mental Readiness Form (MRF-2) - Self-assessment of performance	Questionnaires were completed: Pre-game, during the 1st half, during the 2nd half and post-game during a tournament. Each player evaluated her own performance.	Cognitive anxiety:	Effect of time ($F_{(6,358)} = 6.08$).** Highest pre-game, ↓ to post-game ($ES = 0.72$).** More facilitative toward performance at post-game *vs.* 1st and 2nd half.**
				Somatic anxiety:	Effect of time ($F_{(6,358)} = 4.48$).** Highest pre-game, ↓ to post-game ($ES = 0.48$).** ↓ From 1st to 2nd half of game ($ES = 0.40$).*
				Self-confidence:	Effect of time ($F_{(6,358)} = 2.86$)**. Lowest at pre-game, ↑ significantly to post-game ($ES = 0.36$).**
				Performance:	All three components could explain the amount of variance in performance in 1st ($F_{(12,104)} = 2.5$)** and 2nd half ($F_{(12,106)} = 4.79$).**
Cox *et al.* (2004) Effects of acute 60 and 80% VO_2max bouts of aerobic exercise on state anxiety of women of different age groups across time.	24 active subjects divided in two groups (G1= 18.6 ± 0.7 y) (G2= 40.2 ± 3.4 y) USA	STAI	Questionnaires were completed: Before and at 5, 30, 60 and 90 min following a 33 min aerobic training session on a treadmill or seated passively for the 33 min duration.	Control:	Time effect ($F_{(4,92)} = 3.23$)* for STAI. ↓ From baseline over 5, 30 and 60 min ($\eta^2 = 0.18–0.28$).*
				60% VO_{2max}:	Time effect ($F_{(4,92)} = 13.79$)** for STAI. ↓ From baseline over 5, 30, 60 and 90 min ($\eta^2 = 0.34–0.52$).*
				80% VO_{2max}:	Time effect ($F_{(4,92)} = 18.56$)* for STAI. ↓ From baseline over 30, 60 and 90 min ($\eta^2 = 0.49–0.56$).*
Esfahani & Gheze Soflu (2010) The comparison of pre-competition anxiety and state anger between female and male volleyball players.	82 volleyball players (Age: N/R) Iran	- CSAI-2 - The Spielberger State-Trait Anger Expression Inventory (STAXI)	Questionnaires were completed: 30 min before the university competition.	Females:	↑ Cognitive and somatic anxiety as well as self-confidence (*vs.* males.** ↓ Trait towards anger ($p = 0.39$) *vs.* males. ↑ Expressing anger *vs.* males.**

CSAI-2 = Competitive State Anxiety Inventory; *ES* = Effect Size; G = Group; MRF = Mental Readiness Form; N/R = Not Reported; STAI = State-trait Anxiety Inventory; STAXI = State-trait Anger Expression Inventory; VO_{2max} = Maximal Oxygen Consumption; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 2 (cont.): Research studies on the effect of training or competition on various anxiety questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age, Country)	Questionnaires	Intervention	Results	
Fernandes *et al.* (2013) Factors influencing competitive anxiety in Brazilian athletes.	70 subjects of team and individual sports (24.2 ± 5.1 y) Brazil	- Shortened version of the CSAI-2 (CSAI-2R)	Questionnaires were completed: One hour before the competitions.	Cognitive anxiety: Females: Individual sport: High experience:	Positive correlation with somatic anxiety (r = 0.44)** and negative correlation with self-confidence (r = -0.43).** ↑ Cognitive anxiety* and ↓ self-confidence.** ↓ Levels of cognitive anxiety*, no difference in somatic anxiety and self-confidence. ↑ Levels of self-confidence*, no difference in cognitive of somatic anxiety.
Filaire *et al.* (2009) Psychophysiological stress in tennis players during the first single match of a tournament.	8 tennis players 20.2 ± 1.0 y) France	CSAI-2	Questionnaires were completed: 15 min prior to a match.	Losers:	↑ Somatic and cognitive anxiety and ↓ self-confidence *vs.* winners.*
Gardner *et al.* (2015) The relationship between implicit beliefs, anxiety and attributional style in high-level soccer players.	30 soccer players (24.3 ± 5.2 y) Australia	- The Conceptions of the Nature of Athletic Ability Questionnaire – Version 2 - SCAT - Sports Attributional Style Scale	Questionnaires were completed: 15 min before the training session.	Competitive anxiety:	Significant Related to globalism** and controllability.** Negative events = internality and stability = not significant.

CSAI-2 = Competitive State Anxiety Inventory; SCAT = Sport Competitive Anxiety Test; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 2 (cont.): Research studies on the effect of training or competition on various anxiety questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age, Country)	Questionnaires	Intervention	Results	
Guillén & Sánchez (2009) Competitive anxiety in expert female athletes: sources and intensity of anxiety in national team and first division Spanish basketball players.	84 basketball players 71= First division (23.2 ± 4.0 y) 13 = National team (24.9 ± 3.4 y) Spain	- STAI - Three open-ended questions regarding the causes of anxiety during practice, competition and other sources of anxiety related to participation	Questionnaires were completed: 24 to 48 h prior to a regular game.	Elite: Age: Playing time: Playing experience: Sources of anxiety:	Significantly ↓ anxiety scores *vs.* first division players.* ↔ Effect on either state- or trait anxiety ($p > 0.05$). Significant main effect on both state- and trait anxiety, 30 min or more playing = ↓ state- and trait anxiety.* ↓ Playing time = ↑ Pre-game state-anxiety ↔ Effect on either state anxiety ($p > 0.05$), but ↑ on trait anxiety.* Personal sources had the largest contribution to anxiety experienced during training (57.1%), competition (41.6%) and other sources (55.0%).
Guszkowska (2009) State/trait anxiety and anxiolytic effects of acute physical exercises.	163 recreationally active subjects (16 – 56 y) Poland	STAI	Questionnaires were completed: Just before and immediately after the completion of either aerobic, resistance or mixed exercises.	State-anxiety:	Pre-post differences correlated with pre-exercise anxiety-state values ($r = 0.51$)*, not with trait-anxiety. Pre-exercise values can be associated with post exercise increase.**
Guszkowska & Sionek (2009) Changes in mood states and selected personality traits in women participating in a 12-week exercise program.	39 subjects (27.5 ± 5.6 y) Poland	STAI	Questionnaires were completed: In the first and in last week (3 months later) following regular exercising for 50 min twice a week.	Tension: Trait anxiety:	Positive correlation with trait anxiety ($r = 0.55$).** Negative correlation with vigour ($r = -0.49$).** ↓ In readiness to react with anxiety ($ES = 0.47$).*

ES = Effect Size; STAI = State-trait Anxiety Inventory; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 2 (cont.): Research studies on the effect of training or competition on various anxiety questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age, Country)	Questionnaires	Intervention	Results
Haneishi *et al.* (2007) Cortisol and stress responses during a game and practice in female collegiate soccer players.	18 soccer players (10 starters, 20.2 ± 2.0 y) (8 non-starters, 20.5 ± 1.7 y) Tennessee, USA	CSAI-2	Questionnaires were completed: 30 min before the match (pre-match); 10 min after the match (post-match); immediately before the regular practice (pre-practice) and immediately after the practice (post-practice).	Starters: C was positively and significantly related to cognitive anxiety at pre-practice (r = 0.7).* ↑ Somatic state- (*ES* = 1.04)* and cognitive state anxiety (*ES* = 0.53)* after a match *vs.* practice. Non-starters: ↑ Somatic state- (*ES* = 1.04)* and cognitive state anxiety (*ES* = 0.53)* after a match *vs.* practice. ↑ Self-confidence *vs.* starters post-practice.*
Keikha *et al.* (2015) The relationship between pre-competition state anxiety components and mood state sub-scales scores and the result of among college athletes through temporal patterning.	120 subjects from various sport codes (18 – 26 ± 0.6 y) Malasia	- POMS-Adolescent - CSAI-2	Questionnaires were completed: One week (T1), one day (T2) and 1 h (T3) before competition.	T1: Similar mood and anxiety means between winners and losers. T2: ↑ Vigour, ↓ negative sub-scale. Significant different with T1 and T2.* T2: Losers: ↓ Anger, depression, fatigue and confusion, ↑ tension and vigour *vs.* winners. T3: ↔ Different with T1. Outcome: Influenced by tension, fatigue and vigour at T2.
Kerr *et al.* (2005) Game outcome and elite Japanese women's field hockey player's experience of emotions and stress.	16 hockey players (22.1 ± 2.4 y) Japan	Tension and Effort Stress Inventory	Questionnaires were completed: 10 min before and within 10 min after each game, over 7 games.	Relaxation: ↑ At post-game *vs.* pre-game over the 7 games.** Excitement: ↑ Post-game over the 7 games.** Anxiety: Greater at pre- and post-game for game 1 *vs.* other games. Significant negative linear trend for combined pre- and post-game anxiety.** Significant negative linear trend for post-game ratings, not for pre-game ratings.** Win / lose: ↔ For pre- or post-game values.

CSAI-2 = Competitive State Anxiety Inventory; *ES* = Effect Size; POMS = Profile of Mood States; T = Time point; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 2 (cont.): Research studies on the effect of training or competition on various anxiety questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age, Country)	Questionnaires	Intervention		Results
McDowell *et al.* (2016) Sex-related differences in mood responses to acute aerobic exercises.	26 subjects (20.6 ± 1.5 y) Ireland	STAI	Questionnaires were completed: Immediately before and 10 min after either 30 min passive rest or 30 min vigorous treadmill running	Aerobic Training:	Improved state anxiety ($F_{(1,92)}$ = 12.52)** *vs.* control condition.
Millet *et al.* (2005) Modelling the relationship between training, anxiety, and fatigue in elite athletes.	3 elite triathletes. (Age: N/R) France	Modified STAI A-state and fatigue	Questionnaires were completed: Twice a week over a 40-week season.	Anxiety: Fatigue:	Relationship with training load (r = 0.3).** ↓ at 23 days.** ↑ at 59 days.** Relationship with training load (r = 0.3).**
Oliveira *et al.* (2009) Testosterone responsiveness to winning and losing experiences in female soccer players.	33 soccer players (24.2 ± 4.8 y) Portugal	- MRF - Direct and Indirect Aggression Scales	Questionnaires were completed: 30 min before warm-up as well as before and after the match on the match-day.	Losers: Winners: Hormones *vs.* psychological:	↑ Threat perception and anxiety. ↑ Satisfaction with outcome, ↓ anxiety.** Negative correlation between anxiety and T (r = -0.36, p = 0.06) but not with C.
Sangari *et al.* (2012) Relationship between mental skill and competitive anxiety in female national football players.	16 football players (22 – 27 y) Iran	- Ottawa Mental Skill Assessment Tool 3 (OMSAT-3) - SCAT	Questionnaires were completed: A day and 30 min prior to the competition.	Mental skills: Competitive anxiety:	Significant relationship = mental practice, refocus, fear control, goal setting, focus, self-confidence, psychosomatic, cognitive and mental skill.* ↑ Correlation with self-confidence (r = 0.52)*, ↓ correlation with commitment (r = 0.14). ↑ Relationship with stress control (r = 0.51)* and focus (r = 0.55)* and ↓ relationship with activation (r = 0.16) and imagery (r = 0.22).

MRF = Mental Readiness Form; OMSAT = Ottawa Mental Skill Assessment Tool; SCAT = Sport Competitive Anxiety Test; STAI = State-trait Anxiety Inventory;
↑ = Increased; ↓ = Decreased; ↔ = No changes; * = p < 0.05; ** = p < 0.01

Table 2 (cont.): Research studies on the effect of training or competition on various anxiety questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age, Country)	Questionnaires	Intervention	Results	
Szabo *et al.* (2014) Anxiety and affect in successful and less successful elite female basketball players: in-situ sampling before six consecutive games.	9 successful (26.3 ± 6.1 y) and 11 less successful (21.9 ± 4.1 y) basketball players Hungary	- SCAT - Positive Affect Negative Affect Scale	Questionnaires were completed: Within 30 min prior to the game over six games played at one-week intervals.	Successful:	Significant differences in pre-game anxiety ($F_{(5)}$ = 11.66)* and negative affect across matches.*
				Less successful:	Different levels of positive affect ($F_{(5)}$ = 13.54)* over the matches.
				Team differences:	More successful = ↓ pre-game anxiety (*ES* = 1.2) and negative affect (*p* = 0.12) and ↑ positive affect (*p* = 0.15).
Terry & Munro (2008) Mood and anxiety scores predict winning and losing performances in tennis.	43 tennis players (39.7 ± 9.8 y) Australia	- CSAI-2	Questionnaires were completed: Approximately 10 min prior to each competition.	Anxiety:	Predicted 58.7% correct outcome classifications (λ = .968, x^2 = 18.4).**
				Predictor of outcome:	Somatic anxiety and self-confidence subscales were predictors of the outcome.
				Win:	Associated with ↑ self-confidence and ↓ somatic anxiety.
Van der Pol *et al.* (2015) Autonomy support and motivational responses across training and competition in individual and team sports.	134 athletes from 15 university sport teams Age: N/R United Kingdom	- Autonomy-supportive coaching experience - Intrinsic motivation inventory - Modified sport anxiety scale-2	Questionnaires were completed: Either at the beginning or the end of a training session.	Autonomy:	No main effects on either type of anxiety.
				Somatic anxiety:	Significant interaction with interest in input and type of sport (*SE* = 0.05, *ES* = 0.17).*
				Individual sports:	↑ Interest in input = ↑ somatic anxiety (*SE* = 0.03).*
Varzaneh *et al.* (2011) Relationship between mental skills and anxiety interpretation in female volleyball players.	120 volleyball players (23.2 ± 1.8 y) Iran	- Modified CSAI-2 - OMSAT-3	Questionnaires were completed: 20 – 30 min before the competition.	Anxiety:	Correlated with stress reaction (positive), imagery (negative), focus (negative with self-confidence subscale), mental practice (positive with self-confidence), self-confidence (positive with self-confidence direction), refocus, fear control and commitment (positive with somatic), relaxation (negative with somatic), goalsetting (positive with self-confidence, negative with somatic)*.

CSAI-2 = Competitive State Anxiety Inventory; *ES* = Effect Size; OMSAT = Ottawa Mental Skill Assessment Tool 3; SCAT = Sport Competitive Anxiety Test;
↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

3.2.1.4. **Summary of literature studies on the effect of training/competition on anxiety states in female athletes**

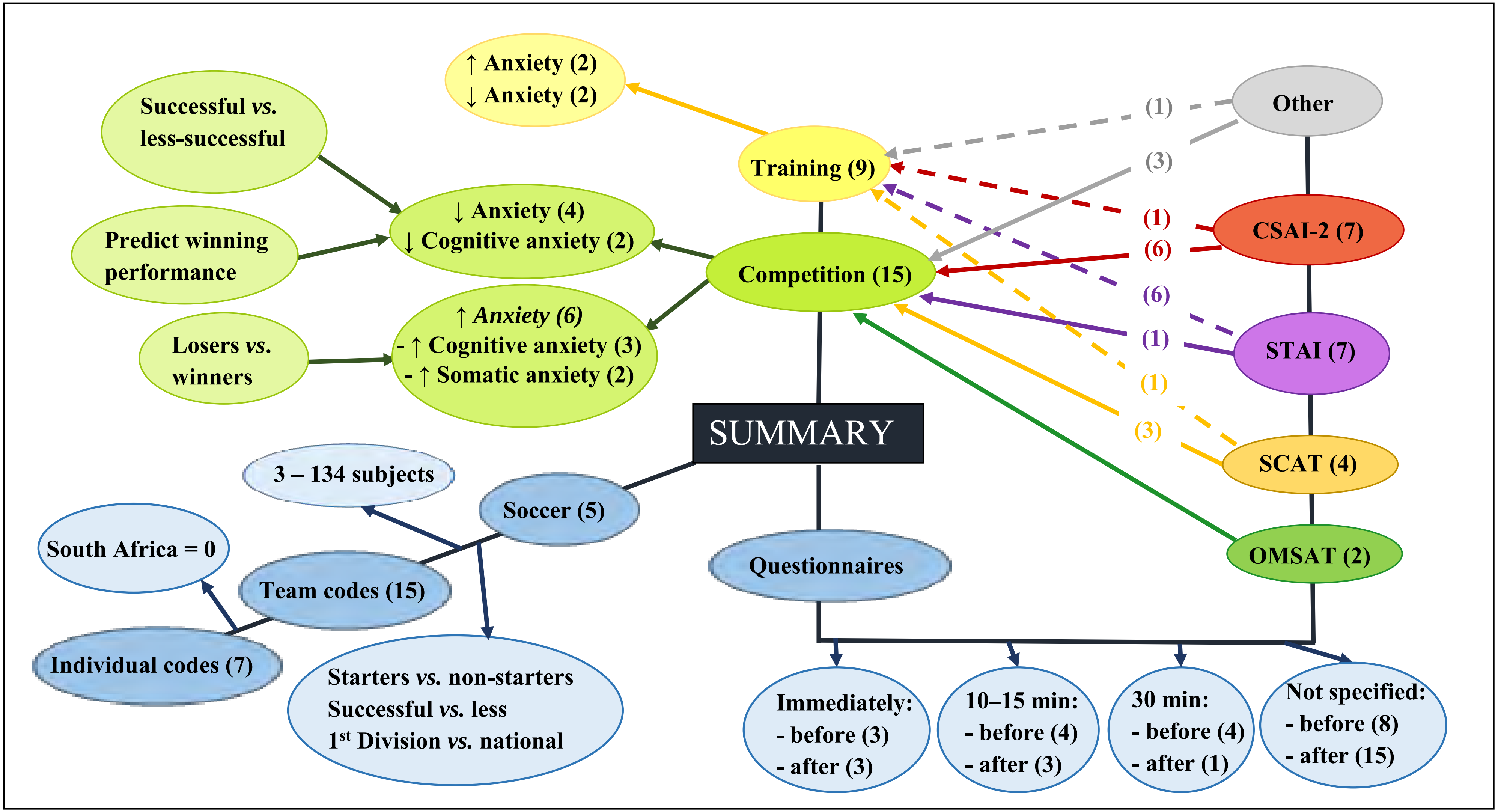

Figure 6. Summary of literature studies on the effect of training/competition on anxiety states in female athletes.

The majority of the studies (6) cited used female soccer players as test subjects, with team sports (soccer, hockey, volleyball and basketball) being the most commonly used population. A few studies did, however, make use of individual sports (recreationally active, individual sport not named, triathletes, tennis).

Samples sizes varied from as small as three to as large as 134 athletes from 15 team sport codes, with no significant differences noted between them. Haneishi *et al.* (2007) divided the subjects into starters and non-starters as used in a soccer match, reporting both starters and non-starters to exhibit higher somatic and cognitive anxiety after a match compared to a practice session, though the non-starters demonstrated higher self-confidence values post-practice compared to the starters ($p < 0.05$). Szabo *et al.* (2014) divided the subjects into successful and less successful basketball players and reported the successful players to display significant lower pre-game anxiety and negative affect / mood across the matches, whereas the less successful players demonstrated varying levels of positive affect / mood. In addition, Guillén and Sanchez (2009) divided the players into first division and elite (national division) basketball players and found the elite players to demonstrate lower anxiety scores compared to the first division players ($p < 0.05$).

A number of questionnaires exist that measure either competitive trait or state anxiety in competitive situations. Among these are the Sport Competition Anxiety Test (SCAT), the Competitive State Anxiety Inventory (CSAI and CSAI-2) and the STAI (Horikawa & Yagi, 2012:1). According to Horikawa and Yagi (2012:1), the STAI questionnaire has proven to be less sensitive in the sport competition context compared to other questionnaires. As seen from the studies above, the most common questionnaire completed was the STAI questionnaire during training regimes, whereas the CSAI-2 questionnaire was popularly implemented in a competition context. This was most probably due to the STAI questionnaire only measuring current and general total anxiety levels, whereas the CSAI-2 measures three anxiety subscales, self-confidence, cognitive- and somatic anxiety. Other questionnaires implemented were the SCAT and Ottawa Mental Skill Assessment Tool 3 (OMSAT-3). Researchers implemented the questionnaires over a wide time period, from as long as 48 h to immediately prior to training and competition. A limitation noticed in the literature is the absence of the specific time indicated for administering the questionnaires before and after the intervention.

Most of the studies cited implemented the STAI questionnaire in conjunction with other questionnaires. The STAI questionnaire demonstrated a correlation with the body sensations questionnaire in recreationally active females for both the state- ($r = 0.31$, $p < 0.05$) and trait-anxiety scales ($r = 0.32$, $p < 0.05$) following an aerobic training session of 6 weeks (Broman-Fulks *et al.*, 2004). The mean scores were lower following the high-intensity training session compared to the low-intensity session (Broman-Fulks *et al.*, 2004), either probably due to a time facet as the high-intensity sessions are shorter, or the linear relationship that exists between training intensity and mood states. Cognitive anxiety demonstrated a positive relationship with somatic anxiety ($r = 0.44$, $p < 0.01$) and a negative one with self-confidence ($r = -0.42$, $p < 0.01$) following various team and individual sport competitions (Fernandes *et al.*, 2013). Further correlations were seen between competitive anxiety and globalism ($p = 0.021$), controllability ($p = 0.027$) (Gardner *et al.*, 2015), self-confidence ($r = 0.515$), stress control ($r = 0.512$) and focus ($r = 0.553$) (Sangari *et al.*, 2012) and between cognitive anxiety and various subscales of the OMSAT-3 questionnaire (Varzaneh *et al.*, 2011).

A positive correlation was reported between the subscales trait anxiety and state anxiety using the STAI questionnaire ($r = 0.48$, $p < 0.001$) (Ziomkiewicz *et al.*, 2015), though this is in contrast with the findings of Guszkowska (2009), who reported no correlation between the sub-scales but rather between the pre-post training state-anxiety scores and the pre-training state-anxiety ($r = 0.51$, $p < 0.001$) scores. In another study done by Guszkowska and Sionek (2009), they reported a positive correlation between trait anxiety and tension ($r = 0.55$, $p < 0.01$) as well as a negative correlation with vigour ($r = -0.49$, $p = 0.002$).

Most of the studies examined the effect of a competition on the anxiety states with the questionnaires completed prior to the competition (15). In the cited literature, six studies reported an increase in anxiety levels following a competition, with primarily increases ($p < 0.05$) in cognitive anxiety and somatic anxiety. Interestingly, Esfahani and Gheze Soflu (2010) observed an increase in self-confidence ($p < 0.005$), whereas Fernandes *et al.* (2013) reported a decrease ($p < 0.01$) in self-confidence as anxiety increased ($p < 0.05$) in a range of female athletes. Regarding the competition outcome, four studies reported their findings, with the losers demonstrating higher levels of tension compared to the winners (Filaire *et al.*, 2009; Keikha *et al.*, 2015), and the successful players showing significantly lower pre-game anxiety levels ($p = 0.04$) with a negative affect across basketball matches ($p = 0.02$) compared to less successful players (Szabo *et al.*, 2014). Also, Terry and Munro (2008:295) concluded that pre-competition anxiety scores can predict up to 58.7% of a tennis outcome, with lower somatic anxiety and higher self-confidence scales being significant predictors of a winning performance.

Moreover, Guillén and Sanchez (2009:416) reported that as the basketball playing time decreased, an increase in anxiety levels took place and *vice versa,* which might be attributed to the fact that the more experienced players received longer play-time, thus experiencing lower anxiety prior to a competition. Dissimilarly, they also found that 30 min or more playing time led to a significant decrease ($p < 0.05$) in both state and trait anxiety levels, which might be due to years of playing experience (Guillén & Sanchez, 2009:416). Furthermore, the elite players perceived their anxiety as more facilitative towards their performance, compared to the non-elite ones perceiving it as debilitative (Guillén & Sanchez, 2009:416).

Four studies reported a decrease in anxiety following a competition, and more specifically, a decrease ($p < 0.05$) in cognitive anxiety, which might be due to the negative linear relationship that exists between cognitive anxiety and performance (Martens *et al.*, 1990:171). Furthermore, Szabo *et al.* (2014) found that successful basketball players demonstrated lower anxiety ($ES = 1.2$) levels prior to a game compared to the less successful players. In addition, Guillén and Sanchez (2009) reported that the basketball players' experience level had an effect on their trait anxiety ($p < 0.05$), but not on their state anxiety ($p > 0.05$). This is in accordance with the results of Fernandes and associates (2013), who found no significant differences in both somatic and/or cognitive anxiety and self-confidence in individual team sports as well as in athletes with more playing experience.

Only a few studies evaluated the effect of an aerobic training session on mood states, with all reporting a decrease in anxiety after training. Studies implementing a training intervention reported an increase in anxiety following the training sessions after applying the STAI questionnaire. Guszkowska (2009) used it during an aerobic or resistance or combination training session, with post-exercise anxiety increases associated with pre-exercise values ($p < 0.00$). Millet *et al.* (2005), on the other hand, evaluated the anxiety levels of three elite triathletes over a 40-week season and reported an increase in anxiety at 59 days, whereas a decrease was noted at 23 days ($p < 0.00$). They concluded that the observed anxiety levels had a positive relationship with the experienced training load ($r = 0.32$, $p < 0.00$). According to Cox and colleagues (2004:173), a 30-min period of aerobic training is often needed to observe a significant drop in state anxiety. If, on the other hand, somatic anxiety is measured, it would be expected to be higher at 30 min post-training, emphasising the importance of the anxiolytic effect of a high-intensity training condition (Cox *et al.*, 2004:173). The researchers conveyed that an acute bout of aerobic exercise (whether training at 60 or 80% VO_{2max}) is sufficient to reduce post-exercise state anxiety (Cox *et al.*, 2004:173).

In a study done by Cox and colleagues (2004), the subjects were exposed to two training sessions, either a 33-min treadmill session at 60% VO_{2max} or a 33-min treadmill session at 80% VO_{2max}. Both training conditions led to a decrease in anxiety, with the lower-intensity (60% VO_{2max}) one demonstrating a decrease from immediately after to 90 min post-training. In conclusion, Bozkus *et al.* (2013:512) reported a negative correlation between anxiety and the experience of female soccer players ($r = -0.218$, $p = -0.01$) and stated that this was an important phenomenon, as performing at top level requires the control of anxiety in order to increase performance.

To conclude this section, the most commonly used anxiety questionnaires were the STAI or CSAI-2 questionnaires, the latter being implemented in competition and the STAI in training situations. The most dominant findings were a decrease in anxiety following a competition, and more specifically, after a winning- compared to an increase after a losing-outcome. Training demonstrated a positive outcome on anxiety, as seen in the majority or studies reporting decreased anxiety levels. Most of the studies only evaluated aerobic training sessions, thus future studies can implement anaerobic training sessions and draw comparisons. Other psychological states frequently measured are the mood states, as these are known to have a strong correlation with sport performance.

3.2.2. *Mood*

Physical activity, and consequently fitness, is widely known to increase an individual's sense of psychological well-being, especially mood states (Byrne & Byrne, 1993:65). In addition to this, a strong association exists between sport performance and mood (Beedie *et al.*, 2000:49; Lowther & Lane, 2002:57). Mood is generally regarded as a long-lasting affect state, which is associated with certain types of feelings that could affect the experience and behaviour of an individual (Scherer, 2005:705). Examples of various mood states include feelings of depression, lethargy, misery and happiness or resilience that may last over a course of a few hours to even a few days (Scherer, 2005:705). According to Lowther and Lane (2002:57), as well as Prapavessis (2000:34), inability to get into the appropriate mood state and control one's emotions (for example anxiety) before and during a competition is related to poor performance.

This was confirmed by Aizawa and colleagues' (2006:326), indicating that as the physical and mental stress of a competition rises, so do the total mood disturbances. According to the profile of mood states (POMS) (McNair *et al.*, 1971), athletes that demonstrate the ability to be more vigorous and less angry, depressed, anxious, fatigued or confused will have a higher chance of success compared to athletes exhibiting the opposite mood states (Prapavessis, 2000:34). Vigour is generally characterised by feelings of cognitive liveliness, energy and physical strength (Nelson & Cooper, 2005:74). If an athlete demonstrates a positive mood state (according to POMS), he will be characterised as having an iceberg profile owing to the one positive state (vigour) lying above the five negative states (anger, anxiety, depression, fatigue and confusion) (Prapavessis, 2000:34). In a review compiled by Prapavessis (2000:35), it was concluded that a large array of research exists regarding the differences between successful and less successful athletes, based on their mood states prior to competition. A meta-analysis study regarding athletic performance and the POMS assessed by Beedie and colleagues (2000:58), reported that a.) athletes as ranked by their achievements do not differ with regard to their mood responses and b.) an athlete's mood response prior to performance is a useful performance predictor. Nevertheless, Hassmén and Blomstrand (1995:305) concluded that pre-game mood scores did not make it possible to predict the outcome of the games observed in their study.

Regarding the ability to handle stressors, Markus and colleagues (2000:338) reported stress-prone participants to exhibit higher baseline scores of tension ($F_{(1,39)} = 42.105$, $p < 0.01$), depression ($F_{(1,39)} = 44.48$, $p < 0.01$), anger ($F_{(1,39)} = 11.956$, $p = 0.00$) and fatigue ($F_{(1,39)} = 15.286$, $p < 0.01$) accompanied by a lower score for vigour ($F_{(1,39)} = 6.723$, $p = 0.01$). In the review compiled by Beedie and colleagues (2000:60) it was found that mood as indicated by tension (p = -0.14), anger (p = -0.02), fatigue (p = -0.04), depression (p = 0.06), vigour (p = 0.22) and confusion (p = 0.11) was a poor predictor of level of achievement. In contrast, Lowther and Lane (2002:61) reported significant agreement ($p < 0.01$) between performance, mood and cohesion scores, claiming that cohesion should relate to mood and performance in the context of team sports. Furthermore, they found significant variations in anger ($p < 0.05$), depression ($p < 0.01$), vigour ($p < 0.05$) and confusion ($p < 0.01$), with no significant difference in pre-competition mood, although mood varied between games (Lowther & Lane, 2002:61). In contrast to these results, a much earlier study (using the 65-item POMS questionnaire) of nine female soccer players (age: 22.0 ±

1.2 y) over a season of 22 soccer matches reported that tension, vigour and confusion scores were higher before the first match of the season compared to any of the remaining 21 matches (Hassmén & Blomstrand, 1995:301–303). Furthermore, the tension and anger scores were lower ($p < 0.01$) after a win than a loss or tie, with the depression and anger scores increasing following a loss and vigour and confusion scores being higher ($p < 0.01$) after a win. Hassmén and Blomstrand (1995:306) concluded that the small differences noticed before the game might be due to the following reasons: a.) the players will still be considered successful compared to the general population as a whole; b.) the players might have helped each other to overcome any negative mood states as they are part of a team, and c.) the coach and players met and might have influenced each other to some extent.

A single bout of physical training (< 30 min) may have various psychological benefits, more specifically regarding mood states, such as a reduction in anxiety or depression (Cox *et* al., 2004; Guszkowska & Sionek, 2009:163; McDowell *et al*., 2016; Rocheleau *et al*., 2004). However, in a study that evaluated the effect of intense training over a week, it was reported that intense training may lead to the appearance of both physical and mental fatigue and that these two types of fatigue may be related (Umeda *et al.*, 2008:902). According to Umeda *et al.* (2008:902), intense training may lead to athletes experiencing a negative psychological state, which is evident in a higher fatigue and lower vigour and total mood score. This may be due to the accumulation of chronic mental and physical fatigue, negative synchronisation of the two types of fatigue (physical and mental), a shift in the timing and appearance of each type of fatigue or the relationship between the two types of fatigue (Umeda *et al.*, 2008:902). This interaction between psychological and physical fatigue was seen in various blood markers analysed and compared to the POMS subscales. They reported that a strong muscle damage anti-inflammatory reaction and energy consumption may lead to mental fatigue (Umeda *et al.*, 2008:902).

The ability of individuals to control their mood involves different neurotransmitter systems such as gamma-aminobutyric acid, acetylcholine, glutamate, dopamine, serotonin, noradrenaline and neuropeptides (Steiner *et al.*, 2003:68). Furthermore, a direct modulatory effect on the binding of the gonadal hormones in the central nervous system takes place as a result of the neurotransmitters being activated (Steiner *et al.*, 2003:68), thereby altering mood states (see figure 4 – Schematic representation of how fatigue in various bodily systems can alter performance). Chase and Hutchinson (2015:13) reported that an individual's fitness level does not affect mood state changes due to the exercise. This was seen in the tension subscale improving in both the fit and unfit groups. A distinction should be made between the various forms of training, as aerobic exercises demonstrated lower confusion scores compared to resistance exercises, which might be explained by the fact that aerobic exercises are easier and less detailed to carry out (Chase & Hutchinson, 2015:13).

Literature studies on various mood questionnaires on female athletes are listed in table 3.

Table 3: Research studies on the effect of training or competition on various mood state questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Questionnaires	Intervention		Results
Aizawa *et al.* (2006) Changes in pituitary, adrenal and gonadal hormones during completion among female soccer players.	9 elite soccer players (20.0 ± 0.4 y) Japan	- POMS Japanese version	Questionnaires were completed: At 18:00 p.m. before the 3-day tournament (six games), during the 2nd game and 3 days after the tournament.	Fatigue: Tension, depression, anger, vigour, confusion:	↑ From pre-match to during the game.* All of the remaining subscales demonstrated an increase from pre-match to during the match ($p > 0.05$) and a decrease from during the match to post-match ($p > 0.05$).
Bateup *et al.* (2002) Testosterone, cortisol and women's competition.	17 rugby players (18 – 22 y) New York, USA	- Aggressive competitiveness - Bonding - Potential challenge - Athlete's skill - Pre-game mental state - Post-game perception	Questionnaires were completed: 24 h before, 15 min before and immediately after five rugby games.	Testosterone: Cortisol:	↑ Pre-game = correlated with being focused ($r = 0.30$)*, unrelated with perception of opposing team. ↑ Pre-game = Significant relationship with bonding ($r = 0.38$)*, but not with aggressiveness. ↑ During-game = Unrelated to winning/losing. ↑ During-game = Associated with greater challenge of the game ($F_{(49)} = 4.378$).*
Brandt *et al.* (2016) Association between mood states and performance of Brazilian elite sailors: winners *vs.* non-winners.	35 sailors (23.0 ± 7.6 y) Brazil	Brunel Mood Scale (BRUMS)	Questionnaires were completed: 30 min prior to a sailing competition, over four competitive occasions.	Overall placing in competition: 2007 Specific competition: Winners: Outcome of competition:	Weak relationship with depression ($r = 0.26$)*, vigour ($r = -0.25$)* and fatigue ($r = 0.28$).* Significant relationship between placing in competition and fatigue ($r = -0.59$)** and tension ($r = -0.90$).** ↓ Depression and fatigue and ↑ vigour *vs.* non-winners.* Significant relationship with ↓ depression ($OR = 0.7$), fatigue ($OR = 0.8$) and ↑ vigour ($OR = 1.3$) = ↑ chances for winning.

BRUMS = Brunel Mood Scale; POMS = Profile of Mood States; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 3 (cont.): Research studies on the effect of training or competition on various mood state questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Questionnaires	Intervention	Results	
Chase & Hutchinson (2015) The effects of acute aerobic exercise versus resistance exercise on mood state.	11 recreationally active subjects (22.3 ± 3.4 y) Miami, USA	- POMS - Short form. - RPE	Questionnaires were completed: After a warm-up, before and after the resistance exercise (three sets of 12 reps for the five major muscle groups at 28% of 1 RM) or aerobic exercise (brisk walking on an incline treadmill at 61% of HR_{max} for exact duration of the resistance session).	RPE: Resistance: Aerobic: Confusion: Vigour:	↔ Between conditions ($p = 0.59$). ↓ Tension ($ES = 1.1$)*, confusion ($ES = 2.3$)*, fatigue ($ES = 0.5$)*, depression ($ES = 0.73$)* from pre- to post-test. ↓ Tension ($ES = 0.8$)*, anger ($ES = 0.6$)*, fatigue ($ES = 0.5$)* and confusion ($ES = 0.6$)* from pre- to post-test. ↑ For resistance *vs.* aerobic exercise pre-values.** ↔ For resistance *vs.* aerobic exercise post-values ($p = 0.25$). ↑ For resistance *vs.* aerobic exercise pre- and post-values ($ES = 0.6$).*
Chennaoui *et al.* (2016) Stress biomarkers, mood states, and sleep during a major competition: "success" and "failure" athlete's profile of high-level swimmers.	3 elite swimmers (22.0 ± 4 y) France	POMS-f (French version)	Questionnaires were completed: Before and after each race over a series.	Failure group: Success group:	↑ Disturbance index ($Z = 2.02$).* ↑ Disturbance index *vs.* the "success" group ($Z = -2.33$).* ↑ Depression, fatigue and confusion and ↓ vigour after the series.** ↑ Anger and depression.* ↓ Vigour *vs.* failure group ($Z = -2.21$).*
Di Corrado *et al.* (2010) Mood states and salivary cortisol in a group of elite female water polo players: before and during the A1 National championship.	15 polo swimmers (22.9 ± 7.3 y) Italy	POMS	Questionnaires were completed: Three times per day for three consecutive days per month.	POMS: Experienced players: POMS & Cortisol:	↔ In the six subscales after the test period in whole group. ↑ In tension-anxiety, anger-hostility and fatigue-inertia.* Negative correlation between tension-anxiety and cortisol ($r = -0.67$).**

ES = Effect Size; POMS = Profile of Mood States; RM = Repetition Maximum; RPE = Rate of Perceived Exertion; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 3 (cont.): Research studies on the effect of training or competition on various mood state questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Questionnaires	Intervention	Results	
Edwards *et al.* (2006) Intercollegiate soccer: Saliva cortisol and testosterone are elevated during competition, and testosterone is related to status and social connectedness with teammates.	18 soccer players (18 – 22 y) Atlanta, USA	- Player rating scale - Individual attraction to the group-social scale of the group environment questionnaire	Questionnaires were completed: Prior to warm-up, 1 h before the game and 15 min after the game.	Overall teammate ratings: Social connectedness:	Pre-game correlated with testosterone ($r = 0.59$).* ↔ with cortisol. Pre-game correlated with testosterone ($r = 0.51$).* Correlated significantly with player rating scale ($r = 0.58$).* No significant correlation with cortisol.
Guszkowska & Sionek (2009) Changes in mood states and selected personality traits in women participating in a 12-week exercise program.	39 subjects (27.5 ± 5.6 y) Poland	- UWIST mood adjective checklist - General self-efficacy scale - Life orientation test	Questionnaires were completed: In the first and in the last week (3 months later) following regular exercising for 50 min twice a week.	Tension: Vigour: Trait anxiety: Optimism:	Positive correlation with trait anxiety ($r = 0.55$).** ↑ From pre- to post-test ($ES = -1.1$).* Negative correlation with vigour ($r = -0.49$).** ↑ From pre- to post-test ($ES = -0.1$)*. Relationship with vigour ($r = 0.43$)** and tension ($r = -0.30$).*
Hassmén & Blomstrand (1995) Mood state relationships and soccer team performance.	10 soccer players (22.0 ± 1.2 y) Sweden	- POMS: Tension [T] Depression [D] Anger [A] Vigour [V] Fatigue [F] Confusion [C]	Questionnaires were completed: Immediately before the warm-up and immediately and 2 h after each match over a season.	Match won: Match lost: Tension: 1st Match:	↓ Tension, anger, confusion, ↔ Fatigue ↑ Tension at 0 and 2 h, ↑ anger, ↔ Fatigue Comparable after a loss or tie ($F_{(4, 32)} = 6.1$).** Significant difference in pre-match scores after a tie compared to a win or lose.* Change over time ($F_{(2,16)}$ = 12.0[T]; 30.1[D]; 22.4[A]; 39.9[V])** and an ↑ over time ($F_{(2, 16)}$ = 35.6[F]; 12.7[C])** over time. Interaction between result and time ($F_{(4,32)}$ = 6.1[T]; 17.5 [D]; 8.7[A]; 6.1 [V]).** ↑ Tension, vigour and confusion *vs.* other 21 matches.

A = Anger; C = Confusion; D = Depression; *ES* = Effect Size; F = Fatigue; POMS = Profile of Mood States; T = Tension; V = Vigour;
↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 3 (cont.): Research studies on the effect of training or competition on various mood state questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Questionnaires	Intervention	Results	
Jones & Sheffield (2007) The impact of game outcome on the well-being of athletes.	8 soccer and hockey players (21.5 ± 3.7 y) United Kingdom	- Symptom checklist - General health questionnaire - POMS – Short form	Questionnaires were completed: 4 – 6 and 10 days after a won/lost. Experimental days: how they felt over the past few days. Control days: how they felt over the last 5 days.	Perception of game: Mood: [D]; [A]; [T]: Vigour:	↑ After a won *vs.* lost.** ↔ Ratings of importance of success in general either after won/lost. Differences after wins and losses ($F_{(12, 376)} = 2.91$).** Small effect of condition [$F_{(2,197)} = 3.98$(D); 4.25(A); 5.36(T)].* ↓ After a won *vs.* a lost.* Large effect of condition ($F_{(2,197)} = 12.14$).** ↓ After a loss *vs.* a win.* ↔ Effect of condition for tension, fatigue & confusion.
Keikha *et al.* (2015) The relationship between pre-competition state anxiety components and mood state sub-scales scores and the result of among college athletes through temporal patterning.	120 athletes from various sport codes (18 – 26 ± 0.6 y) Malaysia	- POMS-Adolescent	Questionnaires were completed: One week (T1), 1 day (T2) and 1vh (T3) before competition.	T1: T2: T2: Losers: T3: Outcome:	Similar mood means between winners and losers. ↑ Vigour, ↓ negative sub-scale. Significant different with T1 and T2.* ↓ Anger, depression, fatigue and confusion, ↑ tension and vigour *vs.* winners. ↔ Significant difference with T1. Influenced by tension, fatigue and vigour at T2.
Kivlighan *et al.* (2005) Gender differences in testosterone and cortisol response to competition.	23 amateur and novice rowers (17 – 31 y) USA	- Behaviour and attitudinal questionnaire (assess dominance, competitiveness and team bonding).	Questionnaires were completed: Before warm-up, 20- and 40 min post-competition.	Testosterone: Cortisol:	↑ At baseline and pre-competition = poorer association with event ranks ($r = 0.49–0.46$).* Novice: ↑ at 20 min post-competition = associated with poorer performance ($r = 0.52$).* ↑ At pre- and 20 min post-competition = associated with interest in bonding and affiliation with teammates ($r = 0.53–0.55$).**

A = Anger; D = Depression; POMS = Profile of Mood States; T = Tension; T1–3 = Time point; V = Vigour; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 3 (cont.): Research studies on the effect of training or competition on various mood state questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Questionnaires	Intervention	Results	
McDowell *et al.* (2016) Sex-related differences in mood responses to acute aerobic exercises.	26 subjects (20.6 ± 1.5 y) Ireland	- Penn State Worry Questionnaire -Brief POMS	Questionnaires were completed: Immediately before and 10 min after either 30 min passive rest or 30 min vigorous treadmill running	Vigour: Aerobic Training:	↑ Post-exercise *vs*. post-control.** ↓ Depression ($F_{(1,86)} = 5.05$)**, fatigue ($F_{(1,86)} = 15.39$)**, confusion ($F_{(1,86)} = 15.39$)** and mood disturbances ($F_{(1,86)} = 36.91$)** *vs*. control condition. Non-significant improvements in worry ($p > 0.07$), tension ($p > 0.19$) and anger ($p > 0.05$) *vs*. control condition.
Oliveira *et al.* (2009) Testosterone responsiveness to winning and losing experiences in female soccer players.	33 soccer players (24.2 ± 4.8 y) Portugal	POMS	Questionnaires were completed: 30 min before warm-up as well as before and after the match on the game-day.	Losers: Winners: Hormones *vs*. psychological:	↓ In mood.** ↑ Mood and satisfaction with outcome. Mood correlated with T ($r = 0.48$)* but not with C. Mood change over the match had a negative correlation with T levels after the match ($r = -0.57$).**
Rocheleau *et al.* (2004) Moderators of the relationship between exercise and mood changes: gender, exertion level, and workout duration.	64 recreationally active subjects (23.0 ± 6.2 y) USA	- POMS - RPE	Questionnaires were completed: Before and immediately after either aerobic or resistance training.	RPE: Mood:	Correlated positively with workout duration ($r = 0.24$).** No correlation with post-exercise negative mood states ($p > 0.2$). Improved after either aerobic or resistance training.* Improved with a ↑ in training duration.*

POMS = Profile of Mood States; RPE = Rate of Perceived Exertion; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

Table 3 (cont.): Research studies on the effect of training or competition on various mood state questionnaires of female athletes.

Authors, Publication Date and Article Title	Subjects (Number, Sport, Age and Country)	Questionnaires	Intervention	Results
Terry & Munro (2008) Mood and anxiety scores predict winning and losing performances in tennis.	43 tennis players (39.7 ± 9.8 y) Australia	BRUMS	Questionnaires were completed: Approximately 10 min prior to each competition.	Mood: Predicted 56.8% correct outcome classifications ($\lambda = .971$, $x^2 = 16.8$).** Win: Higher correct classifications (74.5%) *vs.* losing games (38.3%), specifically with lower anger, confusion, depression and tension. Performance outcome: Anger, confusion, depression and tension = Significant predictors. Tension: Prior to wins = 47th percentile; prior to losing = 67th percentile. Confusion: Prior to wins = 52nd percentile; prior to losing = 70th percentile.
Umeda *et al.* (2008) Effects of intense exercise on the physiological and mental condition of female university judoists during a training camp.	13 judoists (19.1 ± 1.0 y) Japan	POMS	Questionnaires were completed and a blood sample was taken: Before and after the training camp (Camp consisted of 6.5 h of training per day).	Fatigue: ↑ After the camp *vs.* baseline.* Negatively associated with creatine kinase.* Vigour: ↓ After the camp *vs.* baseline.* Positively associated with changes in creatine kinase* and lactate dehydrogenase.* Confusion & depression: Negatively associated with changes in lactate dehydrogenase.*
Zandi & Rad (2013) A comparison of the mood state profiles of winning and losing female athletes.	69 athletes from a range of sport codes. (20 – 30 y) Country: N/R	POMS	Questionnaires were completed: Before and following their respective competitions.	Mood states: Differences between win and lose.** Differences between win and lose before and after competition.* Winning athletes = consistent profile with Morgan's iceberg profile.

BRUMS = Brunel Mood Scale; N/R = Not Reported; POMS = Profile of Mood States; ↑ = Increased; ↓ = Decreased; ↔ = No changes; * = $p < 0.05$; ** = $p < 0.01$

3.2.2.1. **Summary of literature studies on the effect of training/competition on mood states in female athletes**

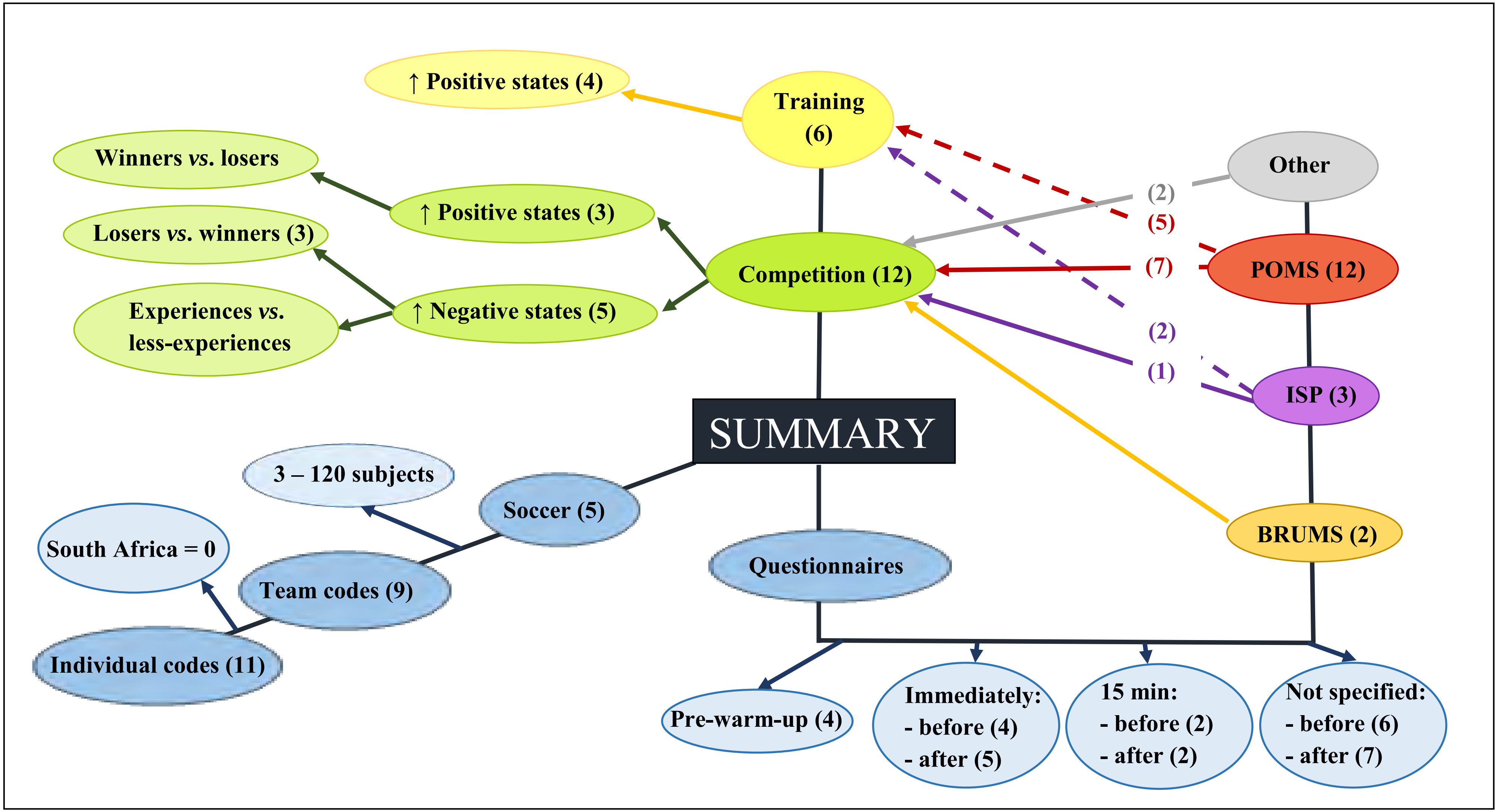

Figure 7. Summary of literature studies on the effect of training/competition on mood states in female athletes.

According to the above literature, five studies used female soccer players as test subjects. In total, nine studies used team sport participants and 11 studies used either individual sport or recreationally active participants. In these studies, the smallest sample included three elite swimmers (Chennaoui *et al.*, 2016) and the largest sample included 120 athletes from various individual and team sport codes (Keikha *et al.*, 2015).

The most popular mood questionnaire implemented was the POMS, implemented in 12 studies. The original POMS questionnaire is a 65-item measure of typical and persistent mood reactions to life situations and has proven to be very popular in the research domain of sport and exercise psychology (Leunes & Burger, 2000:6). However, because of the length and time constraints of the POMS questionnaire, other questionnaires have been developed to measure the same constructs. The Incredibly Short POMS (ISP) of Dean and colleagues (1990) is derived from the original POMS (McNair *et al.*, 1971), proving correlations between $r = 0.67–0.82$ (Dean *et al.*, 1990). The ISP consists of the same six subscales, with one question in each section, thus totalling six questions. Three studies implemented it, with two applying the ISP in a training regime and one during a competition, all reporting a decrease in negative mood states. The most common times for administering the questionnaires were immediately before and after the intervention, though quite a few studies did not specify the time period.

Most studies reported an increase in various negative mood scales following a competition. Chennaoui and associates (2016) evaluated a major swimming competition comparing winning and losing swimmers. They concluded that the defeated group experienced increases in depression, fatigue and confusion and a decrease in vigour, whereas the winning group only showed increases in anger and depression and lower vigour ($p < 0.05$). They conveyed that these results might be due to the high competition load affecting their psychological states (Chennaoui *et al.*, 2016:6). Additionally, the results from Di Corrado and associates' study (2010) demonstrated increases in tension-anxiety, anger-hostility and fatigue-inertia ($p < 0.05$) in experienced players. Regarding the competition outcome, a losing outcome led to a larger increase in tension and vigour levels, whereas another study reported a decrease in vigour levels with no significant difference in fatigue experienced.

In contrast to this, a decrease in tension, anger, confusion, depression and an increase in vigour were reported following a winning outcome in a range of sport codes. This suggests that the positive effects of a winning competition might be larger than the negative effects of a losing competition or that athletes might engage in certain behaviours after either winning or losing a competition (Jones & Sheffield. 2007:62). All of the studies mentioned previously made use of the POMS questionnaire, highlighting the popularity of this questionnaire.

An aerobic exercise session led to a significant decrease in tension ($ES = 0.78$), anger ($ES = 0.59$), fatigue ($ES = 0.53$) and confusion ($ES = 0.57$), as well as an increase in vigour ($ES = 0.62$) and optimism

(*ES* = -0.96) in recreationally active females (Chase & Hutchinson, 2015; Guszkowska & Sionek, 2009; McDowell *et al.*, 2016). The total mood score ($p < 0.05$) also improved as a result of aerobic training and continued to improve as the duration of the exercise increased (Rocheleau *et al.*, 2004). The authors stated that these improvements might be due to the body releasing a natural mood-improving hormone, namely endorphins, or a linear relationship between mood and exertion (Rocheleau *et al.*, 2004:493)

With regard to performance prediction, a significant positive relationship ($p < 0.05$) was seen between a win and a decrease in depression ($r = 0.7$) and fatigue ($r = 0.8$) and an increase in vigour ($r = 1.3$) in female sailors (Brandt *et al.*, 2016). In addition to this, a study on female tennis players reported that mood scores can predict up to 56.8% of a performance outcome, especially a winning outcome (Terry & Munro, 2008:295). In this regard, the subscales anger, confusion, tension and depression are significant predictors of performance outcome, with winning outcomes associated with lower levels of these moods (Terry & Munro, 2008:295).

To summarize, the POMS questionnaire developed by McNair *et al.* (1971) is deemed the most popular mood questionnaire used in the sporting world. During a competition or training, the most common result seen is an increase in the subscale fatigue and a decrease in vigour. With regard to the outcome, “winners” are often characterised by an increase in vigour and excitement, whereas “losers” are characterised by an increase in tension, anger and depression. Both groups demonstrate an increase in fatigue regardless of the outcome.

A constraint that might occur in a sporting context is that though the subscale primarily measures mental fatigue, the participants can evaluate it according to their physically fatigued state. A limitation in the current literature is the absence studies focusing on anaerobic training (such as sprinting or interval training) and the effect thereof on the psychological mood states of females. Most studies primarily focus on the effect of aerobic training or resistance training. In addition, literature can be more thorough in the training descriptions for possible replication thereof. Based on all these findings, a query that arises is whether a relationship exists between hormonal and psychological states and whether these can influence each other.

4. PROBABILITY OF LINK BETWEEN HORMONAL AND PSYCHOLOGICAL ASPECTS

A question that arises when a decline is observed in performance is whether it is due to physiological or psychological aspects (Purvis *et al.*, 2010:444). Is the underperformance due to either psychological (*e.g.* an increase in anxiety) or physical stressors (*e.g.* prolonged fatigue) leading to the activation of the HPA axis, which stimulates cortisol secretion (Dickerson & Kemeny, 2004:355; Raglin & Morris, 1994:47; Varzaneh *et al.*, 2011:19). Concerning psychological aspects, the influence of hormonal changes on human behaviour is difficult to ascertain, as scientists have to manipulate hormone levels to examine the effects thereof on behaviour (Christiansen, 2001:39). Many researchers believe that people’s physiological processes influence the quality of their emotions experienced and that they may be able to alter these emotions (McCraty *et al.*, 2009:14). A question that needs to be addressed when evaluating the link between the psychological and physiological state is the formation of emotions, as emotions are related to both the mind and body (McCraty & Rees, 2009:2) and are known to alter the activity of the body's physiological system (McCraty *et al.*, 2009:15).

4.1. Emotions

The term "emotion" is derived from the Latin term "movere" or "to move", simply implying "energy in motion" (Edwards *et al.*, 2015:998). Currently, the term emotion is seen as the experience of energy moving through the body to generate mental (feelings) and physiological (autonomic nerve system) responses (Edwards *et al.*, 2015:998). On the one continuum, feelings refer to conscious sensations and experiences, whereas on the other, physiological reactions, thoughts and feelings give meaning to emotion (Edwards *et al.*, 2015:998). A relationship exists between various organismic subsystems and emotions (Scherer, 2005:698). These subsystems include the central nervous system (cognitive, neurophysiological, motivational and subjective feeling component), neuro-endocrine system (neurophysiological component), autonomic nervous system (neurophysiological component) and the somatic nervous system (motor expression component) (Scherer, 2005:698). A psychological stressor can affect the physiology by activating the frontal lobes and thalamus, which in turn can lead to the generation of an emotional response (Dickerson & Kemenye, 2004:356). An individual can interpret this response by producing either positive or negative emotions. A technique to organise these positive of negative emotions experienced is drawing an emotion generation model (Gross, 1999:559).

According to this model, certain situations trigger an experimental, behavioural and physiological emotional response, though these responses may be modified to give the emotional response a final form (Gross, 1999:559). This model has five key aspects, namely situation selection, situation modification, attentional deployment, cognitive change and response modulation (Gross, 1999:559, 560). Of importance for this review are the cognitive change and response modulation aspects. The cognitive change aspect refers to an individual altering the emotional significance of a situation by changing the manner of thinking regarding the aspect (Gross, 1999:560). The response modulation aspect is where individuals change their emotional response tendencies as soon as these appear (Gross, 1999:560). Two sub-aspects are emotion-expressive behaviour and physiological response, for example slowing the breathing rate (Gross, 1999:560). Two processes widely known for modifying one's emotion generation as well as attentional control are the bottom-up or top-down processes (Corbetta & Shulman 2002; Ochsner *et al.*, 2009:1327).

4.1.1. *Bottom-up, top-down approach*

As quoted from Ochsner *et al.* (2009:1322), "*Do they (emotions) arise via low-level processes that provide quick, bottom-up affective analyses of stimuli? Or do they (emotions) arise via high-level, top-down cognitive appraisal processes that draw upon stored knowledge?*"

One way to explain the top-down approach, defined previously as the flow of information from higher to lower centres (Corbetta & Shulman, 2002:201), is to instruct a player to think about the outcomes, actions and persons in a given situation (*e.g.* Ochsner *et al.*, 2009:1323). This will initiate the communication pathways from the prefrontal lobe towards the body (thus mind to body, or top-down) (Edwards *et al.*, 2015:998). The brain regions involved during the top-down phenomenon include the left ventral and dorsal lateral prefrontal cortex, bilateral temporal cortex, putamen, bilateral dorsal medial prefrontal and anterior cingulate cortex (Ochsner *et al.*, 2009:1326). In contrast, during the bottom-up phenomenon, the bilateral amygdala, right parietal cortex, occipitotemporal cortex and the lateral prefrontal cortex are involved (Ochsner *et al.*, 2009:1325,1326). During the bottom-up approach, previously defined as information processing from a sensory input towards motor output (Corbetta & Shulman, 2002:201), a player can be instructed to respond naturally to a situation (*e.g.* Ochsner *et al.*, 2009:1323), which will initiate the communication path via the body's reticular, limbic and medullary systems (thus body to mind, or bottom-up) (Edwards *et al.*, 2015:998). This is clearly seen in negative emotions (*e.g.* anger, anxiety, frustration, worry) leading to erratic and varying heart rates, which desynchronise the autonomic nervous system (ANS) and if they persist, tax the nervous system and internal organs and ultimately impede the efficacy of the psychophysiological systems (McCraty *et al.*, 2009:21). On the other hand, positive emotions (*e.g.* vigour, excitement, contentment, love) lead to coherent heart rates and if sustained, can lead to increased synchronisation between bodily systems and the efficacy of the psychophysiological systems (McCraty *et al.*, 2009:22). Therefore, it is important to implement the top-down strategy effectively to reach a coherent state, as a relationship exists between emotional valence and coherence (McCraty *et al.*, 2009:29). Coherence, psychologically, refers to an emotionally balanced and calm yet responsive state to conduct optimal problem-solving, decision-making, attention-focusing, coordination and discrimination and to optimise task performance (McCraty *et al.*, 2009:28). Physiologically, the state of coherence has benefits, including a.) resetting the baroreceptor sensitivity (thus improving respiratory efficiency and short-term blood pressure); b.) increased cardiac output and efficiency in fluid exchange, filtration and absorption between tissues and capillaries; c.) increased vagal afferent traffic (inhibiting the pain signals and sympathetic output); d.) increased temporal synchronisation of the body cells, and e.) improved ability of the cardiovascular system to adapt to circulatory necessities (McCraty *et al.*, 2009:27).

Self-regulation of emotional experiences is a way to maintain positive emotions and thus the state of coherence (McCraty *et al.*, 2009:26). An individual can manipulate the input to the emotion-generative system by implementing antecedent-focused strategies, such as cognitive reappraisal (Chiesa *et al.*, 2013:84). Furthermore, to manipulate the output of the system an individual can implement response-focused strategies such as expressive suppression (Chiesa *et al.*, 2013:84). If an individual can sustain a positive emotion, an increase in the parasympathetic nervous system can take place in response to increased synchronisation in the higher-level brain systems and the ANS (McCraty & Rees, 2009:3). The brain has the ability to become familiar with the rhythmic inputs of various bodily processes; these include the respiratory, muscular tension, facial expression, heart, digestive and hormonal rhythms (McCraty & Rees, 2009:6). The depletion-to-renewal grid is a schematic presentation of the connection between the body and mind and how they affect each other. This is important, as behaviour can alter an individual's reproductive hormone levels and the reproductive hormones can directly affect behaviour (Christiansen, 2001:39).

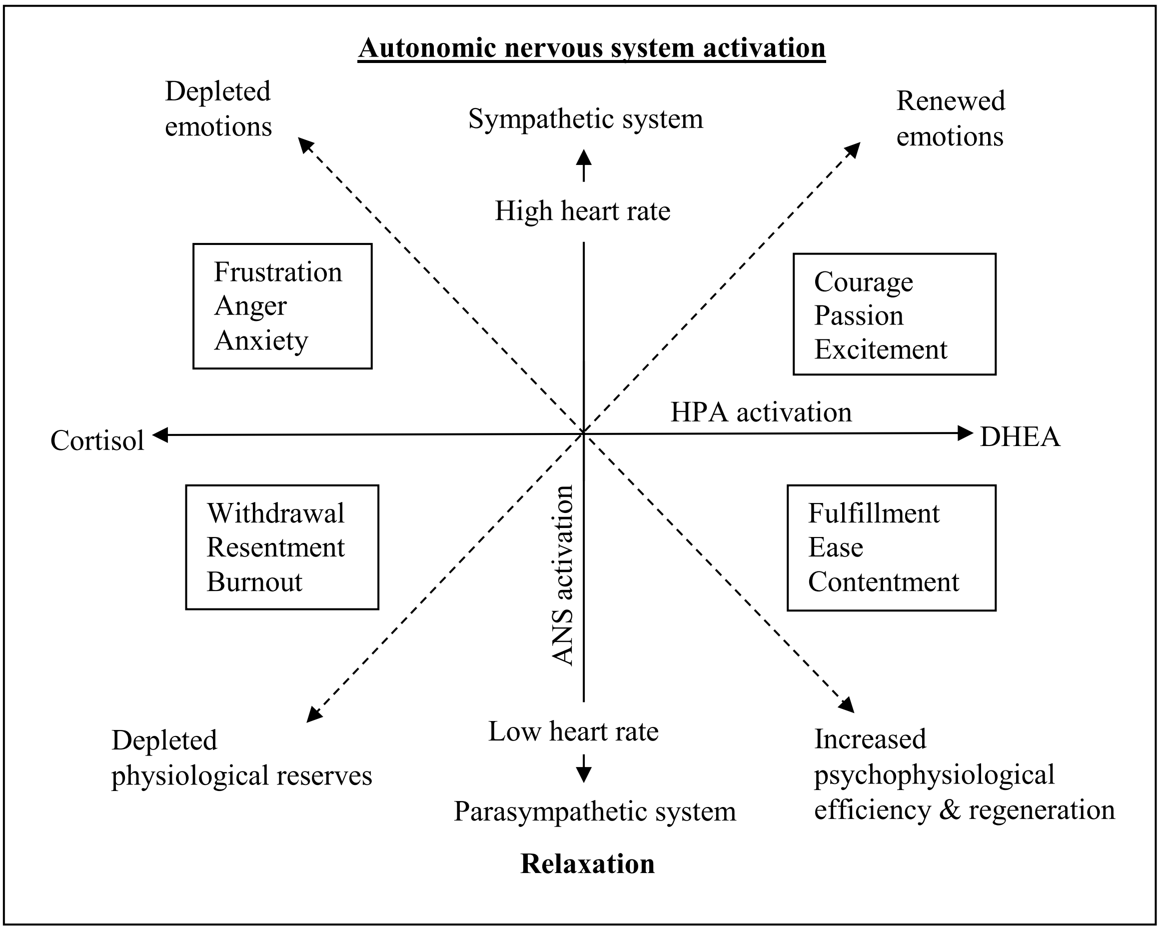

Figure 8: The depletion-to-renewal grid (Adapted from McCraty *et al.*, 2009:29).

According to this grid, sympathetic activation can result in an increase in heart rate, whereas parasympathetic relaxation results from low heart rates (Edwards *et al.*, 2015:999). When an individual experiences depleted negative emotions, a rise in cortisol can occur, while renewing positive emotions is associated with an increase in DHEA (Edwards *et al.*, 2015:999). From this figure, in conjunction with the bottom-up and top-down theories, it is clear that that an individual's physiological state can influence the person's psychological state and *vice versa.*

4.2. Hormones and behaviour

According to Oliveira and colleagues (2009:1056), ample proof exists on the mutual relationship between behaviour and androgens in the competitive sport domain, but nonetheless, it is still regarded as a complex relationship (Christiansen, 2001:39). According to Oliveira and associates (2009:1057), androgen responsiveness is regulated by an individual's perception of the competition outcome. In this regard, the psychological effect of competition, physical training or a combination of the two may result in competition-related increases in salivary cortisol and testosterone (Edwards & Casto, 2013:158). Evidence exists of the dynamic relationship between testosterone, cortisol, competition and sometimes victory and defeat (Edwards *et al.*, 2006:135).

A parallel increase in cortisol and testosterone takes place prior to a competition (Edwards & Casto, 2013:159; Edwards & Casto, 2015:49), with results showing similar rises for men and women, although women may experience an additional rise in both hormones during a match (Bateup *et al.*, 2002:187). This may be due to women adopting a different response pattern when faced with a challenge or stressors due to inherent parental investments (Bateup *et al.*, 2002:184).

Furthermore, the cortisol and testosterone reactions may be related through the players' shared connection to the psychological experience of the match (Edwards & Casto, 2015:49). During a swimming tournament, results showed a positive correlation ($r = 0.9$, $p < 0.05$) between cortisol and vigour at baseline as well as with feelings of fatigue ($r = 0.9$) (Chennaoui *et al.*, 2016:5). This is similar to the result reported by Haneishi *et al.* (2007:585), conveying a positive correlation ($r = 0.70$) between cortisol and cognitive anxiety for the starters, demonstrating that status (start *vs.* non-start) influences the acute cortisol response. In addition, they observed an increase in cortisol levels in both starters and non-starters from pre-game to post-game, with the starters demonstrating the highest cortisol values ($p < 0.05$). Both the starters and non-starters measured higher levels of cortisol, as well as higher levels of somatic and cognitive anxiety after a game compared to a training session (Haneishi *et al.*, 2007:585). Bateup *et al.* (2002:188) stated that two factors were associated with cortisol increase: the extent to which the opposing team was more challenging than expected, and, whether the team won or lost. However, the rise in testosterone observed in their study was unrelated to winning or losing the game (Bateup *et al.*, 2002:188).

Since cortisol is associated with stress, it is of the utmost importance to determine the extent to which cortisol can regulate testosterone reactivity, as stress can manifest itself in many forms (Edwards & Casto, 2015:48). This leads to the assumption that the stressor or challenge sportspeople are faced with will stimulate cortisol release, and in turn testosterone production (Bateup *et al.*, 2002:187). Furthermore, if an increase occurs in players' baseline cortisol levels, leading to a lower testosterone response to stress, an undesirable impact on the testosterone-driven achievement motivation can take place during a stressful or challenging perceived situation (Edwards & Casto, 2015:48). When interpreting the linkage between hormones and the psychological state of females, various additional factors should be taken into consideration, which might affect either or both variables.

4.2.1. *Additional factors to be considered*

4.2.1.1. **Sleep**

As training and the intensity thereof increases, so does an individual's deep slow sleep wave, with a reduction in sleep latencies and fragmentation index (Chennaoui *et al.*, 2016:2). Chennaoui and colleagues (2016:6) reported a negative correlation (r = -0.97) between sleep duration and depression (as measured by POMS) for a group of swimmers during a tournament. In addition to this, they reported that the "failure" group (obtaining the last four places) demonstrated a negative correlation between sleep duration and confusion, as well as cortisol (r = -0.10) (Chennaoui *et al.*, 2016:6). Lastella and colleagues (2014:S126) reported similar findings, with a direct correlation observed between sleep quality and sleep duration (r = 0.4, p < 0.01), fatigue (r = 0.3, p < 0.01), tension (r = -0.21, p < 0.05) and vigour (r = 0.24, p < 0.05). The participants stated that anxiety (21% of the participants), noise (15%), bathroom usage (14%), personal issues (7%) and dreams (5%) were the predominant reasons for night-awakenings. In a study evaluating the effect of nightmares (terrifying and vivid dreams leading to abrupt awakenings) in females, a higher level of stress, depression, anxiousness, sleep problems and somatic symptoms were reported, as well as a blunted or smaller cortisol awakening response (CAR) (Nagy *et al.*, 2015:234). According to the authors, the observation noted in the CAR might be due to the following reasons (Nagy *et al.*, 2015:236):

a.) Nightmare sufferers having a weak physical adaption to anticipated stressors;
b.) A trait-like vulnerability on the HPA axis takes place;
c.) A dysfunctional autonomic regulation of sleep, especially during the rapid eye movement phase, which might be reflected in the HPA response.

A normal sleep cycle usually leads to cortisol increases which is significantly higher than awake levels. In addition, cortisol usually demonstrate similar values during the early part of night compared to being awake, having a significant higher secretion during the latter part of night (Redwine *et al*., 2000:3599). In contrast to this, sleep deprivation can lead to cortisol being higher during both the early and late part of night compared to being awake (Redwine *et al*., 2000:3599). In another study comparing sleep debt (4 h) with sleep recovery (12 h), it was noted that the sleep debt group had short quiescent periods due to the large delay in onset and a raised period in the afternoon and early evening (Spiegel *et al*., 1999:1438). This was reflected in a decrease efficacy of the negative feedback regulation of the HPA axis, showing a 6x slower rate for decreasing cortisol from late afternoon to evening (Spiegel *et al*., 1999:1438). The authors stated that the decrease in sleep was associated with an increase in the degree of sleepiness, thereby increasing the sympathetic activity and afternoon cortisol levels compared to normal sleep cycles.

4.2.1.2. **The cortisol awaking response (CAR)**

The HPA axis has a diurnal rhythm, which is characterised by episodes of high amplitude and short duration (Fries *et al.*, 2009:67). During a normal sleep cycle, cortisol levels increase to their highest level during the second half of the night, peaking in the early morning hours (Fries *et al.*, 2009:67). Fries and colleagues (2009:71) hypothesised that the CAR may activate forthcoming memory representations at awakening, which assists with the orientation about the self in space and time, as well as the anticipation of the demands of the upcoming day. This in turn may cause a fluctuation in the extent of cortisol production from day to day (Fries *et al.*, 2009:71).

According to Kirschbaum and Hellhammer (2000:380), a 50–100% increase in cortisol levels takes place upon wakening in the morning, with the peak levels observed approximately 20–45 min after wakening (Chida & Steptoe, 2008:266). This increase ($p < 0.01$) seen at 30 min after awakening was confirmed by a study of 179 healthy male and female participants (age: 29.6 ± 1.1 yrs) (Kudielka & Kirschbaum, 2003:36). In this study, the participants were further divided into early and late wake-up groups. The researchers found a significant difference ($F_{(2,5,143,9)} = 6.16$, $p = 0.001$) in cortisol levels between the groups, with the early wake-up group demonstrating a significant increase ($p < 0.05$) at 45 and 60 min after waking up compared to the late wake-up group (Kudielka & Kirschbaum, 2003:39,41). The authors claimed that the late wake-up group's lower cortisol levels may contribute to the higher awakening response, which is related to the circadian rhythm (Kudielka & Kirschbaum, 2003:43). According to Chida and Steptoe (2009:272), a negative relationship exists between this increase in CAR and fatigue, burnout or exhaustion ($r = -0.065$, $p = 0.089$). Furthermore, their analysis of the stress a person is subjected to showed a negative association between the increased CAR with depression and hopelessness (p = 0.044). Various factors can influence the CAR, among others an individual's perception of stress, health conditions, sleep (waking times, duration and quality), menstrual cycle phase and OC usage (Fries *et al.*, 2009:69).

4.2.1.3. **Menarche**

Four reproductive hormones (oestrogen, follicle-stimulating hormone (FSH), luteinising hormone (LH) and progesterone) undergo cyclical changes during a normal ovulatory menstrual cycle (Baker & Driver, 2007:613). The production of oestrogens is regulated by the hypothalamic hormones FSH and LH, which in turn are regulated by the gonadotropin-releasing hormone from the hypothalamus (Baker & Driver, 2007:613). During the first day of a normal menstrual cycle, all four reproductive hormones show low levels; from there on, LSH and oestrogens rise and at 16 h before ovulation, LH peaks (Baker & Driver, 2007:613). At the time of ovulation, plasma testosterone and plasma oestradiol levels peak (Christiansen, 2001:41). Following the ovulation phase the hormones decrease and the menstrual cycle takes place, which may be accompanied by women experiencing negative physical and/or psychological symptoms (Baker & Driver, 2007:614). Regarding cortisol secretion during this period, secretion is at its lowest during the follicular phase (until day 4), after which it increases to its peak from day 2 to 0 (menstrual phase), followed by a lower constant secretion during the luteal phase (though higher than follicular phase) (Genazzani *et al*., 1975). Studies show that up to 97% of females experience some form of negative symptoms, with as many as 35% reporting symptoms so severe that these disrupt their social, work and family life (Aganoff & Boyle, 1994:183).

Symptoms include among others mood swings, stress, nervousness, fatigue, oedema, abdominal bloating, water retention, breast tenderness (Derman *et al.*, 2004:203), anger, disgust and sadness (Aganoff & Boyle, 1994:188). Changes in mood, affect and behaviour are related to the cyclic hormonal changes (Steiner *et al.*, 2003:70) and may be a result of the neuroendocrine system fluctuating the gonadal hormones in response to the activation of neurotransmitters (Steiner *et al.*, 2003:68). Furthermore, a high level of androgens can be associated with anovulatory menstrual cycles (Christiansen, 2001:40). In a study evaluating the effect of training during the menstrual cycle phase, a significant difference ($p < 0.01$) was noted between the training and non-training groups regarding their mood states (Aganoff & Boyle, 1994:188). The researchers further stated that females engaging in regular training demonstrated significantly lower levels ($p < 0.01$) of negative mood states such as sadness, hostility, shyness, guilt, anger, shame, disgust and contempt, though no clear physiological explanation exists for this phenomenon (Aganoff & Boyle, 1994:190). The authors assumed that the reason might be that a.) training acts as a distraction from intrusive thoughts or; b.) training improves self-efficacy and the body image or c.) social contact via the group is beneficial (Aganoff & Boyle, 1994:190).

According to Baker and Driver (2007:616), the hormonal variations during a menstrual phase may influence the rapid eye movement (REM) sleep. In addition, a small decrease in REM sleep can take place during the luteal phase, together with an increase in spindle frequency activity (Baker & Driver, 2007:618). According to the authors, previous studies demonstrated that before and during menses there is a decrease in the quality of sleep, although the composition and timing of sleep remains stable across the menstrual cycle (Baker & Driver, 2007:618).

During the low-progesterone follicular phase, a stressful situation may lead to an increase in progesterone (that is a precursor for cortisol synthesis), which in turn may lead to a rise in circulating cortisol (Herrera *et al.*, 2016:102). In another study, a large difference ($ES = 0.8$, $p = 0.05$) was determined between females in the follicular *vs.* luteal menstrual cycle phases (Kudielka & Kirschbaum, 2003:39), though no differences ($p = 0.91$) were observed between the luteal phase compared to the follicular phase with regard to awakening time in the morning (Kudielka & Kirschbaum, 2003:39). During the course of the menstrual cycle, the gonadal steroid hormone production changes, which might have an impact on the cortisol awaking response (Fries *et al.*, 2009:69). However, according to Kudielka and Kirschbaum (2003:44), increased cortisol levels upon awakening cannot be influenced by the menstrual cycle phase.

4.2.1.4. **Contraceptive usage**

Females who make use of OC manipulate their normal menstrual cycles by preventing ovulation (Baker & Driver, 2007:618). Not only do these contraceptives suppress the endogenous reproductive hormones [low ovarian output of oestradiol and progesterone (Herrera *et al.*, 2016:96)], but they also alter an individual's sleep composition, which together with the circadian rhythm, may lead to mood disturbances (Baker & Driver, 2007:618). This in turn can have an influence on an individual's CAR, as previously discussed. In a study conducted on two testing days (14 days apart) on 55 females (age: 19.7 y), OC users demonstrated a significantly lower ($F_{(1,48)} = 5.34$, $p = 0.03$) testosterone level on the first testing day (Liening *et al.*, 2010:11). In addition, the authors reported no significant differences for cortisol or progesterone at either time point; however, the OC users had a higher stability coefficient ($r = 0.81$, $p < 0.01$) in cortisol values than non-OC-using females (Liening *et al.*, 2010:11). This is in accordance with Crewther and colleagues (2015:86) reporting similar cortisol levels for both OC and non-OC users. They did, however, report lower testosterone levels for the non-OC users irrespective of the activity (light or heavy training or club or national competition) performed (Crewther *et al.*, 2015:87). In contrast to this, an increase in cortisol was noticed in OC users compared to non-OC users over two soccer matches (Casto & Edwards, 2016b:22). The authors further reported a significantly lower ($p < 0\ .05$) testosterone level for OC users when a match was won, compared to a losing match demonstrating no significant differences (Casto & Edwards, 2016b:16). This emphasises the importance of recognising the effect of a competition outcome on the psycho-hormonal states of female athletes.

4.2.1.5. **Competition outcome**

As stated previously, the outcome of a match has the ability to affect both hormonal and psychological responses (Sedghroohi *et al.*, 2011:276). Casto and Edwards (2016b:16) reported a significant decrease ($p < 0.01$) in testosterone in females following a winning match compared to a match that was lost. Oliveira and colleagues reported similar results, with losing female players demonstrating a significant rise ($p < 0.01$) in testosterone before a soccer match. However, following the match the losers had a decrease whereas the winners had an increase in testosterone (Oliveira *et al.*, 2009:1060). The authors explained that this phenomenon might be due to the winning team demonstrating higher physical exertion *vs.* the losing team (Oliveira *et al.*, 2009:1061). According to Bateup and colleagues (2002:189), an increase in baseline testosterone or cortisol levels throughout a female rugby match or wrestling match is unrelated to winning or losing. Nevertheless, they found that winning females had higher cortisol levels compared to losing females (Bateup *et al.*, 2002:190). These occurrences might be due to the anticipatory effect before commencement (Oliveira *et al.*, 2009:1061) or the mental pressure and stress during the competition (Sedghroohi *et al.*, 2011:278). In contrast to these results, Sedghroohi *et al.* (2011:277) reported that neither testosterone nor cortisol in the winning and losing team differed significantly ($p = 0.16 – 0.66$) before and after a female basketball match, although the losing team demonstrated higher ($p > 0.05$) testosterone and cortisol levels throughout the match (Sedghroohi *et al.*, 2011:278).

Regarding the psychological aspects, the outcome of the match usually determines the status of the self as well as that of the teammates (Edwards *et al.*, 2006:135). After losing a match, testosterone decreases and the individual will feel the urge to flee and avoid further loss of status (Sedghroohi *et al.*, 2011:278). Conversely, an increase in testosterone takes place as status increases, which in turn increases the individual's behaviour to obtain more status (Sedghroohi *et al.*, 2011:278). Cortisol is known to initiate a catabolic process in the body, as it is a stress hormone (Sedghroohi *et al.*, 2011:278). As a challenge becomes more complex, an increase in cortisol takes place in relation to the increase in the challenge expected ($F_{(49)} = 4.378$, $p = 0.04$) (Bateup *et al.*, 2002:189). If a lower cortisol range is seen in winners, it may be speculated that the individuals managed the challenge of a competition so effectively that a high cortisol prevalence did not interfere with their behaviour (Bateup *et al.*, 2002:190).

In a study undertaken in 1995, it was reported that a match won led to lower tension ($p < 0.05$) and higher vigour scores ($p < 0.01$), whereas a losing match led to a rise in depression and anger scores ($p < 0.01$) (Hassmén & Blomstrand, 1995:301–304). In contrast to these results, Oliveira *et al.* (2007:1059) reported that players' anxiety, mood and aggression did not vary significantly between a losing and winning female soccer match. However, the team that won demonstrated an increase ($p < 0.05$) in mood with a corresponding decrease ($p < 0.01$) for the losers after the match (Oliveira *et al.*, 2009:1059). This study also reported the losing team to experience a greater ($p < 0.001$) perception of threat and anxiety before and after the match (Oliveira *et al.*, 2009:1059). The authors concluded that mood changes may play a role as a psychological modulator and that there is interaction between competition behaviour and androgens (Oliveira *et al.*, 1062).

4.2.1.6. **Warm-up**

Warming up is deemed an important facet of preparing both the body and mind for an upcoming event (Edwards & Kurlander, 2010:606). A few studies reported a rise in cortisol and/or testosterone during the warm-up period prior to either a female soccer (Casto & Edwards, 2016b:16) or a female rugby match (Bateup *et al.*, 2002:187). In contrast to this, Edwards and Kurlander (2010:608) found no changes due to a warm-up prior to a female volleyball or a female tennis match. These observations might be due to one of the following reasons:

a.) Increases in the hormones might be due to the physical exertion of the warm-up (Casto & Edwards, 2016b:20; Edwards & Kurlander, 2010:611);
b.) Increases in the hormones might be due to the psychological effect of preparing for the competition (Casto & Edwards, 2016b:20; Edwards & Kurlander, 2010:611);

i. During the warm-up the athlete prepares his mind and body for the competition (Casto & Edwards, 2016b:19);
ii. Changes might be due to the athletes engaging in behaviours of confidence and strength as they watch and/or are being watched by their opponents (Casto & Edwards, 2016b:19);
iii. The hormones may be influenced by both covert and overt competitive or dominant behaviours (Casto & Edwards, 2016b:19);

c.) Changes might be due to social interactions taking place among teammates and efforts at coordination (Casto & Edwards, 2016b:20);
d.) Females express their aggression more collectively than individualistically (Bateup *et al.*, 2002:188).

4.2.1.7. **Effect of diet**

Individuals often report positive mood states, less stress and the feeling of waking up to a clean slate as a result of a good night's rest (Kate *et al.*, 2017:1). A feature not commonly stated is our food consumption, which influence our mood states by altering the neurotransmitter secretion (Kate *et al.*, 2017:1). Neurotransmitters can act as stimulants of various nerve reactions, with promoting alertness, motor movement, excitement, energy and activity among others (Kate *et al.*, 2017:1). The nutrients within food are precursors for neurotransmitter secretion, and depending on the amount of precursors present, the more or less neurotransmitters are secreted (Kate *et al.*, 2017: 1–2). Dieting is often used to control weight, but may have a negative outcome on various stress indicators (Tomiyama *et al.*, 2010:362–363). The relationship between cortisol, perceived stress and diet was investigated in a number of studies as reported by Tomiyama and colleagues (2010:358). A common method for losing weight is to restrict calorie intake, however this can lead to an increase in cortisol release ($F_{(92,1)} = 8.77$, $p = 0.004$) as well as perception of stress ($F_{(97,1)} = 5.45$, $p = 0.02$) (Tomiyama *et al.*, 2000:362). According to Markus and colleagues, individuals that are stress-prone could have a higher serotonin sensitization due to biochemical adaptations taking place (Markus *et al.*, 2000:334). An increase in cortisol excretion can occur as a result of the increased release of serotonin at the pituitary and periventricular hypothalamus terminals (Markus *et al.*, 2000:334). Furthermore, if an increase in serotonin takes place in the hippocampal structures, the negative feedback control (as previously discussed) over the hypothalamic pituitary axis can also increase (Markus *et al.*, 2000:334).

This is confirmed by a study evaluating the effect of disordered eating (DE) in tennis players (Filaire *et al.*, 2015). They reported higher ($p < 0.01$) cortisol concentrations for the DE group throughout the day as well as after awakening compared to the non-DE group (Filaire *et al.*, 2015:237). Regarding their psychological states, the DE group also demonstrated a higher level of state anxiety ($p < 0.05$) compared with the non-DE group. These results might be due to low calorie dieting stimulating proinflammatory cytokines that in turn, activates the HPA axis (Filaire *et al.*, 2015:239).

Different nutrients interact and influence neurotransmitter release, thus, influencing the quality and quantity of neurotransmitters produced (Kate *et al*., 2017:2). Common nutrients and bioactive factors found in food are dietary amino acids, minerals, vitamins, alcohol, lipids, carbohydrates, neurotransmitters, caffeine, chocolate, and biogenic amines (norepinephrine, dopamine and serotonin) among others (Kate *et al*., 2017:2). Insulin plays a vital role in absorption of carbohydrates by simplifying the transferal process of amino acids from the blood to cells (Kate *et al*., 2017:2). Blood sugar fluctuations are associated with mood and energy changes (Kate *et al*., 2017:2). Two other general dieting methods are to either increase / decrease the carbohydrate intake and to decrease / increase the protein intake. In addition, a carbohydrate-rich, protein-poor (CR/PP) diet may increase the production of serotonin in the brain (Markus *et al*., 2000:333). In the latter study, a significant three-way interaction effect ($F_{(5,35)} = 2.176$, $p = 0.04$) was reported between laboratory stress, diet and proneness to stress, as seen on five subscales of the POMS (Markus *et al*., 2000:337). This three-way interaction originated from the changes observed on the depression sub-scale of the POMS ($F_{(1,39)} = 3.876$, $p = 0.025$) (Markus *et al*., 2000:337). Stress-prone individuals that consumed the CR/PP diet reported unchanged feelings of depression with a slight reduction in depression when faced with stress, however, when consuming the carbohydrate-poor, protein-rich (CP/PR) diet, the feelings of depression increased slightly (Kate *et al*., 2017:2; Markus *et al*., 2000:337). This might be due to carbohydrates increasing the uptake of tryptophan in the brain, thereby enhancing serotonin synthesis and release (Kate *et al*., 2017:2). Furthermore, the stress-prone individuals demonstrated lower cortisol levels when faced with stress whilst on the CR/PP diet ($p = 0.02$), whereas an increase in cortisol was observed when consuming the CP/PR diet (Markus *et al*., 2000:339). A common element consumed when undergoing a stressful time such as a competition, is coffee and sports drinks, both comprising of caffeine (Pritchard *et al*., 2017:2).

Caffeine is a pharmacological substance known to alter cortisol secretion at rest and when faced with various stressors (Lovallo *et al*., 2006:441). However, consuming coffee on a regular basis has positive effects on various health domains, such as alertness and mood (Kate *et al.,* 2017:6) with no influence on cortisol secretion due to tolerating effects (Pritchard *et al*., 2017:4). Over-consumption, on the other hand, suppresses serotonin secretion, leading to depression and negative effects on sleep and wake function (Kate *et al*., 2017:6). Another negative consequence of caffeine consumption and prolonged physical exertion or match play is dehydration. Research has indicated hypo-hydrated athletes to demonstrate significantly higher cortisol responses during moderate (70% VO_{2max}) and high (85% VO_{2max}) intensity training (Maresh *et al*., 2005:768). Managing the state of hydration during training is vital in modulating endocrine responses as fluid balances can alter the anabolism:catabolism relationship (Maresh *et al*., 2005:766), thereby influencing both the physiological and psychological states.

5. SUMMARY

Women's soccer has become popular worldwide, prompting researchers to find improved ways of performance enhancement. Playing at a high level requires players to utilise various physical, physiological and psychological skills optimally. Because of the intermittent nature of the game, fatigue is a huge factor that places both physiological and psychological stress on a player.

Physiologically, the HPA axis reacts to a stressful event by increasing the secretion of cortisol. Cortisol is the primary hormone responsible for the stress response and can lead to various catabolic processes in the body. This includes the inhibition of the inflammatory process and immunity by increasing protein degradation, though cortisol is also involved in various psychological behaviours, and demonstrates the ability to increase negative behaviours in a stressful atmosphere.

Studies on the effect of a competition on cortisol levels have reported significant increases from pre- to post-competition. Most studies measured cortisol by making use of saliva analyses, which are more convenient for the participants. The majority of the studies took a baseline measurement, though the exact measurement time differed from as long as 3 days to an hour prior to the competition. Only a few studies recorded the effect of a warm-up session, reporting cortisol to increase as a result thereof. This might be due to the warm-up having a physiological and psychological preparation phase, activating the HPA axis and increasing cortisol secretion. Following the competition, measurements were taken as rapidly as immediately after to as long as 24 h after the competition. Most studies took a post-sample within 30 min to a few hours, as research has shown that cortisol rises to a peak within 30 min following a stressor. A factor that needs to be considered is the circadian rhythm of cortisol, showing peak increases within the first hour upon awakening as well as direct effects as a result of sleep quality, menarche and OC usage, among others.

Some studies discriminated between winners and losers, successful and less successful participants and starters *vs.* non-starters for either cortisol, anxiety and/or mood evaluations. Dissimilar results were obtained, with one study reporting higher cortisol increases for the winning team and another for the losing team. Furthermore, participants engaged in a longer competition time displayed larger cortisol increases compared to non-starters. This has led researchers to evaluate the effect of various training regimes in determining which conditioning aspects (aerobic *vs.* anaerobic activities) might influence cortisol to a greater extent in competition.

In this regard, studies found higher cortisol increases following high-intensity activities (anaerobic) compared to low-intensity activities (aerobic). They stated that this might be due to different hormonal patterns, depending on the training intensity. In contrast to this, another study reported low-intensity (aerobic) activities to yield higher increases in cortisol, which might be due to the length of a training session increasing as its intensity decreases.

Psychologically, cortisol is involved in behaviours such as aggression, depression, vigour, fatigue and anxiety. Studies have reported positive correlations between cortisol and depression, tension, fatigue and anger and a negative correlation with vigour. Increased anxiety levels (either as a result of cortisol increases or the stressful atmosphere of a match) have proven to be detrimental to performance (as seen from the inverted U-hypothesis). Literature studies have distinguished between various anxiety states and questionnaires when evaluating the effect of a competition or training.

The most popular questionnaire implemented in the sporting domain is the STAI, followed by the CSAI-2. The STAI questionnaire differentiates between the current and ongoing anxiety states, whereas the CSAI-2 discriminates between somatic and cognitive anxiety. Most studies reported increased anxiety levels following a competition, with a few distinguishing between winners and losers. The losers were found to demonstrate higher anxiety and negative mood states compared to winners. Training, on the other hand, has proven either to increase or decrease anxiety and negative mood states, depending on its intensity. High- and low-intensity activities both have the ability to decrease anxiety, though the beneficial effects of low-intensity activities last longer. Researchers have stated that this might be due to endorphins secreted as the training duration increases, or the linear relationship between training duration and mood.

Evaluating the pathway between the body and the mind is important in order to identify a stressor effectively and deal with it. Participants can implement either a top-down approach, thus focusing on psychological aspects (for example the outcome), and consequently reducing the negative effects of stress on the body, or they can implement a bottom-up approach, thus focusing on their physiological and physical states (for example decreasing the heart rate), thus implementing a depletion-to-renewal strategy to influence the psychological states.

Various limitations were identified during the compilation of the literature review. The most important is lack of research on South African female athletes, more specifically on soccer players. The second largest limitation is the scarcity of research evaluating both hormonal and psychological states, more specifically cortisol, anxiety and mood states, in a sport context. Furthermore, there are discrepancies in the collection time of salivary cortisol samples and insufficient reporting on the effect of warm-up or OC usage. Though an array of questionnaires exist, no recommendation on using a specific anxiety questionnaire could be found for either a competition or training set-up. Another limitation is the effect of various training regimes, with studies not distinguishing between the type and intensity of training implemented. Most studies evaluated the effect of aerobic training on either the physiological or psychological states, with only a few discussing anaerobic activities.

Nevertheless, the findings highlight the importance of identifying all the physical, physiological and psychological aspects needed for optimal performance, as fatigue can be detrimental to all of these components. This literature review will enable coaches and sporting staff to implement the correct research techniques to enhance performance, whether a questionnaire (according to their specific needs, *e.g.* match vs training), time points to take measurements (whether a saliva or blood sample) or additional factors to take into consideration. Furthermore, the review will make researchers and sporting staff aware of adequate earlier research studies to compare and assess their results. In conclusion, an adapted version of the pathway of stress as proposed by Ursin and Eriksen (2004:570, 581) is provided below. These adaptions were made from the literature reviewed to provide the reader with a visual summary of the effect of training, competition or a stressor on the psychological and hormonal state of female athletes.

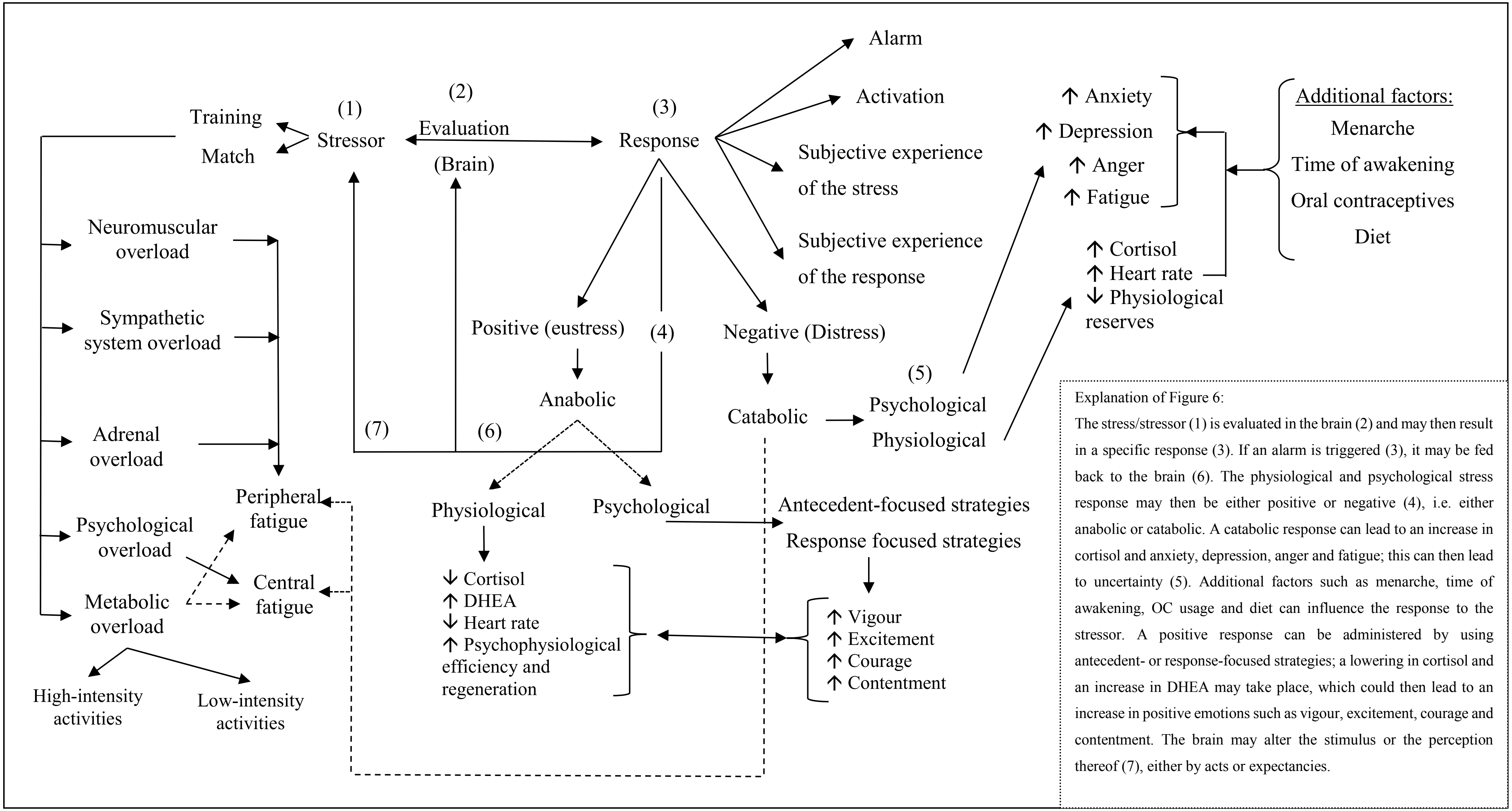

Figure 9: The effect of fatigue as a stressor within the soccer arena on the psychological and hormonal state of female players (Adapted from Lehmann *et al.*, 1998; McCraty *et al.*, 2009:29; Ursin & Eriksen, 2004:570,581).

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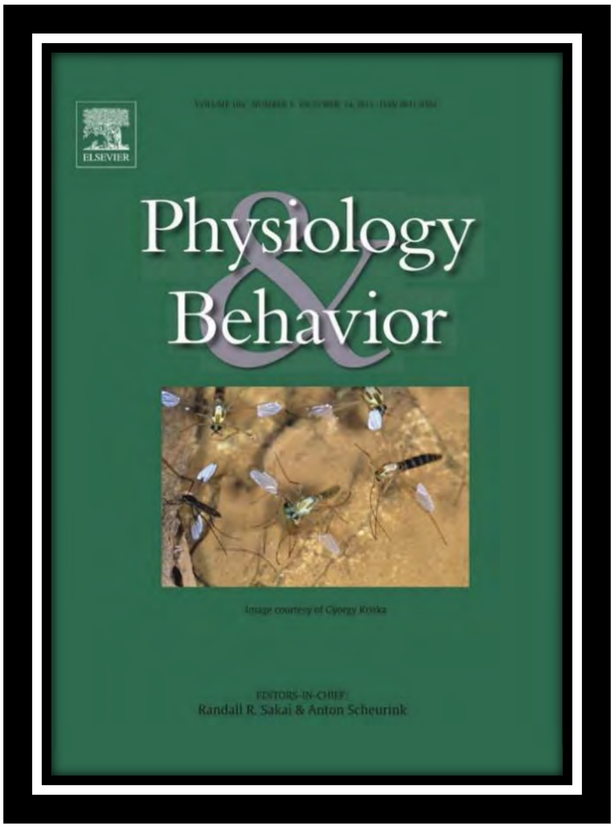
3 THE PSYCHO-HORMONAL INFLUENCE OF ANAEROBIC FATIGUE ON SEMI-PROFESSIONAL FEMALE SOCCER PLAYERS



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The psycho-hormonal influence of anaerobic fatigue on semi-professional female soccer players

Adéle Broodryk[a,*], Cindy Pienaar[a], David Edwards[b], Martinique Sparks[a]

[a] Physical Activity, Sport and Recreation Research Focus Area, Internal Box 494, Faculty of Health Sciences, North-West University, Potchefstroom 2520, South Africa
[b] Psychology Department, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, South Africa

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ABSTRACT

Globally it is assumed that high-intensity activities are the general cause of fatigue experienced during a soccer match. However, little is known about the hormonal and psychological effects of fatigue due to these activities on semi-professional female soccer players. Forty-seven female players (22.0 ± 2.7 y) from a tertiary education institution volunteered for the study. Their cortisol values (saliva sample), anxiety [Spielberger State-trait anxiety inventory questionnaire (STAI)] and mood scores [Incredible Short Profile of Mood states questionnaire (ISP)] were taken an hour and immediately prior to, and 15 min after an anaerobic fatiguing test (AFT). During the AFT, subjects completed a 5-m multiple shuttle run test and their HR_{max}, blood lactate (BLa^-) and rate of perceived exertion (RPE) taken afterwards. Anxiety scores were divided into three categories and mood scores into the Total Mood Disturbances (TMD) and six subscales. The results indicated an increase in cortisol, psychological fatigue and TMD from baseline and/or pre- to post-AFT ($p < 0.05$). Vigour and confusion decreased from baseline and/or pre- to post-AFT ($p < 0.05$). A relationship was seen between state-anxiety and TMD ($r \geq 0.63$, $p < 0.05$) at all three time points, as well as between state-anxiety and HR_{max} ($r = 0.37$, $p = 0.03$). Cortisol and RPE ($r = -0.34$, $p = 0.03$) demonstrated a correlation post-AFT. This is the first study to evaluate the effects of anaerobic fatigue on the hormonal and psychological states of female soccer players. The results suggest that an AFT can be perceived as a physiological and psychological stressor by female players, hence has the ability to influence performance. Altering a player's awareness and anaerobic fitness level might therefore influence both the hormonal and psychological consequences of the stressor, subsequently reducing the experience of fatigue and thereby enhancing performance.

1. Introduction

The popularity of soccer among females has increased worldwide, and since 1960, particularly in South Africa [1]. The game is very erratic in nature, requiring players to have a noble foundation of several psychological and physiological capabilities to allow elite level performance [2]. The game is characterized by both high-intensity (activating the anaerobic energy system) [3] and low-intensity activities (activating the aerobic energy system) [4]. A study by Krustrup et al. [4] reported between 1336 and 1529 activity changes during a female soccer match, with 72–159 classified as high-intensity runs ($18\ km \cdot h^{-1}$) and 9–43 as sprints ($25\ km \cdot h^{-1}$). Muscle biopsies collected following a soccer match demonstrated a 60% increase in muscle creatine-phosphate from baseline, indicating a high turnover rate for the anaerobic energy system [3]. Though players are more involved in low-intensity activities during a match (95.2% of a match accounted for low to moderate intensity activities), the frequent execution of high-intensity activities can lead to performance decrements [4] and fatigue [5]. There are fewer reports in the literature on these activities and, more specifically, its influence on the physiological and psychological aspects of female soccer players.

During the second half of a soccer match, a decline in performance usually occurs [6], as seen from the fewer high-intensity runs [4], sprint speed and the speed and accuracy of shooting and passing [7] taking place. This physical poorer result together with the stressful atmosphere of the game [8], may result in the disruption of physiological and psychological homeostasis [9,10]. Understanding the impact of these high-intensity activities on the physiological and psychological systems, and in so doing gaining information regarding the corresponding stress experience, are important for minimizing adverse consequences [10]. All stressors (whether physical or psychological) follow the same physiological reaction, namely, by activating the hypothalamic pituitary

* Corresponding author.
E-mail addresses: adele.broodryk@nwu.ac.za (A. Broodryk), Cindy.Pienaar@nwu.ac.za (C. Pienaar), edwards.davidjohn@gmail.com (D. Edwards), Martinique.Sparks@nwu.ac.za (M. Sparks).

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adrenocortical (HPA) axis [11]. In turn, the HPA axis stimulates cortisol secretion, which is the main hormone responsible for allostatic stress responses [11–13]. One way to detect the magnitude of the physiological and psychological stress experienced is by assessing a player's hormonal responses, and in particular the cortisol levels [2].

The experience of a perceived stressful experience will result in a rise in cortisol levels [12]. Studies have reported a significant increase ($p < 0.05$) in cortisol values from pre to post soccer match [9,10,12,14]. These levels are higher than those observed during training ($d = 1.52$) [10], which might be due to the stressful nature of a match and the players' experience of it [8]. In another study, a higher cortisol response was noted after a high-intensity interval training session compared to aerobic endurance training session [15]. This might be due to the intensity of the activity as anaerobic exercise metabolites (decreased blood pH level, hypoxia and increased blood lactate [BLa^-]) stimulate the HPA axis [2,6]. Furthermore, cortisol is essential to mobilize energy sources to provide adequate energy to execute the forthcoming activity by elevating blood glucose levels as well as to regulate the inflammatory process afterwards [11]. However, if cortisol production exceeds the quantity necessary, it can act in a catabolic manner [11]. Moreover, the cortisol increases observed following a high-intensity activity [16] have a direct influence on the psychological state of an individual [17].

This interaction is bidirectional, hormones can influence behaviour and behaviour can sometimes influence hormonal concentrations [18]. The HPA axis is involved in affective and cognitive processes and their central nervous system bases, which can lead to the generation of numerous emotional responses [10,11]. On the other hand, psychological stressors can also affect physiology due to these processes, thereby increasing/decreasing cortisol secretion [11]. If a player can maintain a positive mood state, by activating the parasympathetic nervous system, lower cortisol secretion can take place [19,20]. In contrast, negative mood states can activate the sympathetic nervous system, by increasing cortisol secretion [19,20]. In this regard, mental stress is a great stimulus to cortisol production [2] as a strong relationship ($r = 0.7$) between cortisol and cognitive anxiety was seen prior to training [10]. Thus, a high-intensity activity as well as the appearance of a stressor can lead to an increase in cortisol, with the hormone having an effect on the psychological state. Subsequently, physiological influences of stress and mood states may intervene and have rebound effects on performance [16]. This emphasizes the necessity to assess the effect of anaerobic fatigue due to high-intensity activities on the psycho-hormonal state of female soccer players as these factors can intervene to influence one another, and subsequently, performance.

The influence of anaerobic fatigue on the hormonal and psychological performance of female soccer players is of practical interest to sport psychologists, physiologists, and coaches as it is the main cause of performance decline during matches [6]. Female soccer has recently received much attention in South Africa however, an extensive literature review and searches on websites such as PubMed, EBSCOhost, PsychINFO and Sabinet revealed no published research regarding the effect of fatigue on their performance. This is the first study that evaluates the effect of anaerobic fatigue on their hormonal and psychological states. The aim of this research was to discover what effect an anaerobic fatiguing test (AFT) has on salivary cortisol as well as anxiety and mood states, and whether there is a correlation between cortisol and mood or anxiety, in semi-professional female soccer players. This will aid sport coaches, sport scientists and psychologists into identifying whether anaerobic fatiguing exercises may lead to physiological and/or psychological stress in a player and if it influences their performance adversely.

2. Methods

2.1. Experimental approach to the problem

The specific hypothesis under scrutiny was that the AFT will lead to an increase in both cortisol, anxiety and total mood disturbances (TMD) (calculated from the mood questionnaire). Also, a positive correlation between cortisol and TMD, as well as between anxiety and TMD, was postulated. This study was a repeated measures, quantitative research design with a convenient sample. Information was collected by means of a questionnaire and test battery.

2.2. Subjects

Forty-seven female university soccer players (mean age: 22.0 ± 2.7 years; stature: 158.9 ± 5.8 cm; body mass: 55.5 ± 8.1 kg) with mean competitive playing experience of 8.6 years volunteered to take part in the study. Subjects reported to be healthy, and were excluded if they became injured or sick during the time of testing or if they did not complete all tests. The subjects experienced normal day-to-day stressors as they were all enrolled in a tertiary institution and were requested to keep to their academic requirements. The specific testing procedure was compiled in accordance with their training schedule to minimize overloading the players. Their internal training load was monitored by means of heart rate and their rating of perceived exertion after the AFT. During the time of testing they were in their competitive training phase, with average $\dot{V}O_{2max}$ values of 40.8 ± 1.5 ml/min/kg. Ethical clearance was provided by the Health Research Ethics Committee of North-West University (NWU-00055-15-A1). Voluntary, written informed consent was gained from all the subjects and coaches before testing began. An average of 7.8 ± 2.2 h sleep the night prior to testing was reported by the subjects. In addition, the majority of subjects were in their follicular menstrual phase with none consuming oral contraceptives or prescribed medication as testified in the information questionnaire.

2.3. Procedures

The data collection was completed over two consecutive days, two weeks prior to the major tournament of the season. On the first day, written consent was obtained and the testing procedures explained to the subjects. During this period, the subjects were granted the opportunity to ask questions. For testing purposes on the second day, the subjects were advised to sleep at least 8 h and to awake between 6:00 and 7:00 am. Players were advised to have their last meal at least an hour prior to testing. This was done to ensure normal hormonal and psychological baseline values as sleep-duration, -quality, awakening time and diet play a role in both hormonal and psychological responses [16,21,22].

Upon arrival on the second day, the subjects rinsed their mouths with lukewarm water and within 5 min provided the first saliva sample and completed the psychological questionnaires (baseline). Body mass and stature were measured, and the demographic and personal questionnaires completed. They were then fitted with a Fix Polar Heart Rate (HR) Transmitter Belt (Polar Electro, Kempele, Finland) to obtain the maximal HR (HR_{max}) post-AFT. Following a 15-min warm-up period, the subjects provided a second saliva and questionnaire set (pre-AFT) followed by the completion of the 5 m multiple shuttle run test (5-MST) to induce anaerobic fatigue. Immediately after the 5-MST their maximal HR values and rate of perceived exertion according to the Borg scale was recorded and a blood sample collected to measure BLa^-. The last saliva and questionnaires were completed 15 min after the completion of the 5-MST (post-AFT), as peak cortisol levels usually occur 10–30 min following a stress cessation [13].

2.3.1. Saliva sampling

Salivary samples were collected for the assessment of cortisol (nmoll/l) by means of passive drooling into collection tubes [23]. Subjects rinsed their mouths thoroughly with lukewarm water 5 min prior to sample collection to remove any substances that may affect or interfere with the hormonal concentration. Saliva flow was stimulated by chewing on a piece of Para film if needed, and collected through a plastic straw into a 20 ml collection tube. Samples were then stored at 4 ± 1 °C, after which they were sent for analysis by a professional laboratory. The content of saliva was determined from 20 μl saliva samples by using a cortisol saliva luminescence immunoassay. The samples were transferred to a Berthold Luminometer to first calculate the average relative luminescence units, after which it was plotted against the observed concentration to determine the exact content. This method has a non-linear ($r = 1.0$) and linear correlation coefficient ($r = 0.8$) with serum cortisol values, with intra-CV ranging between 0.4 and 1.7% and inter-CV ranging between 0.8 and 1.8% [24]. To minimize the effect of circadian rhythm, samples were collected an hour (baseline) and immediately prior (pre-AFT) to the AFT, with the players being awake and fasting for at least 1 h. Post-AFT samples were collected 15 min after completion of the 5-MST.

2.3.2. Sport psychological questionnaires

2.3.2.1. Mood states. The Incredible Short Profile of mood states (ISP) questionnaire derived from the original Profile of Mood states questionnaire [25] by Dean and colleagues [26] was completed at three time points. The ISP consists of six questions to measure six subscales (anger, depression, anxiety, confusion, fatigue and vigour); correlations of $r = 0.67–0.82$ have been reported between the two questionnaires [26]. The subjects rated the questions on a 5-point Likert scale: 1 (not at all), 2 (a little), 3 (moderately), 4 (quite a bit) and 5 (extremely). Individual scores for each subscale were the value rated per question; the Total Mood Disturbances (TMD) score was calculated by adding the five negative mood states (anger, depression, anxious, confusion, fatigue) and subtracting the positive state (vigour).

2.3.2.2. Anxiety. The State-Trait Anxiety Inventory (STAI) questionnaire was completed to evaluate the subject's level of anxiety over the three time points [27]. The STAI is a 40-item, self-evaluation questionnaire which includes separate measures to determine a subject's state and trait anxiety, respectively. The State-Anxiety scale consists of 20 statements that evaluate how players feel "right now, at this moment" on a 4-point Likert scale: 1 (not at all), 2 (somewhat), 3 (moderate so) and 4 (very much so). The Trait-anxiety scale also consists of 20 statements that assess how people "generally feel" on a 4-point Likert scale: 1 (almost never), 2 (sometimes), 3 (often), and 4 (almost always). Four indicates the presence of high anxiety levels where 1 indicates the absence of high anxiety levels [27]. Anxiety levels were determined by calculating the sum of the different scores. The range of scores can vary from 20 to 80, the higher the score, the greater the perceived anxiety [27]. Internal consistency reliability for trait anxiety is $r = 0.89$ and $r = 0.91$ for state anxiety [28].

2.3.3. Anaerobic fatiguing test (AFT)

The 5-MST was conducted outdoors on a soccer field according to the method of Boddington et al. [29]. This test was implemented as its demands are similar to those experienced during a match [29]. Subjects were familiar with the specific test as they complete the test during physical performance evaluations throughout the season. The subjects were instructed to wear soccer boots and not to pace themselves but to perform with maximal effort throughout the test. The following values were then derived from the results: peak distance (m), total distance (m), fatigue index (W/s), fatigue ratio (%) and average, minimum and maximum power (W). A low fatigue index would be advantageous as it may indicate a rapid recovery whereas a high index might be due to the inability to replenish phosphocreatine stores as well as to remove BLa^- between shuttles.

Immediately after the final repetition, a blood sample was obtained from the left hand's hyperaemic fingertip and transferred into a portable analyser (Lactate Pro, Arkray, Japan) to measure blood lactate (mmol/l). This was done to evaluate whether fatigue, as expected during a match, occurred, as high BLa^- concentrations have been reported following a match, which indicates a high muscle lactate production [3]. Before the collection of the blood sample, the subject's ring finger was cleaned with an alcohol swab and the portable analyser calibrated according to the manufacturer's guidelines. Furthermore, following the AFT the subject's maximal HR achieved was recorded and she was asked to indicate the rate of perceived exertion (RPE) on a 10-point Borg scale [30] as used by Boddington et al. [29]. This was done as research has shown a progressive increase in perceived exertion after each consecutive shuttle [29]. The RPE ($r = 0.92$) as well as HR_{max} ($r = 0.97$) have a high reliability with the specific test implemented [29].

2.4. Statistical analyses

Analyses were conducted using IBM SPSS Statistics (v. 24.0.0.0) [31]. Descriptive statistics (averages, minimum, maximum and standard deviation values) for each variable at every time point were calculated. This was followed by linear mixed model analyses to investigate time point differences with an autoregressive 1 covariance structure. The players were entered as subjects and time points as fixed effects.

The variables were categorized into three rankings (TMD/Anxiety: 0–5/20–39 = low, 6–12/40–50 = average/neutral, 13–18/51–80 = high) for each time point The cortisol values were ranked into three categories according to time of awakening and sample collection time (as adapted from Kirschbaum and Hellhammer [13], the Salimetrics Manual [23] and Westermann et al. [24]) according to the 5% (low = 1), 50% (average/neutral = 2) and 95% (high = 3) percentiles (see Table 1).

The total row score for each ranking of every time point was then expressed as a percentage of the total score for the specific variable for the entire group using cross-tabs with a chi-squared analysis, practical significance indicated by Cramer's V values. Fourth, Pearson's correlation coefficient was used to determine the relationship between the variables (cortisol, TMD, anxiety and AFT variables). A Fisher's r to z transformation was calculated to determine the 90% confidence interval (CI) from the correlation coefficient. The level of significance was set at $p \leq 0.05$. The strength of the correlation was categorized as follows: ≤ 0.01 (trivial), < 0.3 (small), < 0.5 (moderate), < 0.7 (large), < 0.9 (very large) and ≥ 1 (perfect). Effect size (d) was interpreted as: ≥ 0.8 (large), ≤ 0.5 (moderate) and ≤ 0.2 (small) [32].

3. Results

The average results for the AFT were: peak distance: 118.3 ± 7.9 m; total distance: 614.3 ± 41.9 m; fatigue index: 0.018 ± 0.0078 W/s; fatigue ratio: 21.2 ± 7.7%, maximum power: 28.6 ± 4.4 W; BLa^-:16.0 ± 4.8 mmol/l; HR_{max}: 186.2 ± 8.9 bpm; RPE: 7.1 ± 2.2 level. From the 47 subjects, 43 complete saliva sets and psychological questionnaires were obtained. Provided in Table 2 are the

Table 1
The specific ranking for the mean cortisol values (nmoll/l) as adjusted for the time of awakening and saliva collection time.
Adapted from Kirschbaum and Hellhammer [13], the Salimetrics Manual [23] and Westermann et al. [24]).

Time awake: 06:00	Ranking			Time awake: 07:00
Test time point	1	2	3	Test time point
06:00–07:30	≥ 5.1	18.9	≤ 40.2	07:00–08:30
07:30–09:00	≥ 3.6	11.8	≤ 28.4	08:30–10:00
09:00–12:00	≥ 2.1	6.7	≤ 15.7	10:00–13:00
12:00–15:00	≥ 1.8	5.5	≤ 12.1	13:00–16:00
15:00–18:00	≥ 0.9	3.3	≤ 9.2	16:00–19:00

Ranking: 1 = Low; 2 = Average/Neutral; 3 = High

Table 2
Descriptive statistics (± SD) for all variables at the various time points.

Variable	Baseline	Pre-AFT	Post-AFT
Cortisol (nmoll/l)	15.68 ± 2.28*	13.84 ± 1.93*	32.22 ± 3.53
ISP (tension)	2.36 ± 0.15	1.96 ± 0.14	2.45 ± 0.18
ISP (depression)	1.98 ± 0.14	1.61 ± 0.11	1.77 ± 0.14
ISP (confusion)	2.09 ± 0.13*	1.65 ± 0.11	1.66 ± 0.12
ISP (anger)	1.63 ± 0.12	1.35 ± 0.08	1.57 ± 0.14
ISP (vigour)	3.07 ± 0.14*	3.37 ± 0.13*	2.57 ± 0.17
ISP (fatigue)	2.91 ± 0.18*	2.52 ± 0.14*	3.68 ± 0.20
ISP (TMD)	7.47 ± 0.60	5.72 ± 0.45*	8.45 ± 0.56
State-anxiety present	18.76 ± 0.78	17.42 ± 0.83	17.84 ± 0.79
State-anxiety absent	22.22 ± 0.78	22.31 ± 0.93	24.37 ± 1.13

AFT, anaerobic fatiguing test; ISP, incredible short profile of mood states; TMD, total mood disturbances
* Significantly different from post-AFT ($p < 0.05$).

descriptive statistics (± SD) of all variables for all time points.

3.1. Cortisol responses

As seen from Fig. 1, cortisol increased significantly and had a large effect from baseline ($F_{(4198)} = 11.5$, $p = 0.00$, $d = 1.1$) and pre- ($F_{(4198)} = 14.7$, $p = 0.00$, $d = 0.97$) to post-AFT.

3.2. Psychological responses

Fig. 2 illustrates the distribution of the six mood subscales and total TMD score. The mood subscales confusion and fatigue increased and the subscale vigour decreased significantly from baseline to post-AFT ($p < 0.05$, $d \le 0.5$). In addition, both vigour and fatigue decreased as the TMD increased significantly from pre- to post-AFT ($p < 0.05$, $d \le 0.5$).

The cortisol values classified as high (as ranked according to Table 1) were significantly larger than the other rankings at all time points ($p < 0.01$, $V > 0.3$) (Table 3). TMD remained relatively stable with the largest percentage rated as neutral mood states at all three time points. At the first two time points, the second largest contribution of TMD was low, whereas after the fitness test it was high. Furthermore, at all three time points, a more positive anxiety state was reported which is clear from the increase observed from the anxiety-absent subscale. A low total anxiety score was seen at all time points followed by a neutral distribution.

No relationship was seen between cortisol and any psychological parameter. A moderate to large relationship was seen between TMD and state-anxiety at all three time points ($r \ge 0.6$, $p < 0.01$). Trait-anxiety revealed a large correlation with both TMD ($r = 0.5$) and state-anxiety ($r = 0.7$) at baseline ($p \le 0.01$). In addition, cortisol correlated with BLa^- ($r = 0.6$, $p < 0.01$) and negatively with average power output ($r = -0.4$, $p < 0.05$) at pre-AFT and with RPE ($r = -0.3$, $p = 0.03$) and BLa^- ($r = 0.7$, $p < 0.01$) post-AFT. A moderate correlation ($r = 0.4$, $p = 0.03$) was seen between HR_{max} and state-anxiety as well as between TMD and RPE post-AFT ($r = 0.4$, $p < 0.05$).

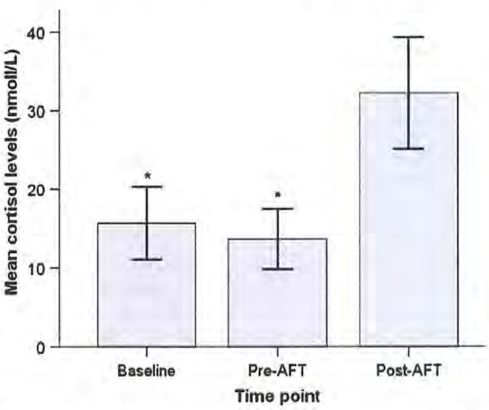

Fig. 1. Mean cortisol (nmoll/l) levels over the three time points. *Significantly different from post-AFT; $p < 0.05$; error bars: 90% CI.

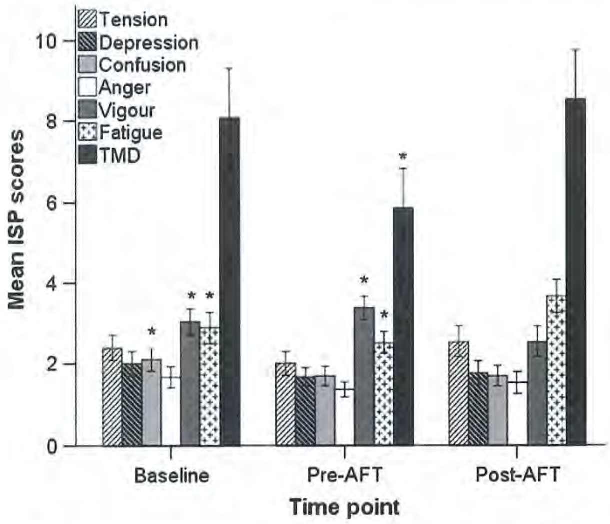

Fig. 2. Mean mood scores for the total and subscales over the three time points. AFT, anaerobic fatiguing test; ISP, incredible short POMS; TMD, total mood disturbances; *Significantly different from post-AFT; $p \le 0.05$; error bars: 90% CI.

4. Discussion

The aim of this study was to determine the effects of an anaerobic fatiguing test on the cortisol and psychological state of semi-professional female soccer players. The authors hypothesized that the AFT will lead to an increase in both cortisol and anxiety and total mood disturbances (TMD). Additionally, positive correlations between cortisol and TMD, and between anxiety and mood states, were postulated after the AFT. The key findings were the significant increase in cortisol, psychological fatigue and TMD as well as a decrease in vigour following the test. Additionally, a correlation was noted between the TMD and state-anxiety, HR_{max} and state-anxiety as well as between cortisol and/or RPE and BLa^- before and/or after the AFT.

The AFT demonstrated that the experience of fatigue post-AFT was similar to a match in terms of the high BLa^-, HR_{max} and RPE values. Krustrup et al. [4] reported peak BLa^- values of 8 mmol/l following an exhaustive incremental running test for female soccer players. Thus, these results signify how anaerobic fatigue (as frequently experienced during a match) can act as a stressor on the body, provoking both physiological and psychological reactions.

4.1. Hormonal results

The cortisol values increased significantly from baseline and pre- to post-AFT and revealed a strong relationship with the average power output and BLa^- of the subjects. This is in accordance with previous research reporting increased cortisol levels either after a female soccer match [33], a high-intensity interval training session [15] or after high-intensity resistance training in recreationally active females [34]. Only one study reported conflicting results, with a trivial (3%) decrease in cortisol levels after an anaerobic training session in active females [35]. One explanation for this increased response might be either the training status of the players, or due to different hormonal response patterns which are dependent on the activity's intensity level (as noticed from

Table 3
The percentage ranking of each variable and 90% confidence interval between the variables at each time point.

Time point	Variable	Ranking			r (90% CI)			
		1	2	3	Cortisol	TMD	State-anxiety	Trait-anxiety
Baseline	Cortisol	26.9	17.1	56.1**	1	0.0 (− 0.2–0.3)	0.0 (− 0.2–0.3)	0.2 (− 0.1–0.4)
	TMD	29	62.1	8.8	–	1	0.6* (0.5–0.8)	0.5* (0.3–0.7)
	State-anxiety	43.2	36.4	13.1	–	–	1	0.7* (0.5–0.9)
	Trait-anxiety	41.3	45.7	13	–	–	–	1
Pre-AFT	Cortisol	28	20.9	51.2**	1	0.1 (− 0.2–0.4)	0.2 (− 0.1–0.4)	–
	TMD	47.8	50.0	2.2	–	1	0.6* (0.5–0.8)	–
	State-anxiety	55.6	25.0	19.4	–	–	1	–
Post-AFT	Cortisol	2.4	11.7	81**	1	− 0.1 (− 0.3–0.3)	0.2 (− 0.1–0.5)	–
	TMD	19.1	66.0	26.3	–	1	0.7* (0.6–0.8)	–
	State-anxiety	36.8	36.8	26.3	–	–	1	–

AFT, anaerobic fatiguing test; CI, confidence interval; TMD, total mood disturbances.
1 = Low; 2 = Average/Neutral; 3 = High.
* $p \leq 0.01$.
** Significantly higher percentage distribution ($p < 0.05$).

the linear relationship between cortisol, RPE, BLa^- and power output) [34]. In this regard, though the players were in their major competition phase, the average $\dot{V}O_{2max}$ values obtained were classified as average, thus leading to fatigue and its negative consequences at an earlier rate. It is therefore assumed that a great stress-response was experienced due to anaerobic fatigue.

Cortisol is recognised as the main hormone responsible for both physiological and psychological stress within the body [13]. Not only are increases in cortisol evident following an acute stressor, but also in anticipation of it [19] as seen from the 51–56% of the pre-values ranked as high, hence the psychological stressor affected the hormonal state [11]. During physical activity, cortisol is vital for the maintenance of plasma glucose [11]. It promotes the breakdown of tissue protein for the formation of amino acids (used then to form new glucose); stimulates the mobilization of free fatty acids and liver enzymes leading to glucose synthesis; and it forces the use of fatty acids as fuel by the tissues [11,36]. However, if cortisol secretion exceeds normal secretion and is greater than its removal [36], it can be responsible for various catabolic processes taking place [16]. In this regard it constrains the inflammatory process and immunity by reducing protein synthesis and increasing protein degradation [16]. These cortisol fluctuations might aid behavioural changes by influencing an interaction component (sensory system, central nervous system or effectors) to behave in a certain way [18].

4.2. Psychological results

The fatigue and confusion subscales increased from baseline to post-AFT whereas vigour decreased from baseline as well as from pre- to post-AFT. In addition, the TMD increased from pre- to post-AFT. Similarly, Oliveira et al. [33] reported an increase in fatigue following a high-intensity training session ($F = 9.77$, $p < 0.01$) in female soccer players. In contrast, another study reported a decline in tension, confusion, fatigue and depression and an increase in vigour from pre- to post-resistance training in recreationally active females [37]. According to the latter study [37], these results might be due to the length of the training session, with longer sessions leading to greater mood improvements (due to the release of the natural mood-enhancing hormones, endorphins) or the linear relationship between mood and exertion level [38]. This linear relationship was confirmed in our results between the TMD and the RPE recorded post-AFT.

Moreover, a positive relationship ($r \geq 0.5$) between TMD and state-anxiety and TMD and the players' rate of perceived exertion was observed. This is in accordance with that found by Millet et al. [39] in professional female triathletes between anxiety and fatigue along with training load ($r \geq 0.3$). Physical exertion influences the somatic anxiety component primarily, and much less the cognitive component [40]. Though no significant correlation was observed between any psychological variable and BLa^- following the AFT, it is important to note their effect on cognitive functioning. This might be due to brain-produced lactate crossing the blood-brain barrier [41]. In this regard, increased BLa^- interferes with the attentional mechanisms [41] and, subsequently, posing the possibility of altering the attentional control system. This then can lead to a shift from a goal-directed to a stimulus-driven mind set [42], increasing the perceived anxiety. Though trait-anxiety (general emotional state) does not play a major role as a determinant of the anxiolytic effect of training [40], state-anxiety, on the other hand, has a strong correlation ($r = 0.57$, $p < 0.001$) with tension-related anxiety and trait-anxiety following training [40]. Though the mood questionnaire measures anxiety only within a subscale, it was able to indicate the current anxiety and RPE state of the players, making it useful for future use in determining the effects of an anaerobic fatiguing test.

4.3. Hormonal and psychological relationship results

Psychologically, cortisol plays a part in behaviour changes such as aggression, arousal, depression and cognitive anxiety [16,17], with high levels observed following peak stressors [19]. The current study found no significant relationship between cortisol and any psychological criterion. This can be explained by hormones not directly initiating changes in behaviour but rather influencing certain stimuli to provoke specific responses [18]. Oliveira et al. [33] also found no relationship between mood and cortisol levels following a female soccer match, whereas Chennaoui et al. [16] testified to a positive correlation between cortisol and fatigue, and depression and confusion ($p < 0.05$) in elite female swimmers. Additionally, a positive correlation was reported between cortisol and anxiety ($r = 0.7$, $p < 0.05$) prior to a female soccer training session [10], whereas a negative correlation between cortisol and tension-anxiety mood ($r = -0.67$, $p < 0.01$) in female waterpolo players [43]. Moreover, lower cortisol levels were significantly associated with a positive affect ($F_{(1901)} = 5.86$, $p \leq 0.05$) and a negative affect with higher cortisol levels ($F_{(1901)} = 6.91$, $p < 0.01$) in females following training [19]. This might be due to either motor activities affecting the two anxiety components (vigour and tension) differently [40], or that mood can mediate the relationship between cortisol and a stressor [19]. In this regard, the magnitude of anxiolytic effects depend on the level of state anxiety prior to exertion [40], which, can have rebound consequences on the psycho-hormonal responses.

Factors such as warm-up, time of awakening, sleep and menstrual phase can influence an individual's physiological and psychological state. Both cortisol and TMD showed a non-significant decrease from baseline to after the warm-up. This highlights the importance of the warm-up period as it is vital for physiological and psychological preparation [12]. As reported previously, the majority of the group were in their follicular menstrual phase, with 12 being in their menstrual phase. Herrera et al. [44] reported cortisol increases in females (when in the follicular menstrual phase) engaged in stressful conditions. Psychologically, the menstrual phase can be linked to emotional and physical fatigue, depression, anger, sadness, disgust, nervousness and mood swings among others, which can have detrimental effects on physical performance [45,46]. No significant relationship was observed between any menstrual phase and cortisol/mood states which might be due to the fatiguing session either acting as a distraction, improving self-efficacy or the social connectedness experienced [45]. Furthermore, Kudielka and Kirschbaum [47] stated that, regardless of the menstrual cycle phase, no influence takes place on the increased cortisol levels expected upon awakening (also known as the cortisol awakening response) or the awakening times.

Furthermore, the subjects were advised to awaken at specific times to minimize the effect of the diurnal rhythm of cortisol, as it peaks within 20–50 min after awakening [48], which could then be detrimental to physical performance by increasing the perceived fatigue [48]. A factor seen to influence this cortisol awakening response and subsequent performance is the quality of sleep, with a dysfunctional autonomic regulation of sleep reflected on the HPA-axis response [21]. In this regard, a negative correlation between sleep duration and cortisol, vigour, tension, fatigue, depression and confusion mood-subscales were reported prior to a competition, ultimately influence performance [16,49].

In summary, the AFT served as a physiological and psychological stressor as seen with the significant differences and relationships taking place. Therefore the authors suggest that emphasis is placed on maximizing a player's anaerobic fitness level to decrease the detrimental effects of it. Furthermore, applying interventions simulating anaerobic fatigue together with various psychological stressors (for example a penalty shoot-out), might aid in altering a player's perception and ability to handle the forthcoming stressor.

5. Conclusion

Though there is ample research on various aspects of female soccer players, few studies are available on the effect of high-intensity activities on the hormonal and psychological state. Furthermore, no research is available regarding African, and in particular South African, female soccer players. This is the first study to evaluate the effect of an AFT on the hormonal and psychological state of a semi-professional South African female soccer team. We have demonstrated that an AFT acts as a physiological and psychological stressor as seen with the significant cortisol and TMD increasing. Moreover, anaerobic fatigue lead to significant increases in the mood aspects of fatigue and confusion as well as a decrease in vigour. Though the players were in their competition phase, it was possible that their fitness levels could have influenced their results. Furthermore, due to the relationship between the fatiguing test with cortisol and psychological states, coaches and sport scientists can focus on maximizing the fitness levels and implement various interventions on how to deal with the fatigue experienced.

Conflict of interests

No conflict of interests took place during the testing period.

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4 THE EFFECTS OF AEROBIC FATIGUE ON THE PSYCHO-HORMONAL STATE OF AMATEUR FEMALE SOCCER PLAYERS

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The effects of aerobic fatigue on the psycho-hormonal state of amateur female soccer players

Running title: Psycho-hormonal effects of aerobic fatigue

Adele Broodryk,[1] Cindy Pienaar,[1] David Edwards[2] and Martinique Sparks[1]

[1] *Physical Activity, Sport and Recreation Research Focus Area, Faculty of Health Sciences, North-West University, Potchefstroom, South-Africa;*

[2] *Psychology Department, University of Zululand, Private Bag X1001, KwaDlangezwa, 3886, South Africa.*

Adele Broodryk

Physical Activity, Sport and Recreation Research Focus Area, Faculty of Health Sciences, North-West University, Potchefstroom, South-Africa

Email: adele.broodryk@nwu.ac.za

ORCID ID: 0000-0001-7396-1775

Tel: +2718 2991804

ABSTRACT

Up to 95% of a soccer match entails aerobic actions that may cause fatigue. Little is known about the effects of fatigue caused by prolonged physical exertion on the hormonal and psychological states of female players. Cortisol values (saliva sample), state anxiety (Spielberger State-trait Anxiety Inventory) and mood scores [Incredibly Short Profile of Mood States (ISP), comprising six subscales and total mood disturbances (TMD)] of 43 female players (aged 22.0 ± 2.7 years) were taken immediately prior to, and 15 minutes after, an aerobic fatiguing test (AFT). During the test, participants completed the Yo-Yo Intermittent Recovery test and their maximal heart rate (HR_{max}), blood lactate (BLa^-) and rate of perceived exertion (RPE) records were taken thereafter. Cortisol increased ($ES = 0.7$, $P < 0.01$) and ISP–confusion and ISP–vigour values decreased ($ES = 0.7$, $P < 0.05$). At pre-AFT, a slight positive relationship between cortisol and the absence of anxiety ($r = 0.3$, $P < 0.05$), and between HR_{max} and ISP–vigour and TMD transpired ($r = -0.4$, $P < 0.01$). TMD consistently demonstrated a strong relationship with all ISP- and anxiety scores ($r > 0.4$, $P < 0.01$). Post-AFT results demonstrated a positive relationship between cortisol and BLa^- ($r = 0.3$, $P < 0.05$), ISP–fatigue with HR_{max} ($r = -0.4$, $P < 0.05$) and ISP–vigour with RPE ($r = -0.4$, $P < 0.05$). This is the first study reporting on the hormonal and psychological effects of an AFT on female soccer players and their corresponding relationships. The results indicate that fatigue caused by prolonged activity may be a greater physiological than psychological stressor, though both may affect soccer performance. We recommend training players to increase their aerobic capacity to ensure maximal quality match-time before fatigue and its subsequent adverse physiological and psychological effects set in.

Keywords: cortisol; mood; anxiety; soccer; exhaustion

Introduction

Soccer is characterised by performing maximally for a prolonged period, with fatigue commonly observed due to the inability to endure the required work-rate for the entire match (Carling, Bloomfield, Nelsen, & Reilly, 2008; Reilly, Drust, & Clarke, 2008). The main activities in soccer are standing, walking, jogging and cruising, with most actions executed at a submaximal exertion level, dominating up to 95% of the work-rate profiles (Carling et al., 2008; Krustrup, Mohr, Ellingsgaard, & Bangsbo, 2005; Robineau, Jouaux, Lacroix, & Babault, 2012). Thus, a large aerobic capacity is needed to maintain high performance throughout a 90-minute match; if not, fatigue may set in either at the peripheral (outside the central nervous system) or central (within the central nervous system) sites of the body, with debilitating physiological and psychological consequences (Ament & Verkerke, 2009; Reilly et al., 2008; Robineau et al., 2012). Physiologically, fatigue (perceived as the “sense of effort”) reflects the dominance of the motor drive from the cerebral cortex to the motor neurons, whereas psychologically it reflects the exercise capacity (Ament & Verkerke, 2009).

It is important when planning training sessions to determine when and why fatigue occurs during a match (Bangsbo, Mohr, & Krustrup, 2006). Fatigue usually rises during the second half of a match and is manifested by a decline in specific playing ability (less distance covered, sprints completed and ball contact) and, ultimately, physical performance (Reilly et al., 2008). There may be several physiological reasons (such as depleted glycogen stores, dehydration, hyperthermia, decreased muscle pH and muscle creatine phosphate concentrations and increased muscle lactate concentrations among others) for this outcome (Bangsbo et al., 2006), although owing to the large load placed on the aerobic system during a match, it is argued that it is here that fatigue is experienced. During prolonged activities (when aerobic glycolysis is the primary energy source), a depletion in glycogen stores and an increase in glucose consumption by the muscle tissues take place (Ament & Verkerke, 2009; Bangsbo et al., 2006). Though the aerobic system is extremely taxed during a match, limited research is available regarding the fatigue experienced during training on the physiological and psychological domains. Hence, examining the effects of fatigue during exercise is an area of concern in studies of the physiology and psychology of sport (Ament & Verkerke, 2009).

A valuable tool for assessing a player’s aerobic capacity is the maximal oxygen consumption ($\dot{V}O_{2max}$) test, but it is not advocated as the best indicator of soccer-specific performance involving intermittent actions

(Svensson & Drust, 2005). Though no field test is able to determine precise match performances, using a validated test might enhance the specificity of the match component being assessed (Svensson & Drust, 2005). One common exercise test used to evaluate and improve the aerobic fitness of soccer players is the Yo-Yo intermittent recovery (YYIR-1) test, which incorporates both anaerobic and aerobic glycolysis (Svensson & Drust, 2005). The YYIR-1 test not only relates to the aerobic fitness and activity patterns involved during a competitive match (Bradley et al., 2014); it is also reliable (r = -0.7–0.8, $P < 0.05$) for evaluating match-related physical capacity and predicts the running performance during a match (Bangsbo, Iaia, & Krustrup, 2008; Bradley et al., 2014; Krustrup et al., 2005).

The effects of exercise on mood are influenced by various factors, among others neurobiological features, player characteristics, features of the exercise and psychological state (McDowell, Campbell, & Herring, 2016; Rocheleau, Webster, Bryan, & Frazier, 2004). Studies have reported increased positive and reduced negative mood (such as tension, anger, fatigue and confusion) and anxiety states following various aerobic exercises (Byrne & Byrne, 1993; Chase & Hutchinson, 2015; Cox, Thomas, Hinton, & Donahue, 2004). Furthermore, for every 30 minutes of training (≥ 60% $\dot{V}O_{2max}$), there is a progressive improvement in mood for various psychological states — including state anxiety, depression, confusion, fatigue and extent of mood disturbances overall (Cox et al., 2004; McDowell et al., 2016). However, when prolonged physical exertion becomes distressing, cortisol is secreted (Dickerson & Kemeny, 2004), which ultimately, might influence mood states.

The secretion of cortisol is modulated by the hypothalamic–pituitary–adrenocortical (HPA) axis (Dickerson & Kemeny, 2004), which is important for normal physiological functioning and cognitive and affective processes (Dickerson & Kemeny, 2004). Physiologically, cortisol is involved in providing energy for muscle tissue by increasing blood glucose levels (Dickerson & Kemeny, 2004) and promoting/inhibiting inflammatory processes (Chennaoui et al., 2016). Psychologically, it is associated with various behaviours such as expression of mood and anxiety (Haneishi et al., 2007). Because of the bidirectional relationship between hormones and behaviour (Nelson, 2017), it is speculated that mood improvements might modify the perception of stressors, subsequently altering cortisol release, and vice versa (Kirschbaum & Hellhammer, 2000; Smyth et al., 1998). This has been reported previously with increased positive mood states reducing cortisol responses ($F_{(1,901)} = 5.86$, $P < 0.05$), compared to negative mood states raising them ($F_{(1,901)} = 6.91$, $P < 0.01$) (Smyth et al., 1998). Furthermore, training has been shown to increase ($P < 0.05$) cortisol secretion by up to 36% (Karacabey et al., 2005), though a larger increase (250%) was described after a soccer match (Haneishi et al., 2007). This indicates the necessity to maximise the match components, and more specifically the aerobic basis, to minimise the incidence of increased negative psychological states and cortisol due to fatigue.

The influence of aerobic fatigue on the hormonal and psychological states of female soccer players is of practical interest in view of its large role during match play, and the importance of these components for maximal performance on the field. Based on an extensive literature review and searches, we found no published research on the effect of an aerobic fatiguing test (AFT) on the psycho-hormonal state of female soccer players in South Africa and, more broadly, in Africa. This is the first study that evaluates the effect of aerobic fatigue on the hormonal and psychological states of female soccer players. The aim of our study was, therefore, first to evaluate the effect of an AFT on the anxiety, mood and cortisol levels of these players; second, to determine what associated relationships prevail between anxiety, mood and cortisol levels and/or the AFT. Such knowledge will aid coaches, sport scientists and sport psychologists in determining whether fatigue due to prolonged physical exertion may lead to physiological and/or psychological stress in players and whether it adversely influences their performance.

Materials and Methods

Participants

Forty-three female university soccer players (mean age: 22.0 ± 2.7 years; stature: 158.5 ± 5.9 cm; body mass: 54.1 ± 6.2 kg; competitive playing experience: 8.2 ± 4.8 years) volunteered to partake. Participants were all healthy at the start of testing and were excluded if they became injured or ill, or did not complete all the tests. They all experienced normal day-to-day stressors as registered students, committed to their academic tasks. The specific testing regime was compiled in accordance with their training schedule (training 3–5 times/week) to prevent overloading the players. Their internal training load was monitored during the testing period by means of their maximal heart rate (HR_{max}) and perceived exertion rate (RPE). During the testing period they were in their competitive training phase, with average $\dot{V}O_{2max}$ values of 41.0 ± 1.5 ml/min/kg. Prior to testing, written consent from all the participants and coaches and ethical clearance from the university authorities (NWU-00055-15-A1) were obtained. An average total amount of sleep of 7.7 ± 2.3 hours were reported the night before the tests on the information questionnaires. The majority were in their luteal menstrual cycle phase, with none consuming any oral contraceptives or prescribed medication as testified in the information questionnaire.

Procedures

The study was completed over two consecutive days, two weeks prior to the main tournament. On the first day, written consent was obtained and testing procedures explained to the participants. During this period, they were granted the opportunity to ask questions. They were advised to obtain a good night's rest (at least eight hours of sleep), to wake up between 6:00 and 7:00 and to have their last meal at least an hour before the start of testing.

Upon arrival on the second day, each student's body mass (BFW platform scale, Adam Equipment Co. Ltd., UK) and stature (Harpenden portable stadiometer, Holtain Ltd., UK) were taken. Following a 15-minute warm-up session comprising aerobic, stretching and sport-specific activities, the participants rinsed their mouths with lukewarm water to remove any food substances, and had to wait for a period of 10 minutes before their saliva was sampled. In this period, they were fitted with a Fix Polar Heart Rate Transmitter Belt (Polar Electro, Kempele, Finland) to monitor the HR_{max} obtained post-AFT, and completed the psychological questionnaires. After 10 minutes they provided a saliva sample (pre-AFT) and completed the AFT with the YYIR-1 test. Immediately thereafter, the players' RPE, HR_{max} and blood lactate (BLa^-) were recorded, with the last saliva sample and questionnaires completed 15 minutes post-AFT.

Saliva sampling

Saliva samples were collected using the passive drool test for the assessment of cortisol (Salimetrics, L.L.C., SalivaBio, 2011). If needed, the participants could chew on a piece of Para film to stimulate saliva flow. Saliva was then collected through a plastic straw into a 20 ml collection vial, after which it was stored in a fridge (at

4 ± 1ºC) and transported to a qualified laboratory for analysis (Salimetrics, L.L.C., SalivaBio, 2011). The cortisol concentrations were determined from 20 µl saliva samples by using a luminescence immunoassay. The samples were transferred into a Berthold luminometer to determine the average relative luminescence units, after which they were converted to exact values by plotting against the cortisol concentrations.
This method has a non-linear (r = 1.0) and linear correlation coefficient (r = 0.8) with serum cortisol values, with intra-CV ranging between 0.4–1.7% and inter-CV ranging between 0.8–1.8% (Westermann, Demir, & Herbst, 2004). The first sample was collected following warm-up (immediately prior to the AFT), and the last sample 15 minutes post-AFT, as previous studies have demonstrated that mean cortisol values peak at 10–30 minutes following a stressor (Kirschbaum & Hellhammer, 2000).

Sport-psychology questionnaires

Mood states

Mood states were evaluated at pre- and post-AFT by using the Incredibly Short Profile of Mood States (ISP) questionnaire derived from the original Profile of Mood States questionnaire (McNair, Lorr, & Droppleman, 1981) by Dean and colleagues (1990). The ISP consists of six questions targeting the same subscales (involving anger, depression, tension, confusion, fatigue and vigour). Correlations between the two questionnaires (r = 0.67–0.82) (Dean et al., 1990), as well as between the six subscales (r = 0.72–0.83), have been reported previously (Bourgeois, Leunes, & Meyers, 2010). The participants rated the questions on a five-point Likert scale: from 1 ('not at all') to 5 ('extremely'). Individual scores for each subscale were the rating indicated per question and the TMD score was calculated by adding the negative and subtracting the positive scales.

Anxiety

The participants' perceived anxiety levels at pre- and post-AFT were estimated from the results of the state–subscale (SAI) of the original State-Trait Anxiety Inventory (Spielberger, 1983). The state–anxiety scale consists of 20 statements evaluating how they feel "right now, at this moment" on a four-point Likert scale, ranging from 1 ('not at all') to 4 ('very much so'). Trait anxiety was not measured, as it evaluates how a person feels "in general". Total anxiety scores were determined by calculating the sum of the different scores as well as the two state-subscales (anxiety – present and absent). The range of scores can vary from 20–80; the higher the score, the greater the anxiety perceived (Spielberger, 1983). Internal consistency has been demonstrated for state anxiety ($r = 0.91$) over a wide range of studies and participants (Barnes, Harp, & Jung, 2002).

Aerobic fatiguing test

A YYIR-1 test was performed to tax the participants' aerobic system maximally to induce aerobic fatigue (Bangsbo et al., 2008). The test was executed as described by Bangsbo *et al.* (2006) to measure the participants' HR_{max} and $\dot{V}O_{2max}$ values while performing intervals over a prolonged period of time. The test was conducted on a flat, clearly marked 20-m stretch of a grass soccer field with the players wearing their soccer boots. The following measurements were taken: total distance covered (m), YYIR-1 level completed, HR_{max}, BLa^- and RPE. There is a high correlation for female soccer players between BLa^- and both the YYIR-1 test ($r = 0.73$, $P = 0.003$), the total distance covered ($r = 0.64$, $P = 0.014$) and the amount of high-intensity running ($r = 0.83$, $P < 0.001$) covered during a match (Krustrup et al., 2005).

Immediately post-AFT, BLa^- (mmol/L) was measured by collecting a blood sample from the left hand's fingertip and transferring this to a portable analyser (Lactate Pro, Arkray, Japan). Prior to collection, the ring finger was cleaned with an alcohol swab and the portable analyser calibrated according to the manufacturer's guidelines. This reading was measured as BLa^- and can be an indication of the degree to which the aerobic and anaerobic glycolysis systems are taxed during the test, and can ensure that the onset of fatigue occurred, as values above 8 mmol/L have been reported for soccer players (Svensson & Drust, 2005). The participants then indicated their RPE on a 10-point Borg scale, with 1 being the lowest and 10 the highest perceived exertion level (Borg, 1973).

Statistical Analyses

IBM SPSS Statistics (v. 24.0.0.0) was used to analyse the data. Descriptive statistics (means, maxima, minima and standard deviation values) were calculated at every time point for each variable. Linear mixed model analyses were then conducted to investigate time point differences with an autoregressive 1 covariance structure. Next, the variables were categorised as three rankings (low, average/neutral and high) as derived from the results for each time point (Spielberger, 1983; Terry et al., 2000). Prior to their ranking, the cortisol values were adjusted according to the awakening and sample collection times (Salimetrics, SalivaBio, 2011; Westermann et al., 2004). All the rankings demonstrated a large correlation with their respective variables at all time points ($r \geq 0.7$, $P \leq 0.01$). The score for each ranking was then expressed as a percentage of the total score at each time point as calculated by chi-squared analysis, with practical significance indicated by Cramer's V values.

Finally, Pearson's rank correlation, rho, was used to determine the relationship between cortisol, TMD; anxiety and AFT. A Fisher's r to z transformation was calculated to determine the 90% confidence interval (CI) from the correlation coefficient. The level of significance was set at $P \leq 0.05$. The strength of the correlation was categorized from ≤0.01 (trivial) to ≥1 (perfect). Cohen's effect sizes (ES) were calculated and interpreted as follows: ≥0.8 (large), ≥0.5 (moderate) and ≥0.2 (slight) (Cohen, 1998).

Results

The average results for the AFT were: Yo-Yo level: 14.1 ± 0.9; total distance: 560.9 ± 212.8 m; HR_{max}: 190.1 ± 8 bpm; BLa^-: 10.9 ± 3.6 mmol/L; RPE: 7.1 ± 1.9. From the 43 participants, 36 complete sets (cortisol and full questionnaires at pre- and post-AFT) were used for analysis, with incomplete sets excluded.

Cortisol results

Cortisol increased significantly (52.2%) from pre- to post-AFT (pre-AFT = 9.9 ± 10.5, post-AFT = 20.7 ± 17.9; $F_{(2,216)} = 5.2$; $P = 0.04$, ES = 0.7). The total proportion of high values increased significantly from 28.2% to 59% ($P < 0.01$, $V = 0.4$).

Psychological results

No significant TMD differences were observed, though the subscales confusion (pre-AFT = 2.1, post-AFT = 1.5, $P = 0.01$, ES = 0.5) and vigour (pre-AFT = 3.1, post-AFT = 2.4, $P = 0.02$, ES = 0.05) decreased significantly (Figure 1). No significant anxiety deviations were seen, although the subscale anxiety–absence was larger than anxiety–present (pre-AFT: –present = 16.5 ± 5.0, –absent = 21.6 ± 6.0, post-AFT: –present =16.2 ± 5.1, –absent = 22.5 ± 6.6). Chi-squared analysis revealed the TMD and anxiety scores as remaining essentially the same ($P > 0.05$). In addition, the total number of high scores increased from 16.4% to 33.3% post-AFT.

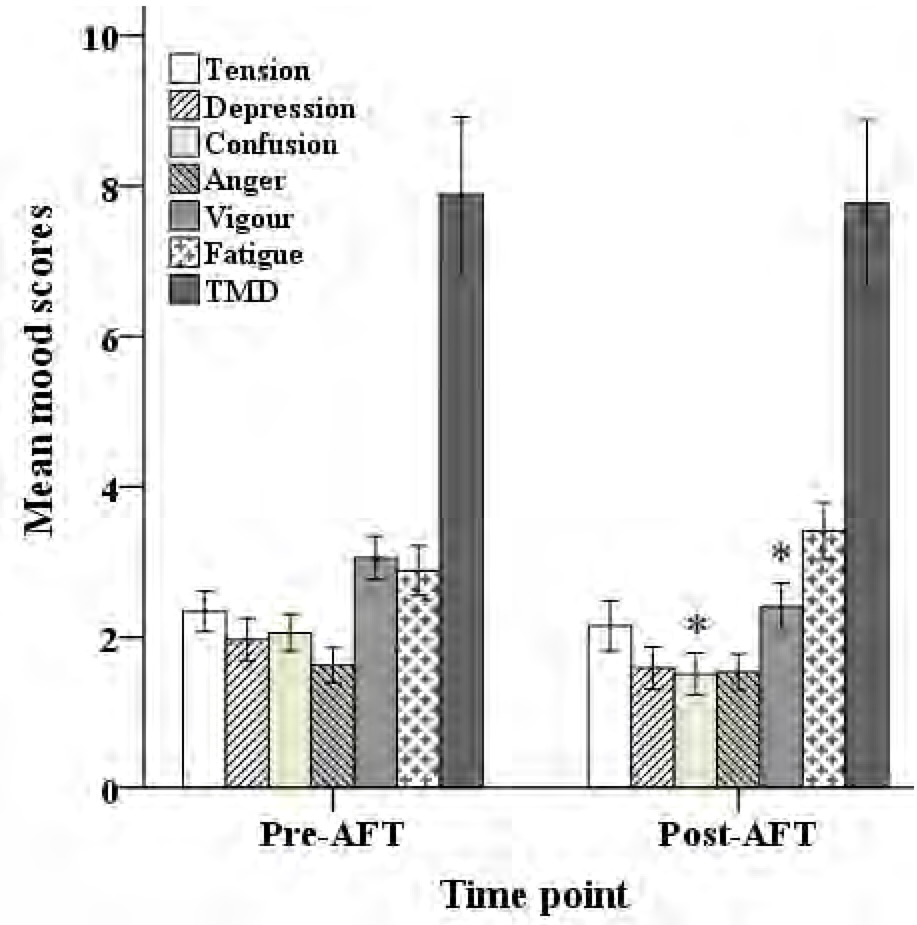

Figure 1. Mood responses at pre- and post-AFT.
* Significantly lower than pre-AFT ($p < 0.05$); CI ± 90%.
TMD, Total Mood Disturbances

Hormonal and psychological relationship results

When evaluating the correlation coefficient (r) at the 90% CI (Table 1), a slight but significant positive relationship was seen pre-AFT between cortisol and the anxiety–absence subscale ($r = 0.3$, $P < 0.05$). TMD correlated strongly with all its subscales as well as anxiety pre- and post-AFT ($P < 0.05$). Following the AFT, a small linear relationship ($r = 0.3$, $P < 0.05$) was derived between cortisol and BLa^-, fatigue with HR_{max}, Yo-Yo level as well as RPE, between ISP–anger and HR_{max} and vigour with RPE ($r = –0.4$, $P < 0.01$).

Table 1. Correlation coefficient (r) at the 90% CI at pre- (□) and post (□)-AFT.

r (CI 90%)	Cortisol	TMD	ISP: Tension	ISP: Confusion	ISP: Depression	ISP: Fatigue	ISP: Anger	ISP: Vigour	Anxiety total	Anxiety absent	Anxiety present
Cortisol	-	-0.1 (-0.2–0.1)	-0.1 (-0.2–0.2)	-0.1 (-0.3–0.2)	-0.1 (-0.3–0.1)	-0.2 (-0.3–0.1)	-0.1 (-0.2–0.3)	0.1 (-0.3–0.2)	0.2 (-0.0–0.4)	0.3* (0.1–0.5)	0.1 (-0.1–0.3)
TMD	-0.2 (-0.4–0.1)	-	0.6** (0.3–0.8)	0.7** (0.6–0.8)	0.8** (0.7–0.9)	0.7** (0.5–0.8)	0.6** (0.3–0.8)	-0.4** (-0.6– -0.2)	0.6** (0.6–0.8)	0.4** (0.2–0.6)	0.7** (0.5–0.8)
ISP: Tension	-0.3 (-0.5–0.0)	0.5** (0.2–0.7)	-	0.5** (0.2–0.7)	0.4* (0.1–0.7)	0.3* (0.1–0.5)	0.1 (-0.1–0.3)	0.3* (0.0–0.5)	0.4** (0.1–0.7)	0.1 (-0.2–0.5)	0.6** (0.4–0.8)
ISP: Confusion	-0.1 (-0.2–0.2)	0.7* (0.6–0.8)	0.2 (-0.1–0.5)	-	0.7** (0.7–0.9)	0.2 (-0.0–0.5)	0.5** (0.4–0.8)	0.0 (-0.2–0.3)	0.7** (0.5–0.8)	0.4** (0.2–0.6)	0.7** (0.6–0.8)
ISP: Depression	0.0 (-0.2–0.3)	0.8* (0.7–0.9)	0.3* (0.0–0.7)	0.7** (0.4–0.9)	-	0.3* (-0.0–0.6)	0.8** (0.5–0.9)	-0.1 (-0.3–0.2)	0.6** (0.5–0.7)	0.4** (0.2–0.6)	0.6** (0.5–0.8)
ISP: Fatigue	-0.2 (-0.3–0.0)	0.5** (0.2–0.7)	-0.1 (-0.4–0.2)	0.2 (-0.0–0.5)	0.0 (-0.3–0.3)	-	0.2 (-0.2–0.5)	-0.4** (-0.7– -0.1)	0.3* (0.0–0.6)	0.2* (-0.1–0.4)	0.4** (0.1–0.6)
ISP: Anger	0.1 (-0.1–0.4)	0.6* (0.3–0.8)	0.2 (-0.2–0.6)	0.5 (0.3–0.7)	0.8** (0.7–0.9)	0.2 (-0.2–0.5)	-	0.0 (-0.2–0.5)	0.4* (0.2–0.5)	0.2 (0.1–0.4)	0.4** (0.2–0.6)
ISP: Vigour	0.2 (-0.0–0.4)	-0.4* (-0.6– -0.1)	-0.1 (-0.3–0.3)	0.0 (-0.3–0.3)	-0.3* (-0.5– -0.1)	-0.4* (-0.7– -0.1)	0.0 (-0.2–0.4)	-	-0.1 (-0.4–0.2)	-0.2 (-0.5–0.1)	0.1 (-0.3–0.3)
Anxiety total	0.2 (0.0–0.4)	0.7** (0.5–0.8)	0.2 (-0.1–0.5)	0.7 (0.5–0.8)	0.6* (0.5–0.7)	0.2 (-0.2–0.4)	0.4* (0.2–0.5)	-0.1 (-0.4–0.2)	-	0.9** (0.8–0.9)	0.9** (0.8–0.9)
Anxiety absent	-0.1 (-0.1–0.4)	0.5** (0.3–0.7)	0.0 (-0.3–0.3)	0.4 (0.2–0.6)	0.4* (0.2–0.6)	0.2 (-0.1–0.5)	0.2 (0.1–0.4)	-0.2 (-0.5–0.1)	0.9** (0.8–0.9)	-	0.5** (0.3–0.7)
Anxiety present	0.1 (-0.1–0.4)	0.7** (0.5–0.8)	0.4** (0.1–0.7)	0.7* (0.5–0.8)	0.6* (0.5–0.8)	0.0 (-0.3–0.3)	0.4 (0.2–0.6)	0.1 (-0.3–0.3)	0.8** (0.7–0.9)	0.4** (0.3–0.7)	-
Yo-Yo level	-0.1 (-0.4–0.2)	0.2 (-0.1–0.5)	0.2 (-0.2–0.5)	-0.2 (-0.4–0.2)	0.0 (-0.3–0.2)	0.3* (0.0–0.6)	-0.1 (-0.3–0.1)	-0.3 (-0.5–0.0)	-0.1 (-0.3–0.2)	-0.1 (-0.4–0.2)	-0.0 (-0.3–0.2)
Maximal HR	0.1 (-0.2–0.4)	0.2 (-0.1–0.5)	0.2 (-0.1–0.4)	0.2 (0.0–0.4)	0.2 (-0.1–0.5)	0.0 (-0.3–0.2)	0.3* (0.1–0.5)	0.1 (-0.2–0.3)	0.2 (-0.1–0.5)	0.2 (-0.1–0.4)	0.2 (-0.1–0.5)
BLa^-	0.3* (0.1–0.6)	0.1 (-0.2–0.3)	0.1 (-0.2–0.3)	0.1 (-0.3–0.4)	0.1 (-0.2–0.4)	0.0 (-0.2–0.3)	0.0 (-0.2–0.3)	0.1 (-0.1–0.3)	-0.1 (-0.3–0.2)	-0.2 (-0.4–0.1)	0.1 (-0.1–0.3)
RPE	-0.3 (-0.5– -0.1)	0.3 (0.0–0.5)	0.2 (-0.1–0.5)	0.0 (-0.1–0.2)	0.0 (-0.3–0.3)	0.3* (0.1–0.5)	-0.2 (-0.4–0.1)	-0.4** (-0.7– -0.1)	0.1 (-0.2–0.3)	0.1 (-0.2–0.3)	0.1 (-0.2–0.4)

□ = 90% CI for various variables Post-AFT; □ = 90% CI for various variables Pre-AFT; BLa-, Blood Lactate; HR, Heart rate; ISP, Incredible Short POMS; RPE, Rate of Perceived Exertion; TMD, Total Mood Disturbances;
** $p < 0.001$; * $p < 0.05$

Discussion

This is the first study to evaluate the effects of an AFT on the cortisol, anxiety and mood states of South African amateur female soccer players. The most significant observation was the increase in cortisol (by 52.2% between pre- and post-AFT) with only two mood subscales (vigour and confusion) decreasing. This suggests that aerobic fatigue, as frequently observed during a match, primarily influences the individual's physiology and its underpinnings rather than the psychological state. In addition, a positive relationship between cortisol and anxiety–absence pre-AFT and between cortisol and BLa^- post-AFT was observed. The mood and anxiety scores correlated strongly at all times, whereas some mood subscale scores correlated with the AFT measurements. We therefore propose that there is a connection between physiological and psychological states when dealing with a physical stressor.

This study made use of an AFT, to induce similar fatigue experienced during a match, and determine the magnitude of its effects. Equivalent fatigue was indeed accomplished, as seen from comparable BLa^- and heart rate results from a similar study evaluating female soccer players (Krustrup et al., 2005). The sensation of fatigue during training is a common experience due to the increased training load and decreased energy stores of the athletes involved (Ament & Verkerke, 2009). These sensations can be physiologically and psychologically related, as exercise affects both a person's physiology and neuromuscular condition (Ament & Verkerke, 2009).

Hormonal responses

The significant cortisol increase is similar to previous reports (Karacabey et al., 2005; McGuigan, Egan, & Foster, 2004), which described a rise following either a 30-minute treadmill run (Karacabey et al., 2005) or after a high-intensity resistance training session, with no significant difference after low-intensity resistance training (McGuigan et al., 2004). A linear relationship between cortisol and physical exertion exists (McGuigan et al., 2004), as evident in the results between cortisol and BLa^- post-AFT. In this regard, training below 60% $\dot{V}O_{2max}$ results in a decrease in cortisol (Crewther, Hamilton, Casto, Kilduff, & Cook, 2015), which might be due to its removal exceeding its secretion by the adrenal cortex (Powers & Howley, 2007). On the other hand, training above 60% $\dot{V}O_{2max}$ causes cortisol increases, either in aiding the metabolism and mobilisation of energy sources to provide sufficient fuel (by promoting fat metabolism or increased blood glucose levels), or as a consequence of its secretion exceeding its removal (Dickerson & Kemeny, 2004; Powers & Howley, 2007). Although the players were in their in-competition phase at the time of testing, their $\dot{V}O_{2max}$ values were rated as average. In addition, the high BLa^- and HR values recorded following the test might indicate a quicker onset of fatigue owing to their training status, which has the ability to influence hormonal response patterns (McGuigan et al., 2004), seen from the correlation between cortisol and BLa^-.

Psychological responses

As seen from the negative correlation between ISP–vigour and RPE, fatigue caused vigour to decrease during the test. The significant reduction in the players' state of confusion might be attributed to them becoming familiar with the specific test, as it was completed regularly during the tournament season. Comparably, a significant decrease in tension, anger, fatigue and confusion was reported following a treadmill session at 61% HR_{max} in recreationally active females (Chase & Hutchinson, 2015). The body releases endorphins (the natural mood-enhancing hormones) during aerobic training, with a particular release threshold reached at higher training loads (Rocheleau et al., 2004). Though no significant anxiety changes were reported, previous research demonstrated reduced anxiety levels following aerobic training (Broman-Fulks, Berman, Rabian, & Webster, 2004; Cox et al., 2004). These dissimilar results from ours might be due to the length of the AFT, as other studies have proposed training for at least 30 minutes to stimulate the release of endorphins, and subsequently provoke improvement in psychological well-being (Cox et al., 2004; McDowell et al., 2016; Rocheleau et al., 2004). Alternatively, as another explanation, the participants had to complete the test until completely fatigued, whereas previous research evaluated a single training session of various intensities. Finally, negative mood states did not increase as postulated, which might have been due to the maintenance of a coherent mood state, as the training session offered a diversion from the tensions of normal life; alternatively, the positive feelings followed the accomplishment of a specific task, the AFT (Rocheleau et al., 2004).

The positive relationship between TMD and anxiety is similar to that reported by Guszkowska and Sionek (2009). They reported a correlation between trait anxiety and various mood subscales (tension and vigour) following training. Though not the sole measurement, an anxiety subscale was examined in the ISP questionnaire, which might explain this relationship. This noteworthy observation makes it possible to implement the questionnaire to indicate a participant's state of anxiety.

Hormonal and psychological relationships

Cortisol is involved not only physiologically in exercise or physical exertion, but also psychologically owing to its mutual relationship with behaviour (Nelson, 2017). Research indicates a link between the higher central nervous system's functioning and the neuroendocrine system, together with the psychological sense of training (Ament & Verkerke, 2009). Our findings demonstrated only a slight positive relationship between cortisol and the absence of anxiety pre-AFT. In contrast, Haneishi et al. (2007) described a higher positive correlation between cortisol and cognitive anxiety prior to training (r = 0.7). However, they did not report the specific training regime, making it possible that their participants anticipated more intense training (Smyth et al., 1998). Our participants, on the other hand, were familiar with the specific test, which reduced their perceived anxiety.

Anxiety questionnaires are primarily developed to detect anxiety changes, although it is plausible that other variables might be picked up, such as cortisol fluctuations. The relationship between behaviour and androgens is widely studied, with researchers believing that our emotions are influenced by physiological processes and vice versa (McCraty, Atkinson, Tomasino, & Bradley, 2009). A possible explanation for this relationship is the bottom-up approach — a stressful situation can result in various negative emotions, leading to erratic heart rates, taxing the nervous system, activating the HPA axis (thereby secreting cortisol) and, subsequently, impeding the psychophysiological system (McCraty et al., 2009).

Conclusions

To our knowledge, this is the first study to evaluate the effect of an AFT on the hormonal and psychological states of female soccer players in South Africa and, more generally, in Africa. The investigation demonstrated that an AFT, such as the Yo-Yo intermittent recovery test, led to an increase in cortisol and decrease in vigour.

We therefore propose that fatigue during a match (brought on by prolonged physical exertion) can act as a greater physiological than psychological stressor. Emphasis could therefore be placed on maximising the aerobic component, as it contributes to both aerobic and anaerobic match performance. The easy administration of the YYIR-1 test together with its soccer-specific nature makes it a useful tool for future evaluations to monitor soccer-specific fitness and hormonal changes. The positive relationship observed between cortisol and anxiety–absence can enable coaches to use the questionnaire to anticipate cortisol responses as a reaction to aerobic fatigue. In addition, we suggest that coaches implement a top-down strategy by using emotional self-regulatory exercises to achieve a coherent emotional state (McCraty et al., 2009), thereby indirectly influencing hormonal responses and their adverse consequences. Coaching staff can also combine match-induced stressors and aerobic fatiguing sessions with various psychological questionnaires to minimize the potential negative effects of cortisol and/or psychological states on match performance.

Our study affirms the negative effects of fatigue due to prolonged activity on the players' psychophysiological state. We believe that aerobic fatigue elicits a greater physiological stress response (which might be due to the depletion of energy stores) than a psychological stress response. We therefore suggest that players and their coaching staff focus on maximising the aerobic component, as it may contribute to longer quality match-time for players before fatigue sets in.

A few potential limitations were identified within the current study. From the 43 subjects that volunteered, only 36 complete sets were analysed. This occurrence might have been due to saliva samples not thoroughly analysed or questionnaires being incomplete. Subjects' acknowledged the SAI questionnaire being lengthy and thereby not completing all the questions. Therefore the authors suggest future studies make use of the ISP questionnaire as a strong correlation was found at all times between the SAI and ISP. It is well known that diet has a large influence over cortisol secretion, thus the subjects' diet prior to testing could have been thoroughly monitored and/or controlled for. Furthermore, a more thorough analysis of their sleep (duration, quality, amount of awakenings and awakening times) can be obtained for plausible explanations on the hormonal and/or psychological states. Furthermore, due to the circadian rhythm of cortisol, the saliva collection times could have been limited to a specific time period to exclude potential outliers. The fitness levels of the subjects' could have been a cofounding factor for the results, leading to a quicker onset of fatigue and slanting the results. Future studies can take into account the current readiness level of the subjects' by making use of questionnaires focusing on their physical and/or psychological inclination. Our study affirms the negative effects of fatigue due to prolonged activity on the players' psychophysiological state. We believe that aerobic fatigue elicits a greater physiological stress response (which might be due to the depletion of energy stores) than a psychological stress response. We therefore suggest that players and their coaching staff focus on maximising the aerobic component, as it may contribute to longer quality match-time for players before fatigue sets in.

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No potential conflict of interest was reported by the authors.

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5 THE EFFECTS OF A SOCCER TOURNAMENT ON THE PSYCHO-HORMONAL STATES OF COLLEGIATE FEMALE PLAYERS

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TITLE: THE EFFECTS OF A SOCCER TOURNAMENT ON THE PSYCHO-HORMONAL STATES OF COLLEGIATE FEMALE PLAYERS

RUNNING HEAD: PSYCHO-HORMONAL EFFECTS OF A SOCCER TOURNAMENT

AUTHORS: **ADELE BROODRYK,[1] CINDY PIENAAR,[1] DAVID EDWARDS[2] AND MARTINIQUE SPARKS[1]**

INSTITUTIONS: [1]PHYSICAL ACTIVITY, SPORT AND RECREATION RESEARCH FOCUS AREA, FACULTY OF HEALTH SCIENCES, NORTH-WEST UNIVERSITY, POTCHEFSTROOM, SOUTH-AFRICA;

[2]PSYCHOLOGY DEPARTMENT, UNIVERSITY OF ZULULAND, PRIVATE BAG X1001, KWADLANGEZWA, 3886, SOUTH AFRICA.

CORRESPONDING AUTHOR:

ADELE BROODRYK

PHYSICAL ACTIVITY, SPORT AND RECREATION RESEARCH FOCUS AREA, FACULTY OF HEALTH SCIENCES, NORTH-WEST UNIVERSITY, POTCHEFSTROOM, SOUTH-AFRICA

EMAIL: adele.broodryk@nwu.ac.za

TEL: +2718 2991804

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ABSTRACT:

Female soccer is becoming ever more popular worldwide. Nonetheless, there exist a gap pertaining to the connection between players' hormonal and psychological responses when playing a tournament, or even a match, and the outcome (victory or defeat). This study evaluates the effects of a week-long tournament on the psycho-hormonal states of collegiate female soccer players. Eight players' (n = 8) cortisol (saliva sample), mood states (incredibly short POMS [ISP]) and state–anxiety (state-subscale of the state-trait anxiety inventory) were assessed one hour prior to and 15 minutes after every match (n = 6). Cortisol increased significantly after most matches, with intra-match differences observed ($p < 0.05$). Match intensity (measured using tracking devices) influenced cortisol secretion, with increased secretion taking place as the intensity intensified. The ISP demonstrated intra-match differences for the subscales fatigue, depression, tension and vigour ($p < 0.05$). Matches lost produced a higher total mood disturbance (TMD) index compared to matches won. Cortisol correlated with the TMD and various mood-subscales prior to a winning outcome, with the ISP correlating at all times with the anxiety scores ($p < 0.05$). In conclusion, these results indicate that physiological and psychological variables combine to contribute to the stress response during a tournament. Focusing on high-intensity activities and minimising fatigue are important, as both are associated with raised cortisol and negative mood states. Lastly, implementing a mood questionnaire over a tournament can be beneficial, as sensitive information on players' hormonal and perceived anxiety states, which subsequently affect performance, can be obtained.

KEY WORDS: Soccer, cortisol, mood, anxiety, competition, women

INTRODUCTION:

As the popularity of female soccer increases globally (25), emphasis is placed on improving the quality of the game (23). Performing at an elite level is not solely due to talent (25), but also depend on high levels of physical, physiological and psychological skills (28). Achieving this desired level frequently exposes young players to various stressors, such as playing multiple matches either over consecutive days or on the same day, sharing accommodation, constant pressure to perform and restricted daily programs among others (35). A typical soccer match involves numerous high- and low-intensity activities, together with technical, tactical and psychological factors (25,28). Research indicated that high-intensity activities lead to various physiological and psychological fluctuations, such as cortisol and negative mood state escalations (7,9,13,31). To fully understand the stress experienced during a soccer match or tournament, the physiological as well as psychological facets should be considered (23). Nevertheless, there is little in current literature on the physiological and psychological stress responses during a female soccer tournament.

When faced with either a physical or psychological stressor, the hypothalamus–pituitary–adrenal (HPA) axis increases the secretion of cortisol (the primary hormone responsible for the allostatic stress response) from the adrenal glands (15,39). The HPA axis is regulated by certain brain regions, creating the ability for cortisol to be involved in numerous physiological and psychological features (5). Cortisol is a vital glucocorticoid for providing fuel, by increasing the blood glucose levels to either sustain an activity or, when faced with a stressor, to activate the fight or flight response (15,26). Cortisol also has the ability to aid in behavioral changes owing to the involvement of the HPA axis in affective and cognitive processes (15,23). In this regard, behaviors reported in the literature include aggression, arousal, addressing challenges (5), depression, cognitive anxiety (10,23) and mental fatigue, among others (11).

During major competitions, players frequently experience stress in various domains, such as communication (coach–player), match demands (intensity and physical fatigue), distractions (the opponents and mental fatigue), and competition areas (anxiety and performance evaluation) (25). Adding to the already experienced exhaustion from a solitary competition, competing in a series of matches over consecutive days may lead to accumulated mental and physical fatigue, which could alter mood states and the stress response (10,43). This, together with the contest for status, results in a stressful atmosphere, which might progressively intensify the apparent anxiety (8,25). Anxiety has the effect to influencing performance either positively or negatively (44). In this regard, an increase in cognitive and somatic anxiety was reported following a match (6), with the losing team experiencing higher anxiety and negative mood states compared to the winners (2,24,27,37). Not only does strong affective states have an influence over emotions, they can also activate physiological systems involved with behavior (39), thereby influencing cortisol secretion (37).

Few studies have assessed the effect of a competition on the hormonal and/or psychological state of female soccer players. In this regard, most reported an increase ($p < 0.05$) in cortisol values post-competition (2,8,19,23). In addition, two studies found no significant differences irrespective of the match outcome (19,37). Research on the psycho-hormonal relationship revealed a positive correlation between cortisol and cognitive anxiety, although it was after a soccer training session (23), with no correlation reported post-match (37). This highlights the necessity to investigate the psychological and hormonal aspects important for soccer performance, as there are differences among sportswomen (23). Unfortunately, measuring cortisol directly is labour-intensive and expensive, making it vital for sporting staff to implement a psychological questionnaire to indicate possible hormonal stress responses. If a relationship transpires between the outcome and/or physical characteristics of a match with cortisol and/or psychological states, interventions can be implemented to increase/decrease either or both variables to enhance their physical performance.

Although an abundance of research is available regarding male soccer players, there is limited published research on the hormonal and/or psychological state of female players (23). There is even less literature on the effect of a soccer tournament on their psycho-hormonal state and whether a relationship exists between these states and the match outcome. The study reported here serves as a basis for future research and to fill the current gap in knowledge by aiding the sporting fraternity to enhance their physical, physiological and psychological match performances. To the authors' knowledge, this is the first study to evaluate the effect of a soccer tournament and the internal and external match loads on both the cortisol and psychological states of collegiate South African female soccer players, and to determine whether a relationship exists between the variables and the outcome of the matches. It was hypothesized that a progressive increase ($d > 0.8$, $p < 0.5$) in cortisol, anxiety and total mood disturbances (TMD) would take place over the course of a tournament. Moreover, a linear relationship was expected between anxiety, cortisol and/or TMD. Following a victory, statistically lower cortisol and TMD responses were expected, whereas higher anxiety, TMD and cortisol responses would follow a defeat. Lastly, it was postulated that matches yielding greater high-intensity loads would have similar cortisol, TMD and anxiety outcomes.

METHODS

Experimental approach to the problem

A repeated measure, quantitative research design over a one-week tournament period was implemented to test the specific hypothesis. Variables assessed prior to and following the six matches played, were their anxiety and mood states (by using two questionnaires measuring each component separately) as well as salivary cortisol concentrations. Some matches were deemed vital in determining the opponents' faced in the play-offs. Internal and external match loads were monitored to provide insights whether any physical influences took place.

Subjects

Eight healthy non-Caucasian collegiate female soccer players (age: 23.1 ± 3.2 y; stature: 158.9 ± 6.4 cm; mass: 54.7 ± 4.2 kg, playing experience: 10.6 ± 5.6 y) from a tertiary institution volunteered to participate in the study. The subjects ranged from midfielders and attackers to defenders (including a goalkeeper) who provided written consent for participation in the study two weeks prior to testing. Ethical approval (NWU-00055-15-A1) from the university's Health Research Ethics Committee and permission from the tournament organizers and coaches were obtained prior to data collection. Subjects reported normal menstrual cycle phases, with the majority of players in the follicular phase at the time of testing and two in the menstrual phase. During the testing period they were in their competitive training phase, with average $\dot{V}O_{2max}$ values of 41.0 ± 1.5 ml/min/kg. As testified on the information questionnaire, none took any oral contraceptives or prescribed medication at the time of testing. Only subjects playing the entire match and remaining free of sickness and injury were included in the study.

Procedures

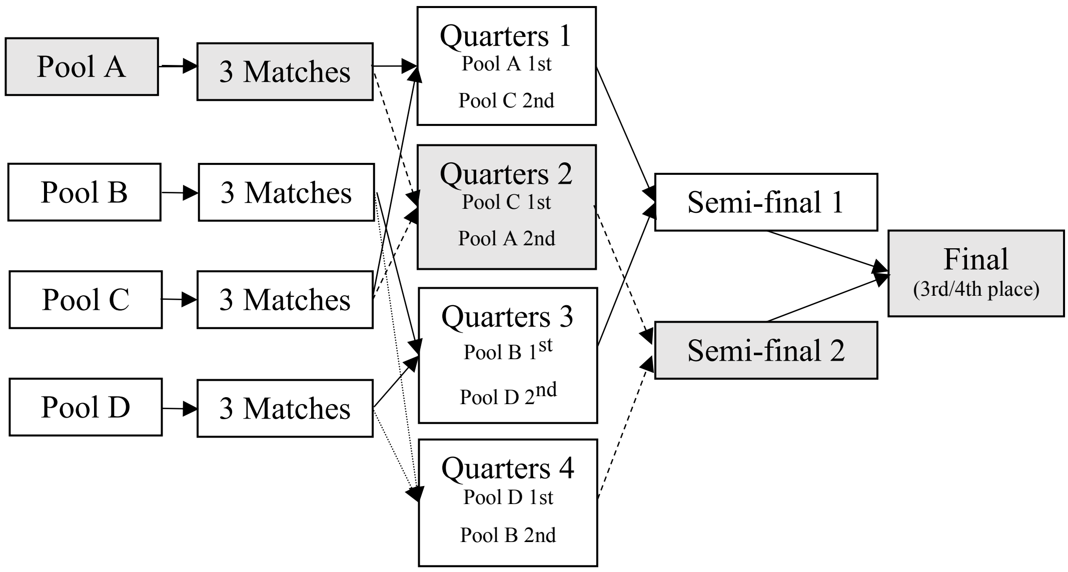

FIGURE 1. Diagram of how the matches were played during the tournament.

Data collection took place over one week, the tournament comprising of six matches with four pools played in a round robin format [three pool matches, followed by quarter-final, semi-final and final matches (Figure 1)]. The final pool match and the quarterfinal were played on the same day (morning and late afternoon).

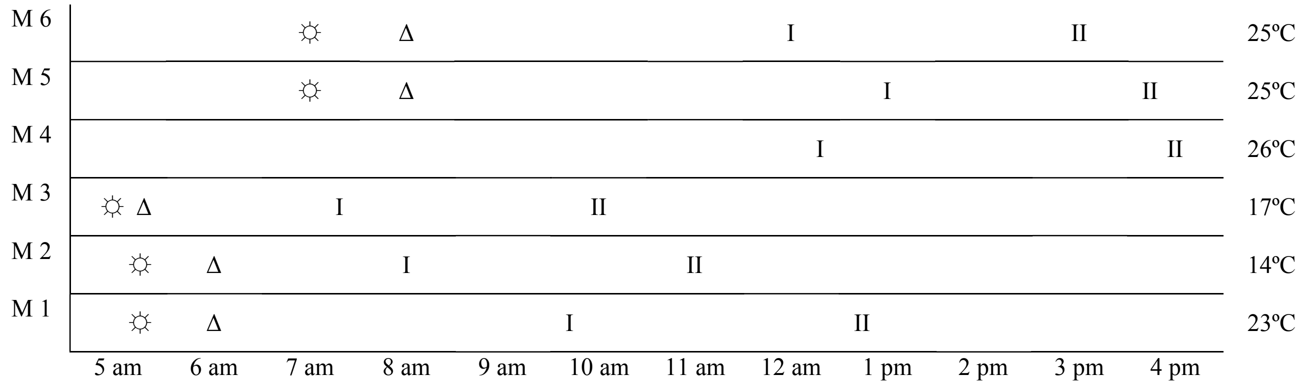

FIGURE 2. Detailed description of the wakening-, breakfast-, 1st and 2nd collection times over the tournament.

M – Match; ºC – Degrees Celsius; ☼ - Awakening time; Δ – Breakfast time; I – 1st collection time; II – 2nd collection time

On the evening prior to the start of testing, all procedures were explained and subjects completed the demographic and personal questionnaires. They were advised to get a full night's rest (at least eight hours' sleep) and to be awake at least an hour before the first data collection (a precise awakening time was specified every evening). Upon awakening on the first day, their body mass (BFW Platform scale, Adam Equipment o. Ltd., UK) and stature (Harpenden portable stadiometer, Holtain Ltd., UK) were recorded. On arrival at the tournament venue each day, the players were fitted with a global positioning system (GPS) and fixed polar heart rate (HR) transmitter belt to calculate the internal and external match loads. An hour prior to each match (immediately before the start of the warm-up), a saliva sample and State-anxiety Inventory (SAI) and Incredibly Short Profile of Mood States (ISP) questionnaires were completed (pre-match). Within 15 minutes after the match, the second saliva sample and completed SAI and ISP questionnaires were collected (post-match). This regime was conducted over five consecutive days for a total of six matches.

Internal and external match loads

Because of the influence of high-intensity activities on the hormonal and psychological states, the subjects' internal (HR) and external (match characteristics) match loads were monitored. On arrival at the sport grounds, each player received a GPS unit that fitted in a catapult support vest. The GPS units sampled at 10 Hz (MinimaxX V4.0, Catapult Innovations, Victoria, Australia) and measured distances covered and velocity data. In addition to the GPS data, an HR transmitter belt (Polar Electro, Kempele, Finland) was used to determine the HR values for every five-second interval during the course of the tournament. All heart rate values above 170 bpm were accepted as high-intensity values. The recordings from the GPS unit and heart rate monitor were downloaded to a computer and analyzed (Logan Plus V4.7.1, Catapult Sports, Victoria, Australia). Only their high-intensity movement patterns – running (4.5–5.3 $m.s^{-1}$) and sprinting (>5.4 $m.s^{-1}$) – were examined, as previous work proposed these as the main factors responsible for impaired match performance (16,30). For a movement to be recorded as an effort, the players had to maintain that velocity for at least one second.

Saliva sampling

Saliva samples were collected for the assessment of cortisol by using the passive drool test (40). The mouth was rinsed thoroughly with lukewarm water five minutes prior to sampling to remove any food substances. If needed, the subjects chewed on a piece of Para film to stimulate flow of saliva, which was collected through a plastic straw into a 20 ml vial. Samples were stored in a freezer (4 ± 1ºC) until analyzed by a professional laboratory. The cortisol content from 20 µl saliva samples were determined by using a cortisol saliva luminescence immunoassay. The samples were transferred into a Berthold luminometer to calculate the average relative luminescence units, which were plotted against the concentration to determine the exact value. There are both linear and non-linear correlation coefficients (r = 0.8–1.0) between saliva and serum cortisol values based on this method. This specific biochemical analysis has an intra-assay variation ranging between 0.4–1.7% and inter-assay variation between 0.8–1.8% (45). To minimize the effect of external factors, players were advised to be awake and fasted for an hour prior to sampling. Owing to the diurnal rhythm of cortisol in the body, the collection and awakening times of each player were logged (45). Post-match samples were collected after 15 minutes, as peak salivary responses occur within 10–30 minutes following a stressor (29).

Sport psychological questionnaires

Incredibly Short Profile of Mood States: The ISP, derived from the original Profile of Mood States questionnaire (32) compiled by Dean and colleagues (14) was completed at pre- and post-match times. Correlations ranging from r = 0.67–0.82 between the two questionnaires have been reported previously (14). The ISP consists of six questions that measure the six mood subscales (anger, depression, tension, confusion, fatigue and vigor). The subjects rated the questions on a five-point Likert scale: 1 ('not at all'), 2 ('a little'), 3 ('moderately'), 4 ('quite a bit') and 5 ('extremely'). Individual scores for each subscale was the rating indicated per question and the TMD score calculated by adding the negative subscales and subtracting the positive scale.

State Anxiety Inventory (SAI): The state-subscale of the original SAI was used to assess the subjects' perceived state anxiety levels at the two points in time over the five days (41). This is a 20-item questionnaire forming a subscale of the original 40-item questionnaire. The SAI evaluates how the subjects feel 'right now, at this moment' on a four-point Likert scale, ranging from 1 ('not at all'), to 4 ('very much so'). A rating of 4 indicates the presence of high anxiety levels whereas 1 denotes the absence of high anxiety levels (41). Anxiety levels were determined by calculating the sum of the different scores. The range of scores can vary from 20–80; the higher the score, the greater the anxiety perceived (41). Internal consistency reliability for state anxiety (r = 0.91) has been reported in previous research (4).

Statistical Analyses

IBM SPSS Statistics software (v. 24.0.0.0) was used for data analyses. Descriptive statistics (means, maximum, minimum, and standard deviation values) were calculated at every time point for each variable. Afterwards, linear mixed model analyses were completed to investigate time point differences with an autoregressive 1 covariance structure. Pearson's rank correlation rho was used to determine the relationship between all variables (cortisol, TMD, SAI and match outcome). A Fisher's r to z transformation was calculated to determine the 90% confidence interval (CI) from the correlation coefficient. The level of significance was set at $p \leq 0.05$. The strength of the correlation was categorized as follows: ≤0.01 (trivial), <0.3 (small), <0.5 (moderate), <0.7 (large), <0.9 (very large) and ≥1 (perfect). Cohen's effect sizes (*d*) were interpreted as large ($d \geq 0.8$), moderate ($d \geq 0.5$) and small ($d \geq 0.2$) (12).

Following this, the cortisol values were graded on a three-point scale (low, average/neutral or high) according to their awakening and sample collection times, based on normal daily variations as reported by Broodryk (7), Salimatrics manual (40) and Westermann *et al.* (45), to eliminate external factors from slanting the results. In addition to this, the ISP and SAI scores were ranked into three categories (low, average/neutral or high) based on norms previously reported (41,42), to determine significant differences regarding the high values between time points. The percentage of the total score, as calculated by chi-squared analysis, for each ranking at every time point was then determined, with practical significance indicated by Cramer's *V* values.

RESULTS

Internal and external match loads

Matches 1, 2 and the quarter-final (penalty shoot-out) were won, match 3 tied, and the semi-final and final (for third place) matches lost. The nature of the victory/defeat did not differ extraordinarily between matches (match 1: 4–0; match 2: 2–0; match 3: 0–0; quarter-final: 1–1, leading to a penalty shootout score of 4–3; semi-final: 0–1, final: 0–2). Moreover, based on the previous year's standings of the opponents, matches 1–3 were against the lower ranked, the quarter-final against more or less an equally ranked team, and the semi- and final against the previous year's first and third ranked teams.

TABLE 1. Descriptive statistics (± *SD*) of the GPS data over the tournament

Variable	Match 1	Match 2	Match 3	Quarter-final	Final	Variance between matches
Total distance (m)	5622.3 ± 841.2	5942.6 ± 540.1	5835.0 ± 615.4	5166.5 ± 719.3	5190.7 ± 883.3	$F_{(4:29)} = 1.1$, $p = 0.4$
LI activities (distance %)	78.9 ± 4.5	77.3 ± 3.3[a]	80.4 ± 3.1	82.4 ± 1.7	82.8 ± 4.3[a]	$F_{(4:29)} = 2.9$, $p = 0.04$*
HI activities (distance %)	14.8 ± 3.3	16.5 ± 2.3[b]	13.5 ± 2.5	11.5 ± 2.0[b]	11.9 ± 3.0	$F_{(4:29)} = 3.0$, $p = 0.04$*
Average HR (bpm)	179.4 ± 15.3[c]	169.4 ± 10.9	165.0 ± 9.2	164.0 ± 7.9	151.4 ± 10.5[c]	$F_{(4:29)} = 5.8$, $p = 0.001$**
HI HR (% total time)	71.4 ± 20.3[d]	54.6 ± 25	45.3 ± 20.7	45.2 ± 19.7	26.7 ± 15.1[d]	$F_{(4:29)} = 4.4$, $p = 0.006$**

bpm, beats per minute; HI, High intensity; HR, Heart rate; LI, Low intensity; *$p < 0.05$; **$p < 0.01$, a, b, c, d = Significant difference between matches

Internal and external match loads are provided in Table 1. Unfortunately, player loads for the semi-final match were lost and, therefore, were not included or used to verify the respective match's results. Seen from Table 1, match 2 demonstrated the least number of low-intensity activities ($p = 0.04$) together with the maximum number of high-intensity activities ($p = 0.04$) compared to the final and quarter-final match respectively. In addition, the final match (played for third place, and lost) demonstrated the lowest percentage time spent in high-intensity HR zones ($p = 0.001$) and lowest average HR ($p = 0.006$) compared to matches one and two (won).

Cortisol results

As seen from Figure 3, no significant differences in cortisol responses (i.e. $\Delta_{cortisol}$ = post-match – pre-match-values) were evident when comparing the various match outcomes, though a significant difference was seen for pre-match values between a victory and a draw ($n_W = 24$, 12.6 nmoll/L; $n_L = 16$, 6.7 nmoll/L; $n_D = 8$, 22.8 nmoll/L, $p = 0.001$).

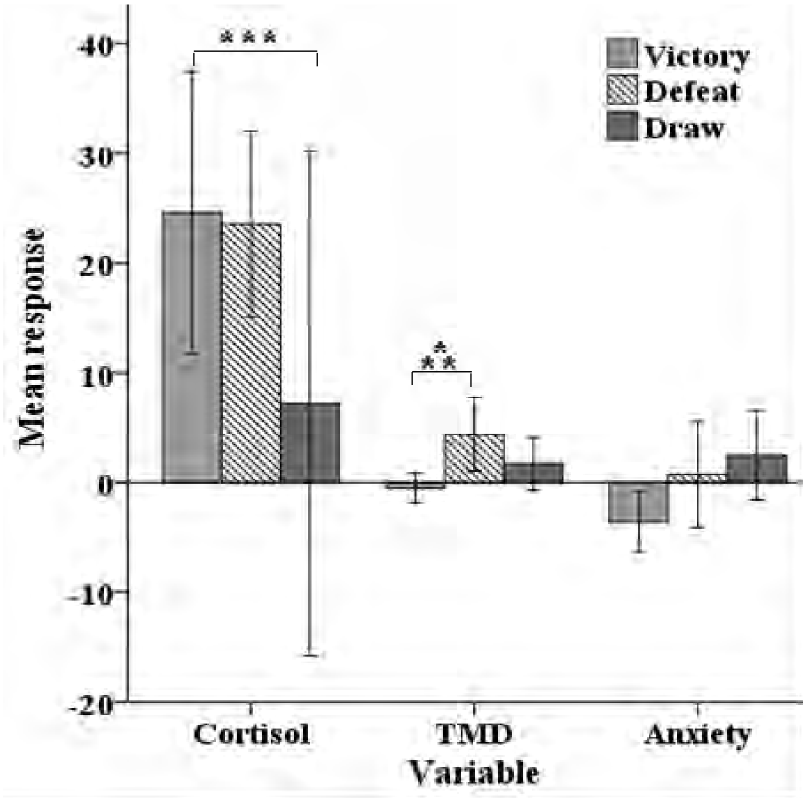

FIGURE 3. Mean overall responses for winning (n = 3), losing (n = 2) and a tie (n = 1) outcomes

* Significant difference in pre/post-values for win/lose/tie ($p < 0.05$), ** Significant change in mood response (Δmood = post – pre-score) for winners *vs.* losers ($p < 0.05$). TMD, Total Mood Disturbances; $CI \pm 90\%$

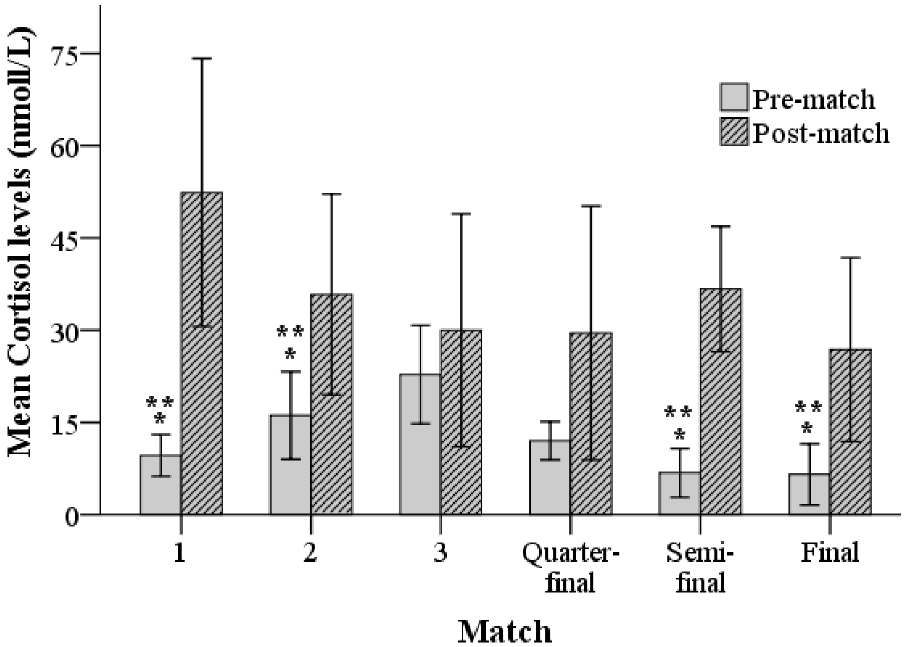

FIGURE 4. Mean cortisol values during the tournament.

* Significantly different from same-day post-match values ($p \leq 0.05$); ** Significantly different from match 3 pre-match values ($p \leq 0.05$); $CI \pm 90\%$

From Figure 4 it is clear that pre- to post-match cortisol increased significantly for matches 1, 2, the semi-final and final ($p < 0.05$, $d > 1.0$). Comparing the pre-match values, match 3 demonstrated significantly higher values compared to match 1 ($p = 0.03$, $d = 1.4$), match 2 ($p = 0.04$, $d = 0.6$), the semi-final ($p = 0.004$, $d = 1.7$) and final ($p = 0.004$, $d = 1.6$) played for third place ($V = 0.5–0.8$).

Psychological results

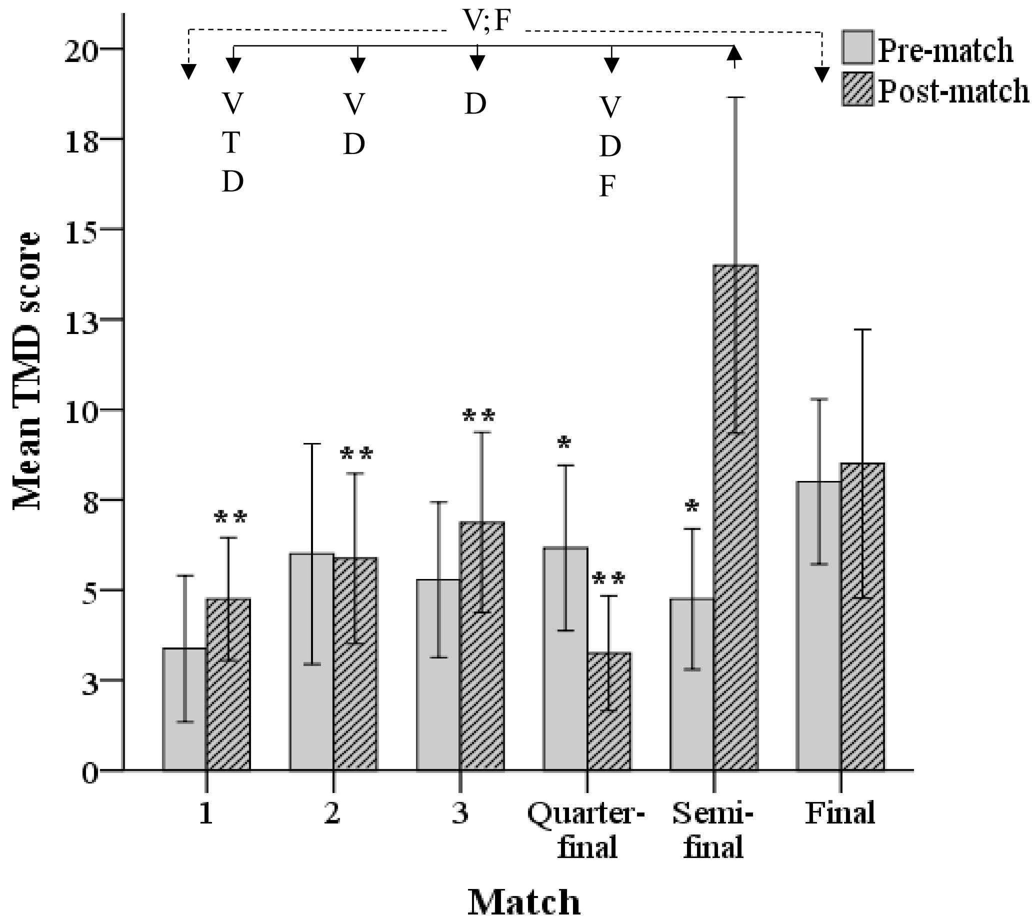

FIGURE 5. Mean TMD scores during the tournament.

* Significantly different from same-day post-match values ($p \leq 0.05$);

** Significantly different from semi-final's post-match values ($p \leq 0.05$).

---► Significantly different in ISP subscales between day 1 and finals (for 3rd place) pre-match ($p \leq 0.05$);

——► Significantly different in ISP subscales between semi-final and post-match indicated ($p \leq 0.05$)

D, Depression; F, Fatigue; T, Tension; ISP, Incredibly Short POMS; TMD, Total Mood Disturbances; V, Vigor; $CI \pm 90\%$

Both the quarter-final (decreased) and the semi-final (increased) matches demonstrated significant pre- to post-TMD changes ($p < 0.05$, $d = 1.1$ and 2.0) (Figure 5). The semi-final resulted in a significantly higher post-match TMD score compared to match 1 ($p = 0.002$, $d = 2.1$), match 2 ($p = 0.008$, $d = 1.7$), match 3 ($p = 0.03$, $d = 1.5$) and the quarter-final ($p < 0.001$, $d = 2.5$). Chi-square analysis revealed the TMD scores graded as 'high' to be significantly higher prior to match 1 compared to the other games, and post-match calculations were significantly higher following the semi-final compared to the other games (60% and 67% respectively, $p < 0.05$, $V > 0.5$).

Regarding the mood questionnaire's subscales, vigor ($p = 0.007$, $d = 1.6$) and fatigue ($p = 0.05$, $d = 1.0$) were significantly higher prior to the final compared to match 1. The post-semi-final match scores were significantly higher compared to pre-match scores for match 1 [vigor ($p = 0.05$, $d = 1.8$), tension ($p = 0.05$, $d = 1.8$) and depression ($p = 0.001$, $d = 2.2$)], match 2 [vigor ($p = 0.01$, $d = 2.0$) and depression ($p = 0.01$, $d = 1.7$)], match 3 [depression ($p = 0.03$, $d = 1.6$)] and the quarter-final [vigor ($p = 0.006$, $d = 2.2$), depression ($p = 0.01$, $d = 2.4$) and fatigue ($p = 0.04$, $d = 1.7$)].
When examining the effect of the match outcome, post-match results demonstrated a significantly higher amount of mood disturbances after losing compared to winning (win: 4.6; lose: 10.9, $p < 0.001$). The mood changes over the tournament (i.e. Δ_{mood} = post-game – pre-game levels) were different between a victory and defeat ($n_W = 21$, -0.6; $n_L = 15$, 4.4; $p = 0.02$), with the losing outcomes having a significantly higher amount of TMD compared to the matches won (Figure 3).

Cortisol versus psychological relationship results

Some correlations were noted for either a winning or a losing match (Table 2). In this regard, prior to a win, there was a moderate to large positive relationship between cortisol and TMD ($r = 0.5$, $p = 0.03$), ISP–tension ($r = 0.6$, $p = 0.006$), –depression ($r = 0.5$, $p = 0.04$) and –fatigue ($r = 0.4$, $p = 0.05$), with no significant relationships post-match. The TMD and SAI-anxiety questionnaire scores correlated moderately to largely prior to and following a victory ($r \geq 0.5$, $p < 0.05$) and a defeat ($r \geq 0.6$, $p < 0.05$). Prior to a defeat, the psycho-hormonal relationships shifted, with a small relationship perceived between cortisol and ISP–confusion as well as ISP–anger ($r = 0.3$, $p < 0.05$), whereas thereafter, cortisol demonstrated only a small negative relationship with ISP–vigor ($r = –0.3$, $p < 0.05$).

TABLE 2. Correlation coefficient for winning and losing outcomes pre- and post-match.

	(r) (90% CI)	TMD	SAI - Anxiety	ISP: Tension	ISP: Confusion	ISP: Depression	ISP: Fatigue	ISP: Anger	ISP: Vigor
Pre-match (won)	**Cortisol**	0.5* (0.0–0.8)	0.2 (-0.3–0.6)	0.6** (0.2–0.8)	0.4 (-0.1–0.7)	0.5* (0.1–0.8)	0.4* (-0.4–1.0)	0.4 (-0.1–0.7)	-0.2 (-0.5–0.2)
	TMD	-	0.7** (0.5–0.9)	0.5* (0.2–0.8)	0.8** (0.5–0.9)	0.8** (0.8–0.9)	0.6** (0.3–0.8)	0.8** (0.5–0.9)	-0.7** (-0.8– -0.5)
	SAI - Anxiety	-	-	0.1 (-0.3–0.6)	0.8** (0.7–0.9)	0.7** (0.5–0.9)	0.3 (0.0–0.6)	0.4 (0.2–0.7)	-0.6** (-0.8– -0.4)
Post-match (won)	**Cortisol**	-0.1 (-0.4–0.2)	0.3 (0.0–0.5)	0.0 (-0.3–0.3)	-0.1 (-0.3–0.2)	-0.1 (-0.4–0.1)	-0.1 (-0.4–0.2)	-0.1 (-0.3–0.1)	0.1 (-0.2–0.4)
	TMD	-	0.5** (0.2–0.8)	0.6** (0.3–0.8)	0.6** (0.3–0.9)	0.7** (0.3–0.9)	0.7** (0.5–0.9)	0.5* (0.3–0.8)	-0.5* (-0.8– -0.2)
	SAI - Anxiety	-	-	0.6** (0.3–0.8)	0.6** (0.2–0.8)	0.5* (-0.0–0.8)	0.1 (-0.3–0.3)	0.1 (-0.2–0.6)	-0.1 (-0.4–0.2)
Pre-match (lost)	**Cortisol**	0.2 (-0.2–0.6)	0.4 (-0.1–0.8)	-0.3 (-0.6–0.0)	0.3* (-0.3–0.9)	-0.0 (-0.3–0.4)	0.5 (-0.1–0.7)	0.3* (-0.3–0.9)	-0.4 (-0.8–0.5)
	TMD	-	0.6* (0.4–0.8)	0.5* (0.1–0.8)	0.5 (0.3–0.8)	0.9** (0.7–1.0)	0.7** (0.5–0.9)	0.6* (0.3–0.9)	-0.3 (-0.7–0.0)
	SAI - Anxiety	-	-	0.3 (-0.1–0.6)	0.6* (0.4–0.8)	0.4 (0.1–0.8)	0.4 (0.1–0.7)	0.3 (-0.1–0.7)	-0.3 (-0.7–0.3)
Post-match (lost)	**Cortisol**	-0.1 (-0.6–0.4)	-0.1 (-0.5–0.3)	0.0 (-0.5–0.5)	-0.4 (-0.7–0.0)	-0.2 (-0.6–0.3)	-0.0 (0.5–0.6)	0.1 (-0.4–0.6)	-0.3* (-0.5–0.6)
	TMD	-	0.7** (0.3–0.9)	0.8** (0.6–0.9)	0.7* (0.0–0.9)	0.8** (0.7–0.9)	0.5 (0.1–0.8)	0.6* (0.1–0.9)	-0.7* (-0.9– -0.4)
	SAI - Anxiety	-	-	0.6* (0.2–0.9)	0.7** (0.4–0.9)	0.5 (0.2–0.8)	-0.0 (-0.4–0.3)	0.7* (0.3–0.9)	-0.3 (-0.8–0.2)

CI = Confidence intervals; ISP = Incredibly Short POMS; SAI = State Anxiety Inventory; TMD = Total Mood Disturbances; * = $p < 0.05$; ** = $p < 0.01$

DISCUSSION

The aim of this study, the first of its kind, was to determine the effect of a six-match tournament on the hormonal and psychological states of collegiate female soccer players. It appeared that physiological and psychological aspects combine in contributing to the significant stress response observed during a tournament. The most significant finding was the acceptability of the ISP questionnaire in indicating underlying physiological and psychological stress prior to and/or following a competition. Furthermore, cortisol increased significantly from pre- to post for most matches. Mood states demonstrated significant fluctuations, also demonstrating variations between matches within its subscales. A significantly higher TMD response was observed following a losing match compared to a win.

Cortisol results

Throughout the tournament, cortisol secretion increased from before to after each match played. The observed increases in cortisol for a single match or over the tournament are similar to other corresponding studies on female soccer (2,8,17–19,23), signifying the physiological and/or psychological stress experienced (8). Physiologically, sample collection time can have a large effect on the cortisol response because of the body's circadian rhythm (23). In this regard, cortisol is known to peak 20–45 min after waking (11) in response to the activation of forthcoming daily demands and memory representations (21). Furthermore, peak cortisol responses take place 10–30 min after a stressor (17), caused by the strong feedback action at the level of the hippocampus, hypothalamus and pituitary gland (29). For this reason, samples were collected an hour prior to and 15 min following a match. Another potential explanation for the cortisol increases might be the physical exertion of each match (26,31), leading to increased haemoconcentration due to fluid shifts, dehydration (8) or the accumulated metabolites (inflammatory response, blood lactate, decreased blood pH level or hypoxia) that stimulate the HPA axis, and consequently, cortisol secretion (26,33).

Psychologically, the stress response can act by two mechanisms: at first a short catecholamine latency takes place, which is dependent on the sympathetic nervous system (thus initiating the fight or flight response); or a slower glucocorticoid response dependent on HPA activation, thereby stimulating cortisol secretion, which is only evident 30 minutes following the stressor (10). The observed cortisol upsurges following the matches cohere with the HPA stress response in contributing to the biosocial model of status, as explained by Jiménez and colleagues (26). Evaluating the games separately or in concurrence, the high cortisol secretion following match 1 might be due to the unfamiliar and ambient environmental conditions of the tournament and/or game set-up (23), decreasing as the tournament progressed and players became acquainted with the environment.

Similar trends were observed for cortisol responses regardless of the outcome. Though further analysis over the specific time slots revealed significant differences prior to the match drawn compared to the winning outcomes. The noteworthy difference between match 3 (which ended in a draw) and the other matches' pre-cortisol levels may again have physiological and psychological underpinnings. Firstly, match 3 saliva samples were collected early, and given the diurnal rhythm of cortisol, could have resulted in the higher values observed (10,45). Secondly, lower ambient temperatures compared to the other days were recorded when collecting the samples, which has a direct influence on cortisol secretion (38). Thirdly, match 3's result had a large influence on the quarter-final playoff, and given the increased cortisol trend observed from matches 1 to 3 as well as the perceived strength of the opposing team (5), there might have been a greater stress response in anticipation of that match (8,23), with the players plausibly sensing some form of discomfort (26). Lastly, the elevated pre-values might have been due to the cumulative effect of fatigue from the previous two matches (match loads of matches 1 —most time spent in high HR zones — and 2 — the maximum number of high-intensity activities), depleting the glycogen stores and ultimately increasing cortisol secretion (3). This is in accordance to a previous study evaluating the effect of a soccer tournament, testifying that the accumulated fatigue due to the congested schedule lead to testosterone and defensive actions decrements (34).

Regarding this, a typical match consists of numerous aerobic and anaerobic activities, which, together with the 90-minute length of a match, can result in greater cortisol reactions compared to other sports (23). A linear relationship between physical exertion and cortisol (33) was evident in our results. Match 2 displayed the maximum distance and high-intensity activities completed, and match 1 the highest amount of time spent in a high-intensity HR zone. Together with significant cortisol increases in these two matches, it is evident that higher cortisol levels are interrelated to high-intensity activities (7,10). In contrast, the quarter-final and final matches revealed the lowest physical exertion (as seen from the internal and external player loads) and cortisol values. The primary role of cortisol is to mobilize energy sources to muscle tissues by increasing blood glucose levels (15). Thus, matches played at a relatively high level of intensity, thus depleting the energy stores and leading to metabolite accumulation (33), might produce greater cortisol reactions. However, this elevated secretion can have a catabolic effect, reducing protein synthesis and inhibiting the inflammatory process (10). Furthermore, the higher cortisol levels might be due to varying hormonal reactions that depend on the intensity level or training status of the players (10,31), which also has a direct influence on psychological states (5). Thus, these observations emphasize the importance of adequate preparation for and recovery during a tournament, to limit the occurrence of fatigue and its adverse consequences.

A large variation was noted in the confidence intervals when evaluating the cortisol responses. This observation might be due to the small sample size, and more specifically, for the match drawn (n = 1; 8 players), leading to hefty disparities. In addition, the player's individual stress reaction (having a direct influence on cortisol responses) could also play a big role, as some players demonstrated a considerable cortisol response either prior to or after a stressor. Though no significant relationships were noticed between any menstrual phase and cortisol, mood or anxiety scores, it is plausible that a slight influence might have taken place as two players were in the menstruating phase, though this influence would have been observed comparing the matches and not pre- to post values as both time periods would elicit higher ranges. Cortisol is known to increase during the menstruating phase, with values being higher during the luteal compared to the follicular phase (22). In addition to this, previous research has specified the negative effects of the menstruating phase on mood states, though acknowledging the beneficial effect of regular training in decreasing these negative states by either acting as distraction, improving self-efficacy or the social connectedness via the team (1).

Psychological results

As reported in comparable studies, there was a decrease in positive mood states for most of the matches (2,10,24,27). In a preceding study, a progressive increase in rate of perceived exertion was noticed as a soccer tournament progressed, with the authors stating that this might be due to increased anxiety levels, psychological stress, more difficult matches and opponents to face as well as accumulated fatigue (35). The literature suggests that mood disturbances follow a linear relationship with the physical and mental stress associated with competition (2). As with the cortisol results, there was a progressive increase in post-match TMD scores from match 1 to match 3, due to tiredness, the significance of certain matches for the playoffs or likely cortisol influence on them. Physical stress can be greatly influenced by the intensity of the match (usually leading to the onset of fatigue) (25), subsequently affecting mood states adversely (43). This could be due to accumulated physical or mental fatigue, adverse management or a change in the incidence of the two types of fatigue, or the relationship between mental and physical fatigue (43).

As in the last-mentioned study (43), match 1 was associated with the smallest TMD increases prior to and after the match (except compared to the quarter-final, though a high pre-score was registered) compared to the rest of the tournament. This observation changed as the players adapted to unfamiliar environmental playing conditions, consequently causing a greater physical and psychological stress reaction (2). The increasing TMD trend (seen from the significant increase/decrease in the subscales fatigue/vigor compared to the final match) could again be due to exhaustion, or because the first match was not an efficient forecaster of the playoffs. The semi-final match resulted in significantly higher post-match mood-subscale scores compared to other matches. The results from the two aforementioned matches (match 1 and the semi-final), support the notion that the match outcome greatly influences mood states, with winning associated with positive, and losing with negative attitudes (27). The lower TMD scores following a victory compared to a defeat might be a result of lower psychological anxiety or stress associated with the victory (8,26). Other studies have reported elevated depression, fatigue and confusion and lower vigor levels following a defeat, with the opposite seen after a win ($p < 0.05$) (10,24,27). According to Haneishi (23), if attention is directed towards arousal (seen from Figure 5 on the ISP subscale — vigor), performance can decline owing to the relationship between perceived psychological arousal and affective-autonomic arousal. No significant differences were noted between pre and post-values for games played early, thereby excluding the possible effect of the ambient cold temperatures on positive affect. Consistent with literature, a bidirectional relationship exists between behavior and hormones (36). Hormones aid in behavioral changes by influencing the input (sensory), integrator (central nervous system) or output (effectors) system (36). Strong emotions, conversely, trigger certain behaviors (such as the fight or flight response), subsequently activating physiological reactions to support the relevant situational behavior (39).

Psycho-hormonal relationships

A moderately positive relationship between cortisol and TMD prior to a victory (as a result of the number of correlations with the various subscales), was observed in this study. Further analysis of this respective questionnaire's subscales demonstrated a positive relationship between cortisol and ISP-tension, depression and -fatigue. Probable reasons either being the anticipation of matches, perceived strength of the opponents (5), the experimental team's former performances and fatigue. Anticipation to a stressor is known for increasing cortisol secretion and perceived anxiety (23,37). High depression scores were recorded prior to the quarterfinal match, which might be due to players expecting an undesirable result as the team have never qualified for a semi-final in a major tournament. In addition, a possible explanation for the observed correlation between cortisol and fatigue, might be the physical exertion of the matches played previously, increasing physical fatigue. Mentally, it is plausible that stress, anticipation or poor sleep increased cortisol and subsequently, exhausted the players (11). This could perchance clarify the non-significant relationships between cortisol, anger and confusion preceding a winning outcome. During further inspection, the highest negative scores were recorded prior to the quarter-final match played. Again plausibly, due to them having negative prospects over the approaching game.

Additionally, the mood subscales ISP–anger as well as ISP–confusion demonstrated a small positive relationship with cortisol prior to a defeat, suggesting that irrelevant and intrusive thoughts, adding to the stress response and increasing cortisol, had an impact on their match characteristics (20). Lastly, a slight negative relationship between cortisol and ISP–vigor was seen after matches lost. With only the last two matches ending in defeat, the author suggests that fatigue along with the rising cortisol levels might have contributed to the players feeling less energetic.

Increased mood and anxiety states between and prior to matches may have adverse effects on cortisol secretion, subsequently having an undesirable effect on physical match characteristics. As noticed from our results, the ISP questionnaire correlated with cortisol as well as the Spielberger SAI questionnaire throughout the tournament. Firstly, given the length of the anxiety questionnaire, administering the ISP questionnaire is beneficial, as adequate time is not always available for completing the entire survey. Furthermore, cortisol analysis is expensive and time-consuming, hence administering the ISP questionnaire during a competition, and in particular prior to one, is advisable, as vital information on the physiological and psychological stress responses might be indicated.

CONCLUSION

This is the first study to evaluate the effect of a week-long tournament and its outcome on the psycho-hormonal states of collegiate female soccer players in South Africa, and more broadly, Africa. It appeared that both physiological and psychological variables combine (as seen in the increase in cortisol and negative mood state) to contribute to the stress-hormone response observed during a single competition. The cortisol and mood state results could be a reflection of anticipation of the game's outcome, the perception over the opponents' strength, their own respective failures, the circadian rhythm of cortisol, or the influence of exhaustion and/or physical exertion. Matches demanding the highest internal and external load, thus making the greatest demand on the players, demonstrated similar cortisol and TMD increases. Our results signify the importance of adequate physical and mental preparation, as fatigue – either due to a single match or over consecutive days – can accompany various detrimental physiological and psychological consequences on elements vital for competition. Though an abundance of literature is available on the physical (internal and external loads) characteristics of a female soccer match, limited research is available on the consequences and relationship of cortisol and psychological states.

The relationship recorded between mood states and cortisol prior to and after a competition was consistent with previous studies. In addition, the TMD scores demonstrated significant correlations with the state-anxiety scores at all times, implying the adequacy of the ISP questionnaire in indicating underlying anxiety states during a tournament. The authors suggest that coaching staff implement a mood questionnaire prior to a competition, as essential physiological and psychological information could be collected. Moreover, improving the players' mood states may be beneficial to influence their cortisol and perceived anxiety positively.

Competing at a high level may necessitate that matches be played over consecutive days, increasing the experience of fatigue and a stressful atmosphere (35). Further studies regarding the psycho-hormonal effect of a female soccer tournament are therefore needed to verify the results presented here. Furthermore, testing a larger population would prove valuable, not only to exclude outliers slanting the statistics, but also to determine dissimilarities between starters, non-starters, positions and duration of play time (as found in previous research (23)). Recorded weather temperatures varied during collection times, and as previous research has indicated, it has a direct influence over cortisol secretion and positive effect. Future studies should pose a question to their participants to indicate their affective states towards ambient conditions. As noted from the timeline, matches were played at various time points, making direct comparisons difficult as cortisol follows a diurnal rhythm, peaking during the early mornings and then sloping as the day pass. Although the current study did evaluate and rank each value according to sample collection time, future studies can try to evaluate matches completed at precise same times to make more direct comparisons possible. Though the strength of the opponents were determined by the previous year's standings, future studies can pertain the players' perception over the opponents' strength as well as their perceived exertion over each match to compare to previous research (34,35).

PRACTICAL APPLICATIONS

The present study provided a novel examination of the psycho-hormonal influence of both a single soccer match and a tournament on collegiate female players. The findings suggest that a high physiological and psychological stress response takes place preceding a match, permitting coaches and players to appreciate the total stress accompanying high-level competition. Although high-intensity activities contribute only a small percentage of the total effort during a match, their influence on cortisol and negative mood states is immense. Maximizing physical, physiological and psychological states, and mitigating the effects of physical and mental fatigue present a challenge for coaches. Thus, quantifying the physical demands and their subsequent hormonal and psychological effects during a match is important. Cortisol measurements are considered important for their physiological and psychological consequences, though, unfortunately, they are expensive and labor-intensive. Therefore, discovering their possible responses in other ways is deemed important. There was a strong relationship between the mood and anxiety questionnaire as well as with cortisol either prior to or following a competition. It is therefore proposed that the ISP questionnaire be administered, as it can provide significant information on the physiological and psychological stress response. In conclusion, maintaining a coherent mind set and heightening a positive mood state, might decrease over-secretion of cortisol and anxiety accumulation, thereby, indirectly enhancing vital physical match characteristics.

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6 SUMMARY, CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

1. SUMMARY

The purpose of this study was threefold: firstly, to determine the effect of an anaerobic fatiguing test on salivary cortisol and the psychological states of amateur female soccer players, and whether a relationship exists between these variables and/or the fatiguing exercise; Secondly, to determine the effect of an aerobic fatiguing test on salivary cortisol and the psychological states of amateur female soccer players, and whether a relationship exists between these variables and/or the fatiguing exercise and lastly, to determine the effects of a tournament and the match outcomes (win/lose) on salivary cortisol and the psychological states of amateur female soccer players, and whether a relationship exists between these variables and/or the match outcome. This will provide players and coaching staff with the necessary information to identify the main components responsible for fatigue during a match and its consequences, and how to implement interventions or preventive measures to ensure optimal performance.

Chapter 1 provided a brief summary of the problem underlying the research questions, objectives and hypotheses of the study, as well as a conceptualised framework and structure of the thesis. From the research questions posed, it was clear that a repeated measure, quantitative research design would be the most accurate protocol for testing the identified hypotheses. From this chapter it was acknowledged that further research was needed to evaluate the effect of fatigue on various aspects of female soccer players in order to maximise performance. Among these aspects were the physical (aerobic and anaerobic fitness), the hormonal (cortisol) and psychological (anxiety and mood) state of the players as a result of either training or a match. Therefore, the effect of fatigue (as a result of either training or a match) on the psycho-hormonal states of female soccer players should be examined further. This led the researcher to conduct a thorough literature review of the effect of various forms of fatigue (whether during training or competition) on the psycho-hormonal states of female athletes.

A literature review entitled: "The influence of fatigue on the hormonal and psychological aspects of female athletes" was covered in chapter 2. This section commenced with a brief overview of soccer in South Africa and the various components that the match comprises. A conceptualised model, to indicate the shortcomings in the South African female soccer domain and how the review would aid in overcoming and addressing those shortcomings and add to the gap in the current literature, was provided. The first aim of the literature review accentuated fatigue as a stressor (as commonly observed during a female soccer match), together with the various facets (neuromuscular, physical, physiological and psychological) comprising fatigue. The second aim of the review was to examine the available literature on females in the competition domain, during either various forms of training or competitions. The outline included both the hormonal and psychological aspects of female athletes and the critical facets of each. The first aspect (hormonal states) included a brief overview of cortisol — as it is the main stress-response hormone — with its physical and psychological consequences.

Following this, cortisol was briefly discussed in conjunction with testosterone, as both are regarded as important androgens, followed by the psychological influences of cortisol. The second aspect (psychological states) was divided into two sections, namely anxiety and mood states. The first subsection (anxiety) was introduced citing its importance for performance by emphasising two theories that are important for interpreting anxiety. This was then trailed by the types of anxiety and the various factors influencing it (namely type of sport, level of competence and competition, gender, thinking styles and the interpretation of anxiety). The second subsection, mood states, explained the importance and influence of these states on performance.

This led to the first main purpose of the chapter, namely to provide the reader with an overview of the available literature on the effect of training or competition on the hormonal, anxiety and mood states in female athletes. The available literature of the past 10 years (since the commencement of the study: 2005–2017), with regard to the study subjects, the nature of the intervention as well as the findings concerning the effect of these hormonal and/or psychological components was discussed. From the literature found (32 publications on hormonal states, 23 on anxiety and 16 on mood states), various results were obtained and conclusions drawn. This was included in an illustrated as well as written summary of the available literature.

Overall, cortisol is a popular hormone assessed within the sporting domain in either individual or team sport participants. The most prominent finding reported are an increased cortisol response as a result of competition. Studies evaluating the effect of various forms of training revealed contrasting results. In this respect, low-intensity training yielded increases and decreases in cortisol values. Compared to high-intensity training, lower intensity training yielded lower cortisol responses. Most studies found an increased cortisol response after high-intensity training, though a few reported a decreased response, whereas others reported none. The specific training procedures not specified in the cited literature conceded that cortisol increases as a result of training.

Testosterone was the ancillary hormone assessed most frequently, with a positive correlation between testosterone and cortisol being reported. This might be due to the adrenal glands secreting both hormones, or the glands and ovaries responding similarly during a stressor.

The exact time of sample collection in the cited studies varied to a large degree. All the studies took a measurement prior to testing to serve as baseline, though the time period was inconsistent. Some studies took a measurement from as long as three days to 24 hours before testing to serve as baseline. Other studies took a sample upon awakening, one to two hours to 15 minutes before and immediately prior to the training or competition. The most popular collection time was one to two hours prior to testing, and immediately thereafter. With regard to the post-test samples, samples were taken from immediately after to 12–48 hours post-test, though no significant differences were observed between collection times. Cortisol follows a diurnal rhythm, peaking within 50 minutes after awakening and 15 minutes following a stressor; thereafter it slopes downwards.

Only a few studies took into consideration external factors (such as warm-up, experience level, gender, sleep and wakening times and menarche among others) that might contribute to cortisol changes. A few examined the effect of the warm-up period because of its significance for physical and psychological preparation. They reported a rise in cortisol responses, which might be beneficial, as cortisol is vital for normal physiological functioning, such as regulating blood glucose levels and providing energy for subsequent activities. In addition, a few studies reported on their participants' oral contraceptive usage and/or menstrual phases. This is deemed vital, as it might contribute to physiological and psychological changes, which, ultimately, can influence performance. Less than 10% of the cited studies examined cortisol alongside psychological states. These studies reported either a positive or negative correlation between cortisol and anxiety prior to training/competition. In addition, correlations were reported between mood subscales and cortisol during a competition, among others fatigue, depression and confusion.

Due to the influence of anxiety on performance, it is a vital component to evaluate in the sporting domain. Several of the studies cited evaluated its effect during a competition and a training session. Contrasting results were reached, with competitions increasing the perceived overall, somatic and cognitive anxiety levels. Of interest is that winners had lower anxiety levels and these lower levels predicted a winning outcome. Pre-competitive anxiety states were able to predict up to 60% of the competition outcome (victory/defeat), with the subscale self-confidence the most important contributor. The studies evaluating the effect of training yielded equal amounts of contrasting results, reporting either an increase or a decrease in anxiety as a result of training. Quite a few questionnaires were implemented in the cited studies, the most popular ones being the CSAI-2 in a competition set-up and the Spielberger STAI in a training set-up. Protocols varied concerning the implementation of the questionnaires. The time of completion of the questionnaires ranged from immediately to 30 minutes before and after training/competition. However, the majority of studies did not specify the exact collection times before and after the intervention. When specifying the exact collection times, the most popular times were 10–30 minutes prior to, and 0–15 minutes after the intervention, again with no significant differences observed as a result thereof.

Mood is known to fluctuate as a result of physical exertion, having a subsequent influence on sport performance. Though it is therefore considered an important facet, the literature search yielded the lowest number of studies on the effect of mood, compared to anxiety and cortisol, on training/competition. Most studies examined the mood states in a competition domain, with the majority reporting increases in negative mood states either as a result of the competition outcome (defeat) or the experience of the participants (less experienced). A victory, on the other hand, demonstrated increases in positive mood states. Most studies examining training reported increased positive mood states as a result, though a limitation was seen regarding the intensity specification. In this regard, most studies evaluated aerobic training sessions with non-anaerobic training sessions and a few did not specify the training set-up.

The most popular questionnaire implemented, regardless of the training or competition set-up, was the POMS. Unfortunately participants reported this to be a lengthy questionnaire, leading to researchers compiling shortened versions. Completion of the mood questionnaires prior to and following testing took place from 15 minutes before training or matches to immediately before the activity, again with several studies not reporting the specific point in time. In addition, a few studies took into account a warm-up period, as questionnaires were completed prior to and after training/competition.

Certain shortcomings were, however, identified in the literature review regarding the effect of training/competition on the hormonal and psychological states of female athletes. In this regard, sample sizes differed largely, from as small as three to 134 participants. A major limitation on the scope of this study was the small number of studies evaluating soccer players, with not a single research study on any sport code conducted on a South African or even an African population, thus making future comparisons difficult. A second shortcoming in the available literature is that the specific training protocol is not specified, more specifically whether the training regime comprised high- or low-intensity activities. Most studies implemented aerobic training sessions, with none reporting on anaerobic training for the psychological measures. Several studies did not specify the intensity and type of training. Thirdly, a large number of studies did not specify the specific points in time for completion of the psychological questionnaires and saliva sampling. Sample/questionnaire collection times varied widely among studies, from as long as three days to immediately prior to the activity, and immediately to 48 hours after testing, again making future comparisons difficult. In addition, only the studies on the hormonal states took baseline measurements, which can be regarded as important, as this is the optimal state to which the participant should return. Moreover, only a few studies evaluated the effect of a warm-up session on either the cortisol or mood states, with none taking the perceived anxiety states into account.

Furthermore, an immense gap was identified in studies examining both the hormonal and psychological states and whether relationships exist between them and/or performance. This is important for future researchers and the sporting fraternity to draw conclusions on what can be implemented successfully to enhance performance.

Lastly, a limitation was found regarding various factors influencing the hormonal and psychological states, such as contraceptive usage, menstrual cycles and sleep, among others.

This led the researcher to investigate the link between the hormonal and psychological aspects. This section was characterised by two important approaches, namely the bottom-up and top-down attentional control processes. These terms are easily defined as the flow of information either from the higher to lower centres, thus mind to body (top-down), or communication from the body upwards to the mind by responding naturally (bottom-up). It is considered essential, as emotions have the ability to influence physiological systems, and *vice versa.* A schematic representation of the depletion-to-renewal grid was provided to provide the reader with insights into how these systems can influence each other.

The last section was compiled according to the final gap identified in the literature, namely the additional factors influencing both the hormonal and psychological states that should be taken into consideration either by future researchers or the sporting fraternity. Factors that have been identified as playing a role in both hormonal and psychological assessments were **a)** the effect of sleep, as poor sleep quality has been demonstrated to have a negative influence on mood and cortisol responses; **b)** the cortisol awakening response: cortisol follows a diurnal rhythm, peaking within the first 50 minutes upon awakening and then sloping downwards, with increased cortisol responses demonstrating negative correlations with mood states; **c)** menarche, as the various menstrual phases have different hormonal and psychological effects on an individual; **d)** contraceptive usage, which has an influence on an individual’s sleep patterns, cortisol awakening response and hormonal levels; **e)** competition outcome, where a positive outcome is generally correlated with lower cortisol and negative mood states compared to a defeat, **f)** the effect of warm-up, as this period is deemed important for both physiological and psychological preparation, and **e)** the influence of diet on both the hormonal and psychological states.

From the available research into the hormonal and psychological responses to either training or competition in female athletes, the following research questions were posed:

- What is the effect of an anaerobic fatiguing test on salivary cortisol and the psychological states of amateur female soccer players, and does a relationship exist between these variables and/or the fatiguing exercise?
- What is the effect of an aerobic fatiguing test on salivary cortisol and the psychological states of amateur female soccer players, and does a relationship exist between these variables and/or the fatiguing exercise?

- What is the effect of a tournament and the match-outcomes (win/lose) on salivary cortisol and the psychological states of amateur female soccer players, and does a relationship exist between these variables and/or the match outcome?

These research questions were discussed in article format in Chapter 3, Chapter 4 and Chapter 5.

The first article, as presented in Chapter 3, entitled: "The psycho-hormonal influence of anaerobic fatigue on semi-professional female soccer players", was published in the *Physiology & Behavior* journal. It is assumed worldwide that the fatigue observed during a soccer match is generally caused by frequent execution of high-intensity activities. However, limited research is available about the psychological and hormonal consequences of fatigue due to these high-intensity activities on female soccer players. Therefore, the purpose of this article was to investigate the effects of an anaerobic fatiguing test (AFT) on the cortisol, mood and anxiety states of semi-professional female soccer players. Forty-seven female players (22.0 ± 2.7 years) from a tertiary institution volunteered to take part in the study. Their cortisol values (saliva sample), anxiety (Spielberger State-trait anxiety inventory questionnaire) and mood scores (ISP) were taken an hour before and immediately prior to, and 15 minutes after an AFT. During the AFT, a 5-m multiple shuttle run test was completed, with HR_{max}, blood lactate (BLa^-) and rate of perceived exertion (RPE) taken immediately thereafter. The results indicated increased cortisol, psychological fatigue and TMD responses from baseline and/or pre- to post-AFT ($p < 0.05$). Vigour and confusion scores decreased from baseline and/or pre- to post-AFT ($p < 0.05$). In addition, a moderate relationship was observed between state-anxiety and TMD ($r \geq 0.6$, $p < 0.05$) at all three time points, as well as between state-anxiety and HR_{max} ($r = 0.4$, $p = 0.03$). Cortisol and RPE ($r = –0.3$, $p = 0.03$) demonstrated a trivial negative correlation at post-AFT. This is the first study to evaluate the effects of anaerobic fatigue on the psychological and hormonal states of semi-professional female soccer players. The results suggest that anaerobic fatigue can be perceived as a physiological and psychological stressor, hence having the ability to influence performance. In conclusion, altering a player's perception to fatigue and the anaerobic fitness level might have an impact on both the hormonal and psychological consequences of the specific stressor, consequently reducing the experience of fatigue and in doing so enhancing performance.

The second research question was posed in Chapter 4, consisting of the subsequent article compiled according to the guidelines of the *Journal of Sports Sciences* and titled: "The effects of aerobic fatigue on the psycho-hormonal state of amateur female soccer players". Since a soccer match entails up to 95% of aerobic activities, it might contribute to the fatigue experienced during a match. Unfortunately, little is known about the effects of fatigue due to extended physical effort on the psychological and hormonal states of female soccer players. Therefore, cortisol values (saliva sample), state anxiety (state subscale of the Spielberger's SAI) and mood scores (ISP), comprising the original six subscales and TMD, of 43 female players (aged 22.0 ± 2.7 years) were taken immediately prior to and 15 minutes after an AFT. During the AFT test, participants completed the Yo-Yo Intermittent Recovery test, and their HR_{max}, BLa^- and RPE were recorded thereafter. Cortisol increased ($F_{(2,216)} = 5.2$; $p < 0.01$, $d = 0.7$, $V = 0.4$) and ISP–confusion and ISP–vigour values decreased ($p < 0.05$, $d > 0.5$). At pre-AFT, a slightly positive relationship between cortisol and the absence of anxiety ($r = 0.3$, $p < 0.05$), and between HR_{max} and ISP–vigour ($r = -0.3$, $p < 0.05$) and TMD ($r = 0.3$, $p < 0.05$) transpired. TMD consistently demonstrated a strong relationship with all ISP and anxiety scores ($p < 0.05$). Post-AFT results demonstrated a relationship between cortisol and BLa^- ($r = 0.3$, $p < 0.05$), ISP–fatigue with HR_{max} ($r = 0.3$, $p < 0.05$) and ISP–vigour with RPE ($r = -0.4$, $p < 0.01$). This is the first study examining the psychological and hormonal consequences of an AFT on female soccer players and the corresponding relationships. The results indicate that fatigue due to prolonged physical exertion may be a greater physiological than psychological stressor, though both may influence soccer performance. We therefore recommend training players by increasing their aerobic capacity, ensuring maximal quality match-time before fatigue, and its subsequent adverse physiological and psychological effects, set in.

The last chapter consisted of the answer to the third research question in article format according to the guidelines of the *Journal of Strength and Conditioning Research*, entitled: "The effects of a soccer tournament on the psycho-hormonal states of collegiate female players." Female soccer is becoming more popular worldwide, as seen from an increase in competitive matches taking place. However, there is a gap in the literature on players' hormonal and psychological responses when playing a match, especially a tournament format, and the final outcome (victory or defeat). This is the first study to evaluate the effects of a week-long soccer tournament and its outcome on the psycho-hormonal states of collegiate female players. Eight players' cortisol (saliva sample), mood states (ISP) and state–anxiety (the state subscale of the state-trait anxiety inventory) were assessed one hour prior to, and 15 minutes following every soccer match. Cortisol increased significantly after most matches, with intra-match differences observed between all matches ($p < 0.05$). Matches demonstrating most high-intensity activities produced the largest cortisol increases. The TMD demonstrated a significant time effect, with intra-match differences observed for the subscales fatigue, depression, tension and vigour ($p < 0.05$). Prior to a victory in the group stages, cortisol was significantly lower compared to a match that was drawn, with no significant responses observed between a win and a loss. Mood states, on the other hand, demonstrated a significant response (Δ_{mood} = post-values – pre-values), with a defeat, resulting in a larger TMD index compared to a victory. A moderately positive correlation was recorded prior to a victory, between cortisol and ISP–tension, ISP–fatigue, ISP–depression and TMD ($p < 0.05$). Before a defeat, there was a moderate relationship between cortisol and ISP–confusion or ISP–anger and with ISP–vigour post-match ($p < 0.05$). TMD significantly correlated with state-anxiety at all time points ($p < 0.05$). In summary, the players' physiological and psychological capacities combined in contributing to the stress response commonly observed during a tournament. Therefore, focusing on high-intensity activities and their effects is vital, as they are associated with raised cortisol and negative mood states. In brief, monitoring mood states over a tournament can be beneficial, as profound hormonal and anxiety information, which can subsequently affect performance, can be collected.

2. CONCLUSIONS

The conclusions drawn from this study are presented in accordance with the set hypotheses from Chapter 1:

Hypothesis 1: *"The anaerobic fatiguing test will result in a statistical significant increase (p < 0.05) in cortisol, anxiety and mood states of amateur female soccer players. Also, a positive correlation between cortisol and total mood disturbances, and between anxiety and total mood disturbances are hypothesised. Furthermore, a positive relationship is expected between the anaerobic fatiguing exercise variables (maximal heart rate and blood lactate levels) and cortisol, as well as the negative mood states."*

The first hypothesis of the study is partially accepted, as a significant increase in cortisol and TMD did take place due to the fatiguing test, with no difference observed for anxiety scores. However, in contrast to the hypothesis, no significant relationship between cortisol and any psychological measurement ($p > 0.05$) was seen. Similar to the hypothesis, a strong positive correlation was seen between the mood and anxiety scores at all time points. Regarding the fatiguing test, a positive relationship was observed between cortisol and BLa^- ($r = 0.6$, $p < 0.01$) as well as power output ($r = -0.4$, $p < 0.05$) prior to testing. Following the fatiguing test, a positive correlation was again seen between cortisol and BLa^- ($r = 0.7$ $p < 0.01$) and RPE ($r = -0.3$, $p = 0.03$), as well as between TMD and RPE ($r = 0.4$, $p < 0.05$).

Hypothesis 2: *"The aerobic fatiguing test will result in a statistical increase (p < 0.05) in salivary cortisol and anxiety states, as well as a significantly negative effect (d ≥ 0.8) on the mood states of amateur female soccer players. A positive relationship is expected between anxiety and cortisol or mood states and a negative relationship is expected between cortisol and mood states. Furthermore, a positive relationship is expected between the aerobic fatiguing exercise and positive mood states, with no relationship expected for cortisol and anxiety."*

The second hypothesis is partially accepted, as a significant increase ($p < 0.01$, $d = 0.7$) in cortisol as well as in the mood subscales confusion and vigour ($p < 0.05$, $d > 0.5$) took place, though no significant differences were noted for the anxiety results. In addition, there was a relationship between cortisol and the absence of anxiety ($r = 0.3$, $p < 0.05$) prior to the testing, and between cortisol and ISP-fatigue afterwards. The mood and anxiety scores also demonstrated strong correlations at both pre- and post-AFT ($p < 0.05$). Regarding the fatiguing test, relationships were seen between HR_{max} and TMD prior to ($r = 0.3$, $p < 0.05$), and between HR_{max} and ISP-fatigue ($r = 0.3$, $p < 0.05$), cortisol and BLa^- ($r = 0.3$, $p < 0.05$) as well as between ISP-vigour and RPE ($r = -0.4$, $p < 0.01$) post-test.

Hypothesis 3: *"A progressive increase (d > 0.8, p < 0.05) in cortisol and anxiety, together with a decrease in positive mood states, will take place over the course of the tournament. Moreover, a positive relationship is expected between anxiety and cortisol or mood states and a negative relationship between cortisol and mood states. A victory will result in statistically better mood states and lower cortisol responses, whereas a losing outcome will result in higher anxiety, negative mood states and cortisol."*

The third hypothesis is partially accepted, as the results yielded significant cortisol and TMD increases over the course of the tournament ($p < 0.05$), with no changes seen in the anxiety scores. Furthermore, as hypothesised, a positive relationship was seen between anxiety and mood states across the tournament ($r \geq 0.5$, $p < 0.05$), though no correlation was seen between cortisol and anxiety or mood states across the tournament. The only relationship seen between the hormonal and psychological states was regarding a victory or defeat. Concerning this, prior to a victory a positive relationship was found between cortisol and the TMD as well as the mood subscales tension, fatigue and depression ($r \geq 0.4$, $p < 0.05$). Prior to a defeat, a positive correlation was seen between cortisol and the mood subscales confusion and anger ($r \geq 0.3$ $p < 0.05$), whereas thereafter a trivial negative correlation was seen between cortisol and vigour ($r = -0.3$, $p < 0.05$). Equivalent to the hypothesis, a losing outcome did indeed result in significantly higher negative mood states ($p < 0.05$), with no significant changes observed for cortisol or anxiety.

Taken collectively, female soccer has become popular globally, with match fixtures and training loads increasing at domestic and international level. It is vital to identify the components imperative during a match, and how these change during the course thereof, to maximise performance and decrease the occurrence of fatigue. A typical match comprises high- and low- to moderate-intensity activities, hence maximising these components during training is essential. A match or tournament can be very stressful for the players, which may be due to the physical, physiological or psychological aspects associated with the match. One stressor commonly mentioned by players is the prevalence of fatigue (whether mental or physical), usually setting in during the second half of the match and having detrimental physical performance consequences. The onset of fatigue may be due to a range of factors, physical exertion during a match being the most common reason.

In this regard, it was clear from the results that a match and/or tournament caused an increase in cortisol (the main stress hormone) as well as negative mood states. Though a typical match is primarily dominated by a low work-rate profile (thus the aerobic system), the frequent execution of high-intensity activities (thus the anaerobic system) is generally perceived as the major contributor to the fatigue experienced. This was seen in the results, with the matches of more high-intensity bouts demonstrating larger cortisol responses. When evaluating the effect of an AFT, these observations were also seen, with cortisol and negative mood states increasing significantly. Therefore maximising the anaerobic component during training is vital. Various factors, however, can influence either cortisol or psychological states, as previously stated. Among these are sleep quality and quantity, time of awakening, diet, menarche, oral contraceptive usage and warm-up. During the first outcome, as mentioned in this study, baseline measurements (cortisol and psychological states) were evaluated and compared to the pre-fatiguing values to determine whether any of the above-mentioned factors played a significant role and had to be corrected. No significant changes or correlations between the factors or variables were seen, therefore this time point measurement was excluded in subsequent objectives.

As a match involves the aerobic energy system 95% of the match time, evaluating the extent to which this contributes to fatigue and the results thereof was deemed important. The AFT also demonstrated a cortisol increase, though only certain subscales of the mood state questionnaire differed, with the overall mood score remaining more or less the same.

When comparing the two fatiguing tests, both demonstrated similar cortisol increases when controlled for their own baseline (aerobic 52.2% *versus* anaerobic 55.8% increase); however, the anaerobic test revealed larger increases in the perceived negative mood states. This was also noticed in respect of the fatigue indicators, with BLa^- demonstrating a 32% higher response following the anaerobic test. Because of the mutual relationship between cortisol and behaviour, and the ability of a player to modulate it, it can be considered important either to change the perception of anaerobic fatiguing tests, or maximise the anaerobic capability in order to minimise the negative mood states and subsequent cortisol increases.

The mutual relationship between cortisol and behaviour documented in literature led the researcher to investigate whether there were any relationships between cortisol, anxiety, mood and/or the AFTs and tournament outcomes. Only the AFT demonstrated a relationship with an anxiety-subscale prior to the tests, though BLa^- correlated with cortisol during both tests. Furthermore, the anxiety and mood state questionnaire correlated at all time points, thus one can assume that with an increase in negative mood states, an increase in perceived anxiety might take place.

Lastly, though not in the scope of this study, changing the perception of a player prior to a match is important. According to the results, a defeat resulted in significantly higher negative mood states, with quite a few psycho-hormonal relationships observed regardless of the outcome, and though prior to a victory a linear relationship could be seen between cortisol and mood states.

3. CONTRIBUTIONS OF THIS THESIS

The research performed for this particular thesis contributes the following new information to the existing body of knowledge in the literature:

1. To the authors' knowledge, this is the first study that evaluated two fatiguing tests as well as a tournament involving female soccer players in South Africa. Therefore this study serves as a baseline for future researchers as well as the sport practice for evaluating this population.
2. In addition, no other research could be found that evaluated the effects of cortisol and mood states following a fatiguing test in South Africa. Only a few other investigations were found that evaluated both the hormonal and psychological aspects of female athletes, but none examined the effects of an anaerobic and aerobic fatiguing test on both the hormonal and psychological aspects of female athletes. Therefore the current study contributes to the field of the psycho-hormonal states of female athletes since:
    - 2.1. The study demonstrated that anaerobic and aerobic fatiguing tests, as commonly administered to soccer players, are adequate to cause a physiological and psychological homeostatic disruption.
        - 2.1.1. An anaerobic fatiguing test causes a greater disruption compared to an aerobic fatiguing test in both the physiological and psychological fields.
        - 2.1.2. Measuring blood lactate can be useful to indicate underlying cortisol levels during aerobic and anaerobic fatiguing tests as a relationship exists thereafter.
        - 2.1.3. Implementing the ISP questionnaire can be useful to indicate players' underlying anxiety state, as well as their perception of how exhausted they feel after both an aerobic and an anaerobic fatiguing test.
3. Only a few studies were found that evaluated a tournament involving female athletes, but none completed on a South African population. Thus, this thesis can serve as a baseline for future researchers and the sporting fraternity to evaluate the effect of a week-long tournament, its outcome and the internal and external loads placed on the players, on the psycho-hormonal states of female players. Therefore the present study adds to the field of the psycho-hormonal states of female athletes since:
    - 3.1. The authors found that a week-long tournament leads to significant hormonal and psychological interferences, increasing during each match.
    - 3.2. Matches deemed vital for playoff rankings resulted in higher cortisol secretion, suggesting that both a physiological and a psychological component were involved.
    - 3.3. The intensity of the match yielded similar cortisol reactions, with an increase in secretion noticed as the intensity amplified, suggesting that a linkage existed between exertion level and cortisol secretion.

3.4. The outcome of a competition had a larger effect on the psychological than on the hormonal states, with a defeat causing a decline in positive mood states.

3.5. Prior to a competition the ISP questionnaire can be administered, as it might pick up underlying hormonal and anxiety states, making it believable to attempt to influence mood states prior to a match, to effect performance enhancement.

4. LIMITATIONS AND RECOMMENDATIONS

The study provides support for the investigation of various fatiguing factors associated with a female soccer match. Unfortunately, the findings suggest certain shortcomings that need to be considered when interpreting the results of this study.

- Outliers among the soccer players that completed the soccer tournament could have pulled the ANOVA results skew because of the rather small sample size used ($n = 8$). A larger sample size would therefore be advisable.
- Furthermore, evaluating a large sample size during a tournament and taking into consideration positional differences might be beneficial for future research.
- The players were only advised on their sleeping, drinking and training level, which might have influenced either the hormonal or psychological results. Therefore, future research should clearly monitor a participant's lifestyle and training habits in order to correct and minimise the possible effects of cofounding variables of female soccer players.
- Furthermore, clearly monitoring their sleep patterns and sleep quality would be beneficial in minimising negative mood states and increased cortisol levels usually observed. In this regard, future studies can take samples on a neutral day prior to testing or competition to serve as individual baselines for comparing future results.
- The players only reported the date of their last menarche and oral contraceptive usage. It is possible that the information provided was falsified or inaccurate or that the players made use of other contraceptive devices. Future studies can collect the menarche dates of the past two months to clearly calculate the precise menstrual phase in which they currently are. Furthermore, a list of possible contraceptive aids can be listed for the players to tick.
- The current stress levels experienced by the players were unreported. This is deemed important, as the participants were students at a tertiary institution and were advised to adhere to all academic requirements, subjecting them to various stressors.
- Only two questionnaires were completed, one assessing the mood and one the anxiety states. The state-trait anxiety inventory questionnaire is a popular modality for assessing acute (state) and chronic (trait)

anxiety levels. In addition, previous research has shown this modality to be generally used during a training regime, though not during a competition. Therefore future studies can implement the mentioned as well as other commonly used questionnaires during competitions to compare and analyse results.

- Future research can evaluate shorter questionnaires, as lengthy questionnaires can lead to participants providing false information (i.e. recalling their last answers or completing the questionnaire in a rush).
- Research on soccer players has demonstrated differences in playing positions. Further research may take this into consideration in providing position-based interventions regarding the specific tests implemented and stressors to which they are exposed.
- The warm-up period is a critical period for the physiological and psychological preparation prior to training and competition. Further research is needed for investigating whether it has a positive effect on the psycho-hormonal states of female athletes.

In spite of the shortcomings identified, this study provides a basis for future research in the area of the effects of various fatiguing tests and a tournament on the psycho-hormonal states of female soccer players. This is due to the fact that no other studies could be found that evaluated two types of fatiguing tests together with a tournament and its outcome on cortisol, together with the mood and anxiety states and whether any relationships take place as a result thereof on amateur female soccer players. According to the authors' knowledge, this is the first study to report on the effects of fatigue (aerobic and/or anaerobic) as well as a tournament and its outcome (victory/defeat) on the hormonal (cortisol) and psychological (mood- and anxiety) states and the psycho-hormonal relationship thereof on amateur female soccer players.

APPENDIX

APPENDIX A METHODOLOGY

1. *Empirical investigation*

1.1. Study design

This study formed part of a bigger project entitled: “Investigating performance indicators and injury risk factors for the development and performance of female soccer players”. The design of the study was a repeated measure, quantitative research design. Information was collected by using a questionnaire and a test battery. Each team was tested at its respective university’s training facilities. The objectives of the study were explained to players, after which they were provided with a consent form to complete prior to the first testing opportunity. The final assessments (saliva and questionnaires) took place during the University Sports South Africa (USSA) National Football Club Championships, two weeks after the collection of the physical testing data. Ethical approval was obtained for both the main and sub-study from the Ethics Committee of the North-West University (NWU-00055-15-A1).

1.2. Test subjects

The study was performed by recruiting 50 university-level female soccer players representing either the NWU or the Tshwane University of Technology (TUT), at the USSA National Football Club Championships in 2016. The coaches of the respective teams were recruited by means of a scheduled meeting and the players were approached thereafter. Because of the high number of female players participating in the particular tournament, as well as the total number of players per team, a limitation was placed on the number of sampled players during the tournament. Players were divided into three groups, namely defenders, attackers and midfielders. The coaches provided a written consent form for the testing of their respective teams.

2. *Procedures*

2.1. <u>Demographic and general information questionnaire</u>

Soccer players’ demographic and personal information (gender, ethnicity and age) was collected by means of a demographic and general information questionnaire prior to testing. Players’ exercising habits, injury incidents, competing levels and years of participation were also determined with this questionnaire. Furthermore, information on the use of oral contraceptives, prescribed medication, the date of their last menarche as well as the quality of sleep (and reason for this) during the past 24 hours (h) was asked.

2.1.1. Sport psychological skills

The psychological questionnaires aided in assessing the players' psychological state prior to and after the various fitness tests and matches. A qualified sport scientist with specifically a psychological background assisted while the players completed the questionnaires as well as with the data analyses. The questions in the questionnaires were shuffled for each testing session to prevent players from recalling their previous answers.

a) State-Trait Anxiety Inventory

The State-trait Anxiety Inventory (STAI) questionnaire was implemented to determine the players' anxiety prior to and after the specific tests (Spielberger, 1983). The questionnaire was thoroughly explained in English as the players were enrolled as English (speak, read and write) students at their respective institutions. The STAI is a 40-item, self-evaluation questionnaire, which includes separate measures to determine a players' state and trait anxiety respectively.

The State-anxiety scale (STAI-1) consists of 20 statements that evaluate how players feel "right now, at this moment" on a four-point Likert scale: one (not at all), two (somewhat), three (moderately so) and four (very much so). The Trait-anxiety scale (STAI-2) also consists of 20 statements that assess how people "generally feel" on a four-point Likert scale: one (not at all), two (somewhat), three (moderately so) and four (very much so). Four indicated the presence of high anxiety levels, whereas one indicated the absence of high anxiety levels (Spielberger, 1983). The STAI-1 was implemented at all time points (baseline on the first day, 1 h before and 15 minutes (min) after the fatiguing tests and matches), whereas the STAI-2 was used only at baseline on the first day of testing, as research has shown that individuals demonstrating higher trait-anxiety scores tend to report higher state anxiety as well (Horikawa & Yagi, 2012:1). Anxiety levels were determined by calculating the sum of the different scores. The range of scores can be from 20 to 80; the higher the score, the greater the anxiety indicated (Spielberger, 1983). Internal consistency reliability for state anxiety ($r = 0.91$) has been reported (Barnes *et al.,* 2002:610).

b) Profile of Mood States

The Incredibly Short Profile of Mood States (ISP) of Dean and colleagues (1990) was used to assess the players' profile of mood states (POMS). The ISP was completed at baseline on the first day of testing (to serve as reference point) and then 1 h before and 15 min after the tests and matches. The ISP is derived from the original POMS questionnaire (McNair *et al.,* 1971), proving correlations to vary by $r = 0.67–0.82$ between the two questionnaires (Dean *et al.,* 1990). The original POMS questionnaire is a 65-item measure of typical and persistent mood reactions to life situations and has proven to be very popular in the research domain of sport and exercise psychology (Leunes & Burger, 2000:6). The internal consistency of the POMS ranges between $r = 0.87$ and $r = 0.92$, with a test-retest reliability ranging between $r = 0.68$ and $r = 0.74$ (McNair *et al.,* 1971).

The POMS comprises six subscales, tension-anxiety, anger-hostility, vigour-activity, depression-dejection, fatigue-inertia and confusion-bewilderment. However, owing to the length of the POMS, the ISP questionnaire was used, which comprises the same six subscales. Players rated the respective questions on a five-point Likert scale with individual responses ranging from "Strongly disagree"; "Disagree"; "Neutral" and "Agree" to "Strongly agree".

2.1.2. Physiological tests

2.1.2.1. Salivary sample collection: Cortisol

Not only is blood sampling an expensive and labour-intensive measurement (Cardinale & Varley, 2017:56), but the venepuncture can be very stressful, which can be counterproductive in stress research (Kirschbaum & Hellhammer, 2000:379). Fortunately, salivary cortisol concentrations correlate ($r \geq 0.90$) with the amount of free cortisol in the blood (Kirschbaum & Hellhammer, 2000:380), making salivary measurements ideal and more suitable for the study outcomes.

To minimise the effect of circadian rhythm, players were advised to wake up between 6:00 and 7:00, as peak levels of cortisol usually occur early in the morning approximately 30 min after awakening (Kirschbaum & Hellhammer, 2000:380). Salivary samples were collected for the assessment of cortisol by using the passive drool test (Salimetrics, 2011:6). Players had to have been awake and fasting for at least an hour prior to their arrival at the field to allow the cortisol peak that occurred upon wakening and as a result of eating breakfast to decrease and stabilise (Kirschbaum & Hellhammer, 2000:380). Only on the first day of testing, baseline salivary samples were collected upon arrival at the field to serve as the starting point for subsequent values. Players were tested at the same time of day on the physical testing days (two consecutive days) to prevent discrepancies with regard to weather, temperature, their biological rhythm and diet. Following a warm-up session, a saliva sample was collected (second sample on day one, first sample on day two). A third saliva sample was collected 15 min after the specific fatiguing tests on both days, as maximal cortisol levels are reached 10 – 30 min after a stressful event (Kirschbaum & Hellhammer, 2000:381). During the tournament, samples were collected an hour prior to and 15 min after each match, regardless of the time of day.

The players were advised not to eat, drink, chew gum or brush their teeth for at least an hour before sampling. The mouth was rinsed thoroughly with lukewarm water 5 min prior to sample collection to remove any food substances. Saliva flow could be stimulated by chewing on a piece of Para film if needed. Saliva was collected through a plastic straw into a 20 ml collection vial and were stored in a freezer (4 ± 1ºC) until analysed by a professional laboratory.

The salivary cortisol content was determined from 20 µl saliva samples by using a cortisol saliva luminescence immunoassay. The samples were first transferred into a Berthold luminometer that calculated the average relative luminescence units, after which the results were plotted against the concentration. This method has a non-linear (r = 1.0) and linear correlation coefficient (r = 0.8) with serum cortisol values, with intra-CV ranging between 0.4 and 1.7% and inter-CV ranging between 0.8 and 1.8% (Westermann *et al.,* 2004).

2.1.2.2. Blood sample collection: lactate

Krustrup and associates (2005:1247) reported peak BLa^- values of 8 mmol following an exhaustive incremental running test for female soccer players. An increase in blood lactate is known to occur as peripheral fatigue develops (Ament & Verkerke, 2009:392). Thus, determining the players' blood lactate following the fatiguing exercises revealed the level of fatigue they experienced after each exercise.

Immediately after the various fatiguing tests, a blood sample was collected by means of a single finger prick on the left hand by a qualified sport scientist who had received training in the collection of blood samples. Thus, a total of two samples were collected per player over the course of two days. All personnel wore protective disposable gloves and a new pair was worn after each blood sample collection. Before the collection of the blood sample, players' fingers were cleaned with an alcohol swab and the portable analyser was calibrated. A portable analyser (Lactate Pro, Arkray, Japan) was used to measure the BLa^- in mmol/L. The analyser was used according to the manufacturer's guidelines. A single blood droplet was collected on the portable analyser and a measurement provided within 10 seconds (sec) after the capturing. The needle of the finger prick apparatus and the disposable gloves were dispelled into an anatomical waste bin after each measurement had been taken. After a lactate reading had been given by the lactate analyser, the used blood lactate strip was also dispelled into the portable biodegradable waste container. The disposable waste container was collected by the manufacturer, as per instructions.

2.1.3. <u>Anaerobic fatiguing test</u>

The 5-metre shuttle run test (5-m MST) was conducted outdoors on a soccer field according to the method of Boddington *et al.* (2001), as determined for female field hockey players. This test has an intra-class correlation coefficient ranging from 0.98 to 0.74 for the total, peak and delta distance and fatigue index (Boddington *et al.,* 2001:225). However, Boddington *et al.*'s study completed the test on a rubberised indoor track, whereas the current study will simulate it on a soccer field. Players were instructed not to pace themselves but to perform with maximal effort throughout the test. The layout of the test consisted of six cones placed 5 m apart in a straight line to cover a total distance of 25 m. Players started the test at the first cone and, upon an auditory signal, sprinted 5 m to the second cone, touched the cone with their hands and returned to the first cone. Players then sprinted 10 m to the third cone and back to the first cone, etc. Players continued to sprint back and forth until a period of 30 sec had elapsed. After the first bout, players were given a 35-sec rest period before the start of the second repetition. During this recovery period, they were instructed to walk back to the starting position. Verbal instructions were given to inform players of the recovery time remaining between each sprint. Players had to repeat this activity six times. The distance covered by each player was determined to the nearest 2.5 m during each 30-sec shuttle, which was used to determine the average total distance covered by each player. The data recorded during the test was then categorised as follows (Boddington *et al.,* 2001:224):

Peak Distance = The greatest distance covered during a 30 sec shuttle;

Total Distance = The total distance covered during the 6 x 30 sec shuttles;

Delta Distance = The difference between the longest and shortest test shuttle distance;

Fatigue Index = Calculated according to the following formula

$$= \frac{\left[\frac{(\text{Shuttle 1} + \text{Shuttle 2})}{2} - \frac{(\text{Shuttle 5} + \text{Shuttle 6})}{2}\right]}{\frac{(\text{Shuttle 1} + \text{Shuttle 2})}{2}} \times 100.$$

Immediately following the 5-m MST, the players had to indicate their rate of perceived exertion (RPE) on the Borg Scale (Borg, 1973). This scale consists of 10 statements that indicate their perceived exertion. This served as verification whether the test was fatiguing, as a previous study reported a correlation between RPE and BLa^- ($r = 0.63$, $p < 0.05$) or percentage HR peak ($r = 0.60$, $p < 0.05$) following a small-sided soccer match (Coutts *et al.,* 2009:82).

Furthermore, a blood sample was obtained from the left hand's fingertip to measure BLa^- while the player was seated, as described in 2.1.2.2. The reason for this test was to evaluate whether the anaerobic test accomplished exhaustion in the players.

Ten minutes after the 5-m MST, the STAI-1 and ISP questionnaires were completed and after 15 min a second saliva sample was collected by using the passive drool method previously described.

2.1.4. Aerobic fatiguing test

A Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1) ($r = 0.95$) (Bangsbo *et al.,* 2008) was performed during the two-week period prior to the tournament to evaluate soccer players' endurance levels. According to Krustrup and colleagues (2005:1245), the Yo-Yo IR-1 test can serve as a good predictor of a female soccer player's ability to perform high-intensity running during a match ($p < 0.05$). The Yo-Yo IR1 test was executed as described by Bangsbo *et al.* (2006:4) to measure the players' maximal heart rate and maximal oxygen consumption while performing intervals over a prolonged period of time. The Yo-Yo IR1 was conducted on a flat, clearly marked 20-m stretch on a soccer field while all players wore their soccer boots. Players were warmed up before commencement of the test as previously described. Three lines 20 m and 5 m apart were marked by using cones. A pre-recorded, commercially available compact disc (CD) was played on a CD player. Players were required to run back and forth between the lines and to pace themselves so that their arrival at the end of the 20-m stretch coincided with the signal emitted by the CD. Players had to touch the marked lines at either end of the 20-m stretch with one foot as the signal sounded from the CD. Each player received a short 10-sec active rest period after each 40 m (2 x 20 m runs) during which they walked back and forth over a 5-m stretch. The test started at a speed of 10 km/h and the speed progressively increased until the test was terminated if the player voluntarily dropped out or could not make it to either end-mark of the 20-m distance within the given signal time in two successive shuttles. The result noted was the distance covered at the point where the player could not maintain the required speed of the test. The players were verbally encouraged to perform maximally during each assessment. After the players had finished the test, they were asked to indicate their RPE according to the Borg Scale (Borg, 1973).

The Yo-Yo IR1 was performed while the players were fitted with a Fix Polar Heart Rate Transmitter Belt (Polar Electro, Kempele, Finland). Immediately following the Yo-Yo IR1 test, a single blood sample was obtained from the left hand's fingertip as described in 2.1.2.2. to measure BLa^- while the player was seated.

Ten minutes after the Yo-Yo IR1 test the STAI-1 and ISP questionnaires were completed and after 15 min a second saliva sample was collected.

2.1.5. <u>Match variables</u>

The following was done to serve as verification of the expected outcomes (fatigue):

A global positioning system (GPS) unit sampling at 10 Hz (MinimaxX V4.0, Catapult Innovations, Victoria, Australia) was fitted to the upper back of each player prior to the start of each soccer match by using a harness supplied by the Catapult manufacturers. Players were already familiar with the GPS units, as they had completed minor matches and leagues with it previously. In addition to the GPS-derived values, a Fix Polar Heart Rate Transmitter Belt (Polar Electro, Kempele, Finland) was used to determine the heart rate for every 5-sec interval during the course of the tournament, which consisted of six matches. The last-mentioned equipment allowed the researcher to obtain data regarding the distance covered, velocity and heart rates of each of the players. The recordings from the GPS unit and heart rate monitor were downloaded to a computer and analysed by software (Logan Plus V4.7.1, Catapult Sports, Victoria, Australia). GPS Doppler data was used during analyses of the GPS variables. The following movement categories for female soccer players were used: running (4.5–5.3 $m.s^{-1}$) and sprinting (>5.4 $m.s^{-1}$) (Dwyer & Gabett, 2012:820). For a movement to be recorded as an effort, players had to maintain that velocity for at least 1 sec. HR values above 170 bpm were computed as high-intensity values (Casamichana & Castellano, 2010:99).

Each player was instructed to sleep at least 8 h during the evening and morning prior to the different testing sessions. They were requested to abstain from ingesting any drugs or participating in strenuous physical activity that could influence the physical or physiological responses of the body for at least 48 h before the scheduled tests.

The following independent variables were requested and/or monitored for each player:

- Adequate hydration by supplying and monitoring their fluid intake;
- Diet monitoring by providing them with a sandwich prior to each test;
- Eight hours of sleep prior to the test (questionnaire);
- No use of supplementation;
- Oral contraceptive usage (questionnaire);
- No consumption of alcohol;
- No smoking;
- Being fitted with heart-rate monitoring belts throughout the training session to ensure that the fitness tests were adequate in exhausting the players;
- Remaining injury-free;
- Completing all the physical and physiological tests;
- Completing 100% of the total match time during the tournament.

3. Testing procedure

Before commencement of the testing session, players had the opportunity to complete a familiarisation session where all the tests were done in the correct order.

The testing sessions, involving the psychological and physiological assessments, took place over two consecutive days. The two teams were tested on separate occasions at their respective universities' facilities. The second testing period took place at a major university soccer tournament within 2 weeks of the initial testing period.

The hard copies of the data recorded on the first two testing procedures will be stored for a minimum of 7 years in a secure safe in the project leader's office, accessible only to the primary researchers, after which it will be destroyed by means of a paper shredder. This includes the reports received from the laboratory responsible for the stress hormone analysis. All participants' data are stored under their participant number and their personal information is secured in a password-protected file.

The electronic data recorded by the GPS units and heart rate monitors was downloaded to a password-protected personal laptop immediately after each match and a back-up was made on a CD and will be stored for a minimum of 7 years in a secure safe in the project leader's office, accessible only to the primary researchers, after which it will be erased from the laptop and the CD will be destroyed.

The blood samples will not be stored, as the values will be displayed within 10 sec following capturing. The Pro Lactate strips used for the blood sample analysis will be discarded in special medical containers and removed by professional personnel immediately after testing.

The project leader will ensure and monitor that all the data is properly captured in a password-protected document and only handled by the personnel involved in the study. The project leader will also provide the reports of the testing sessions to the coaches and players individually.

4. Statistical analyses

The Statistical Consultation Services of the NWU determined the statistical methods and procedures for the analysis of the research data. The Statistical Data Processing package (Statsoft Inc., 2014), available on the NWU network was used to process data. Firstly, descriptive statistics (means, standard deviations, minimum and maximum values) of each variable were calculated.

This was followed by a linear mixed model analysis to investigate positional differences with an autoregressive 1 (AR 1) structure. The different players were entered as subjects and time point as the fixed effects.

Pearson's rank correlation (rho) was then used to determine the relationship between cortisol, anxiety, mood state or the fatiguing session or match outcome. A Fisher r to z transformation was calculated to determine the 90% confidence interval from the correlation coefficient. The level of significance was set at $p \leq 0.05$. The strength of the correlation was categorised as follows: ≤0.01 (trivial), <0.3 (small), <0.5 (moderate), <0.7 (large), <0.9 (very large) and ≥1 (perfect). Effect sizes were calculated for differences between variables for all times to determine the practical significance of all the values. Effect size (d) (expressed as Cohen's D-value) was interpreted as follows: a d of more or less 0.8 as large, a d of more or less 0.5 as moderate and a d of more or less 0.2 as small (Cohen, 1998).

Thirdly, the variables were categorised into three rankings (TMD/Anxiety: 0–5/20–39 = low, 6–12/40–50 = average/neutral, 13–18/51–80 = high) for each time point. The cortisol values were ranked into three categories according to time of awakening and sample collection time (as adapted from Kirshbaum and Helhammer, 2000, the Salimetrics Manual, 2011 and Westermann *et al,* 2004) according to the 5% (low = 1), 50% (average/neutral = 2) and 95% (high = 3) percentiles. The total score for each ranking at every time point was expressed as a percentage of the total score using cross-tabulations with a chi-square analysis; the practical significance was indicated by Cramer's V values.

Objective	Independent variable	Dependent variable	Inferential statistics
To determine the effect of an aerobic fatiguing test on the salivary cortisol of amateur female soccer players	Aerobic fatiguing test (Section 2.1.4)	Salivary cortisol (Section 2.1.2.1)	Repeated measures ANOVA (Linear mixed model) Chi-square analysis Cohen's effect sizes
To determine the effect of an aerobic fatiguing test on the psychological state of amateur female soccer players	Aerobic fatiguing test (Section 2.1.4)	Psychological questionnaires (Section 2.1.1)	Repeated measures ANOVA (Linear mixed model) Chi-square analysis Cohen's effect sizes
To determine whether a relationship exists between cortisol, anxiety, mood states and/or an aerobic fatiguing test of amateur female soccer players	Aerobic fatiguing test (Section 2.1.4)	Salivary cortisol, psychological questionnaires and AFT variables (Section 2.1.1, 2.1.2.1 & 2.1.2.2)	Pearson's rank correlation Fisher r to z transformation
To determine the effect of an anaerobic fatiguing test on the salivary cortisol of amateur female soccer players	Anaerobic fatiguing test (Section 2.1.3)	Salivary cortisol (Section 2.1.2.1)	Repeated measures ANOVA (Linear mixed model) Chi-square analysis Cohen's effect sizes
To determine the effect of an anaerobic fatiguing test on the psychological state of amateur female soccer players	Anaerobic fatiguing test (Section 2.1.3)	Psychological questionnaires (Section 2.1.1)	Repeated measures ANOVA (Linear mixed model) Chi-square analysis Cohen's effect sizes
To determine whether a relationship exists between cortisol, anxiety, mood states and/or an anaerobic fatiguing test of amateur female soccer players	Anaerobic fatiguing test (Section 2.1.3)	Salivary cortisol, psychological questionnaires and AFT variables (Section 2.1.1, 2.1.2.1 & 2.1.2.2)	Pearson's rank correlation Fisher r to z transformation
To determine the effects of a soccer tournament and the outcome (win/lose) on the salivary cortisol and the psychological states of amateur female soccer players	Match outcome (Section 2.1.5)	Salivary cortisol and psychological questionnaires (Section 2.1.2.1 & 2.1.1)	Repeated measures ANOVA (Linear mixed model) Chi-square analysis Cohen's effect sizes
To determine whether a relationship exists between cortisol, anxiety, mood states and/or match outcome on amateur female soccer players	Match outcome (Section 2.1.5)	Salivary cortisol, psychological questionnaires and match outcome (Section 2.1.1, 2.1.2.1 & 2.1.5)	Pearson's rank correlation Fisher r to z transformation

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APPENDIX B ETHICAL APPROVAL FOR LARGER PROJECT

NORTH-WEST UNIVERSITY ®
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Ethics Office
Tel: 018-299 2092
Fax: 018-299 2088
Email: Minrie.Greeff@nwu.ac.za

2 November 2015

Dr M Sparks
PhASRec

Dear Dr Sparks

HREC APPROVAL OF YOUR AMENDMENT REQUEST

Ethics number: NWU-00055-15-A1

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Project title: Investigating performance indicators and injury risk factors for the development and performance of female soccer players

Project leader/supervisor: Dr M Sparks

Student: A Stauss

Application type: Amendment – Add several tests

Risk level descriptor: Minimal

You are kindly informed that at the meeting held on 21 October 2015 of the HREC, Faculty of Health Sciences, the aforementioned was approved.

The period of approval for this project is from 02/11/2015 to 31/12/2017.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).

The HREC requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the protocol or other associated documentation must be submitted to the HREC prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form.

A progress report should be submitted within one year of approval of this study and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC must be notified if the study is temporarily suspended or terminated. The progress report template is obtainable from Carolien van Zyl at

Carolien.VanZyl@nwu.ac.za. Annually a number of projects may be randomly selected for an external audit.

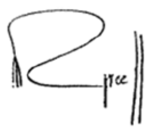
Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC complies with the South African National Health Act 61 (2003), the regulations on Research with Human Participants of 2014 of the Department of Health and Principles, the Declaration of Helsinki, 2013, the Belmont Report and the Ethics in Health Research: Principles, Structures and Processes (SANS document).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Ethics Office at Carolien.VanZyl@nwu.ac.za or 018 299 2089.

Yours sincerely



Prof Minrie Greeff
HREC Chairperson

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2015 Applications\Applications 09 - 21 October 2015\NWU-00055-15-A1 (M Sparks-A Strauss)\NWU-00055-15-A1 (M Sparks-A Strauss) - AL\NWU-00055-15-A1 (M Sparks-A Strauss) - AL.docm
02 November 2015

File reference: 9.1.5.3

APPENDIX C ETHICAL APPROVAL FOR PHD THESIS

NORTH-WEST UNIVERSITY ®
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Web: http://www.nwu.ac.za

Faculty of Health Sciences
Health Sciences Ethics Office for Research, Training and Support
Health Research Ethics Committee (HREC)

Tel: 018-285 2291
Email: Wayne.Towers@nwu.ac.za

28 February 2017

Dr C Pienaar
Human Movement Sciences-PhASRec

Dear Dr Pienaar

APPROVAL OF YOUR APPLICATION BY THE HEALTH RESEARCH ETHICS COMMITTEE (HREC) OF THE FACULTY OF HEALTH SCIENCES

Ethics number: NWU-00055-15-A1 Investigating performance indicators and injury risk factors for the development and performance of female soccer players

Kindly use the ethics reference number provided above in all correspondence or documents submitted to the Health Research Ethics Committee (HREC) secretariat.

Study title: The psycho-hormonal influence of fatigue on amateur female soccer players

Study leader/supervisor: Dr C Pienaar

Student: A Broodryk

Application type: Sub-study

Risk level: Minimal

You are kindly informed that your application was reviewed at the meeting held on 10/03/2016 of the HREC, Faculty of Health Sciences, and was approved on 28/02/2017.

The commencement date for this study is 28/02/2017 dependent on the conditions indicated below. Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years when extension will be facilitated during the monitoring process.

After ethical review:

Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC, Faculty of Health Sciences (if applicable).

The HREC, Faculty of Health Sciences requires immediate reporting of any aspects that warrants a change of ethical approval. Any amendments, extensions or other modifications to the proposal or other associated documentation must be submitted to the HREC, Faculty of Health Sciences prior to implementing these changes. Any adverse/unexpected/unforeseen events or incidents must be reported on either an adverse event report form or incident report form at Ethics-HRECIncident-SAE@nwu.ac.za.

A monitoring report should be submitted within one year of approval of this study (or as otherwise stipulated) and before the year has expired, to ensure timely renewal of the study. A final report must be provided at completion of the study or the HREC, Faculty of Health Sciences must be notified if the study is temporarily suspended or terminated. The monitoring report template is obtainable from the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-Monitoring@nwu.ac.za. Annually a number of studies may be randomly selected for an external audit.

Please note that the HREC, Faculty of Health Sciences has the prerogative and authority to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.

Please note that for any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC, Faculty of Health Sciences. Ethics approval is required BEFORE approval can be obtained from these authorities.

The HREC, Faculty of Health Sciences complies with the South African National Health Act 61 (2003), the Regulations on Research with Human Participants (2014), the Ethics in Health Research: Principles, Structures and Processes (2015), the Belmont Report and the Declaration of Helsinki (2013).

We wish you the best as you conduct your research. If you have any questions or need further assistance, please contact the Faculty of Health Sciences Ethics Office for Research, Training and Support at Ethics-HRECApply@nwu.ac.za.

Yours sincerely

Prof Wayne Towers
HREC Chairperson

Prof Minrie Greeff
Ethics Office Head

Current details: (13210572) C:\Users\13210572\Documents\HREC\HREC - Applications\2016 Applications\Applications 02 - 10 March 2016\NWU-00055-15-A1 (C Pienaar-A Broodryk)\NWU-00055-15-A1(C Pienaar-A Broodryk)-AL\NWU-00055-15-A1(C Pienaar-A Broodryk)-AL.docm
6 March 2017

File reference: 9.1.5.3

APPENDIX D PARTICIPATION INFORMATION LEAFLET AND CONSENT FORM

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Health Research Ethics Committee
Faculty of Health Sciences
NORTH-WEST University
(Potchefstroom Campus)
2015 -10- 02

HREC Stamp

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR UNIVERSITY-LEVEL FEMALE SOCCER PLAYERS

TITLE OF THE RESEARCH PROJECT: INVESTIGATING PERFORMANCE INDICATORS AND INJURY RISK FACTORS FOR THE DEVELOPMENT AND PERFORMANCE OF FEMALE SOCCER PLAYERS

REFERENCE NUMBERS: NWU-00055-15-A1

PRINCIPAL INVESTIGATOR: DR. MARTINIQUE SPARKS

ADDRESS: BUILDING K3, CNR OF THABO MBEKI & MEYER STR, POTCHEFSTROOM

CONTACT NUMBER: 018 299 1770

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00055-15-A1)** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

- *This study will be conducted at your home training field at the university and at the annual University Sports South Africa (USSA) tournament and will involve us measuring your body composition (eg. Mass, skinfolds etc.), flexibility as well as you performing two fitness tests, a speed test, a stability test and a skills test with experienced health researchers trained in Sport science. You will also be asked to complete two psychological questionnaires and an injury questionnaire. We will also monitor and analyse all of your matches during the USSA tournament. Thirty-eight participants will be included in this study.*
- *The objectives of this research are:*
  - *to determine the anthropometric profile according to player position of university-level female soccer players, thus developing a player profile for each position describing body mass, height, stature and fat percentage;*
  - *to determine the relationship between technical skills and the aerobic- and anaerobic fitness characteristics of university-level female soccer players, thus determining whether your technical skill performance will be influenced by your level of aerobic- and anaerobic fitness;*
  - *to determine the position specific internal and external match demands of university-level female soccer players by making use of global positioning system (GPS) analyses, thus using GPS technology to provide information such as your heart rate and the amount of time you spend at different intensity efforts performing activities such as standing, walking, jogging and running.*
  - *to determine the effect of an aerobic fatiguing test on the salivary cortisol levels and the psychological state of amateur female soccer players;*
  - *to determine the effect of an anaerobic fatiguing test on salivary cortisol levels and the psychological state of amateur female soccer players;*
  - *to determine the effects of the match-outcome (win/lose) and fatigue as a result of a soccer match on salivary cortisol levels and the psychological state of amateur female soccer players*
  - *to determine the injury history and incidence in university level African female soccer players.*
  - *to determine the intrinsic risk factors associated with lower limb injuries in university level African female soccer players.*

Why have you been invited to participate?

- *You have been invited to participate because you are female and represent your university in soccer*
- *You have also complied with the following inclusion criteria: you are currently injury free and you are part of the USSA squad.*
- *You will be excluded if: you become injured or ill at any time during the project or if we are not able to test you 2 weeks before the USSA championship starts.*

What will your responsibilities be?

- *You will be expected to:*
  - *Complete the informed consent form before the first day of testing. Informed consent will be obtained by an independent person not directly involved in the study.*
  - *Complete a questionnaire that pertains to your demographic information, playing and injury history on the first day of testing.*

- *You will be instructed not to engage in strenuous exercise for at least 48 hours before the fitness testing session. The research team will guide the coaches towards which activities will be allowed to engage in before testing. The research team will also consult with coaches beforehand to inform them of the intended dates of testing, so that they can plan their training program in such a way to accommodate the testing and necessary rest before testing commences.*
- *Conduct assessments for body composition. Various measurements will be taken using a skinfold calliper by Level 2 International Society for the Advancement of Kinanthropometry (ISAK) certified anthropometrists at a private designated testing area. This will include measurements of body mass, stature, skinfolds, breadths, girths and lengths and will require you to wear minimal clothing. You are allowed to strip down to as much clothing as is comfortable to you. These measurements will be taken at a private enclosed location. The flexibility in your legs and ankles will also be tested in this area.*
- *While you wait to be measured an injury history questionnaire will be completed for you by a Biokineticist. You will also be asked to complete two psychological questionnaires and provide a saliva sample before the onset of the physical testing*
- *Conduct tests for speed (40 m sprint test) and stability. The tests will be explained to you after which you will engage in a general warm-up of 10-15 min consisting of low-intensity jogging followed by static and dynamic stretching. You will then conduct the tests for speed and stability. You will be allowed to wear you soccer boots to complete these tests.*
- *You will then perform the technical skills test (consisting of dribbling, passing and control), followed by the aerobic fitness test (Yo-Yo Intermittent recovery test level 1 (Yo-Yo IR1)). During the execution of the aerobic fitness test you will be asked to run while being fitted with a portable gas analyser apparatus to measure you expired air as you run. This will require a harness to be fitted to your chest as well as a mask over your head and face. The mask will not hinder your breathing and you will be able to breathe normally. There will also be a heart rate belt fitted across your chest to monitor your heart rate during the test. After the completion of the aerobic fitness test, you will rate how tired you are and a blood lactate concentration [LA] will be taken from the fingertip of the left hand by means of a finger prick test. After blood sampling, you will again complete the skills test. You will not be allowed to recover between the technical skills test and the aerobic test, because we want to examine the effect of exhaustion on soccer skill performance. After the final test you will complete two psychological questionnaires for a second time and thirty minutes after the last test another saliva sample will be taken.*
- *Engage in explosive power (Vertical jump) test, technical skills and anaerobic fitness tests on the second day of testing. The tests will be explained to you after which you will perform general warm-up of 10–15 min consisting of low-intensity jogging followed by static and dynamic stretching. You will also be asked to complete two psychological questionnaires and provide a saliva sample before the onset of the physical testing. You will then conduct the explosive power test, technical skills test (consisting of dribbling, passing and control) and the anaerobic fitness test (repeated sprint ability test (RSA)). During the execution of the repeated sprint ability test, you will be required to sprint shuttles of 5 meters over a distance of 20 meters. After the completion of the anaerobic fitness test, you will rate how tired you are and a blood*

lactate concentration [LA] will be taken from the fingertip of the left hand by means of a finger prick test. After blood sampling, you will again complete the skills test. You will not be allowed to recover between the technical skills test and the anaerobic test, because we want to examine the effect of exhaustion on soccer skill performance. After the final test you will complete two psychological questionnaires for a second time and thirty minutes after the last test another saliva sample will be taken.

- *Be assessed at the USSA championship during the matches. You will be asked to wear a GPS sampling unit. This is a vest that is worn underneath your playing shirt, it is lightweight and will not obstruct your play in any way. Again a heart rate belt will be fitted across your chest to monitor your heart rate during the match. Before commencement of the tournament, you will be allowed to wear the GPS harness with the unit and heart rate (HR) transmitter belts during a practice session to familiarize yourself with units, to ensure you do not feel uncomfortable on the first day of the tournament.*
- *During the tournament saliva samples will be collected and the STAI and POMS questionnaire completed 1 hour before- and 30 minutes after the match, independent of the time of day that the match will take place. Following the soccer match, players will be instructed to indicate their RPE and complete the two questionnaires. Thirty minutes after the match the saliva samples will be collected.*

Will you benefit from taking part in this research?

- *The direct benefits for you as a participant will be that you will have access to your results by means of a personal report provided to you within 4 weeks after the final testing period. In the case of any immediate or unanticipated incidental findings occurring during the time of testing, you will be informed. The data gathered could enable you as player and your coach to compile specific and effective conditioning programs for the different player positions (attackers, midfielders and defenders) that will prepare you for the demands of soccer matches, consequently resulting in an improvement of performance. You could also benefit in terms of your own health by using results provided to you to improve your body composition and physical condition.*
- *The indirect benefit will be a broadening of specialist sport science knowledge with regards to female soccer, which can be transferred to the larger soccer community. This includes knowledge in the field of physical and physiological profiles and match demands of university level female soccer players. Workshops will be held after the completion of the project to empower not only university-level coaches, but also developing coaches to construct effective training programs for their female soccer teams.*
- *Scientists will gain understanding into the different intrinsic risk factors that are associated with lower limb injuries and how to minimize such injuries in future.*
- *Two Biokineticists will also travel with your team during the tournament to assist with strapping and massaging before and after matches.*

Are there risks involved in your taking part in this research?

- *The risks in this study are:*
  - *Physical discomfort: No severe physical stress beyond the risk encountered in normal life and everyday training are anticipated. All the skills and fitness tests are movements that you regularly do in your training program. You will be thoroughly warmed up before taking part in any of these tests. Should you*

experience pain or injury at any point you can stop the test and the physiotherapist or team doctor will first be allowed to examine you and assess your injury or discomfort.

- *Physical exhaustion: The Yo-Yo IR1 test and RSA test are maximal performance tests that need to be done until complete exhaustion. However, you are free to stop the test if you experience any light headedness, shortness of breath or headaches. An Automated External defibrillator (AED) with a qualified operator will also be at all testing opportunities if the need to use the apparatus arises.*
- *Social stress: No negative influence due to the presence of other players and the coaching staff are anticipated, due to you being accustomed to participate in a group and in front of the coaching staff and spectators. A clinical psychological consultant will be available for debriefing in case of any emotional reactions.*
- *Blood sample collection: Some discomfort might be experienced when the finger prick is done for blood sample collection. The procedure will be thoroughly explained to you and you will be free to withdraw if you are not comfortable with continuing. It will be a single prick on your finger taken by qualified scientists. Extreme pain is not expected. The finger prick test will be done on both the first and second day of testing. The needle of the finger prick apparatus as well as the disposable gloves will immediately be dispelled into an anatomical waste bin after each measurement taken. After a lactate reading is given by the lactate analyser the used blood lactate strips will also be dispelled into the portable biodegradable waist container. All the material will be disposed immediately after the collection of the data. The disposable waist container will be collected by the manufacturer, as per instructions.*
- *Salivary sample collection: No severe physical stress beyond the risk encountered in normal life and everyday training are anticipated. Saliva will be collected through a plastic straw into a 20 ml collection vial. The saliva sample will only be used to do hormonal analysis; no other analyses will be done.*
- *Psychological stress: A Sport Psychological Consultant will be available for debriefing in case of any emotional reactions experienced.*
- *Injury at the championship: You will not be exposed to additional risk due to the research conducted, in addition to that related to playing the game. Trained paramedics arranged by the tournament directors will be present at the tournament in case of any injuries.*

➢ *The benefits outweighs the risk*

What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

➢ *You might feel uncomfortable during the anthropometric measurements where body mass, stature, skinfolds, breadths, girths and lengths will be measured as you will be required to wear minimal clothing and the measurements might result in discomfort. The measurements will be taken by two experienced ISAK Level 2 certified anthropometrists. You will be measured alone in a private designated and enclosed area and will be allowed to strip down to as much clothing as is comfortable for you. Testing will be done taking your privacy into consideration*

➢ *Should you have the need for further discussions after the physical or psychological discomfort which might be experienced during the testing* *procedures, an opportunity will be arranged for you to consult with the team doctor, physiotherapist or clinical psychologist.*

Who will have access to the data?

- *Anonymity will be partial to protect you as individual. Confidentiality will be ensured by assigning a code to you only known by the researchers. Complete confidentiality cannot be ensured, as the research will be conducted in a group setting and the researchers cannot ensure confidentiality by other group members who might disclose information outside the research setting. In the final dataset it will not be possible to identify you. Reporting of findings will be anonymous and confidential by not using any individual identifiers in any publications resulting from this study. Only the researchers involved in this study will have access to the data obtained.*

What will happen with the data/samples?

- *This is a once off collection that will only take place during 2015 and the data will be fully analysed here in South Africa by the Statistical Consultation Services of the NWU.*
- *The hard copies of the data recorded on the first three testing procedures will be stored for a minimum of 7 years in a secure safe in the project leader's office, accessible only by the primary researchers, after which it will be destroyed by means of a paper shredder. The electronic data recorded by the GPS units and heart rate monitors will be downloaded to a password protected personal laptop immediately after each match and a back-up will be made on a compact disc (CD) and stored for a minimum of 7 years in a secure safe in the project leader's office, accessible only by the primary researchers, after which it will be erased from the laptop and the CD destroyed.*
- *The salivary samples will only be used for stress hormone analysis and no other analyses will be conducted on those samples. These analyses will be done at a professional laboratory (Ampath), guaranteeing confidentiality.*

Will you be paid to take part in this study and are there any costs involved?

No, you will/will not be paid to take part in the study but refreshments will be provided during and after the testing. You will not have any additional travel expenses during the times that this procedure will be conducted, as the first 3 days of testing will be conducted during normal practice times when you will be at the training field. The final testing to be conducted at the USSA tournament will also not lead to any additional travel expenses for you, as these expenses for travel, accommodation and meals will already have been paid for by the respective universities. There will thus be no costs involved for you, if you do take part.

Is there anything else that you should know or do?

- You can contact Dr. Martinique Sparks at 018 299 1770 if you have any further queries or encounter any problems.
- You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2089; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.

- You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

- The findings of the research will be shared with you 4 weeks after the final testing period. We will be sharing the findings with you by providing you with a personal report regarding your performance scores. You are welcome to contact us regarding the findings of the research. Findings with regards to the game analyses and playing position profiles will be shared with the coaching staff, however these results will be given as group statistics with no individual players identified.

Declaration by participant

By signing below, I .... agree to take part in a research study titled: Investigating performance indicators and injury risk factors for the development and performance of female soccer players

I declare that:

- I have read this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions to both the person obtaining consent, as well as the researcher and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) .... on (*date*) .... 20....

....

Signature of participant **Signature of witness**

Declaration by person obtaining consent

I *(name)* .... declare that:

- I explained the information in this document to ....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter.

Signed at (*place*) .... on (*date*) .... 20....

....

HREC General WICF Version 3, March 2015 Page 8 of 9

Signature of person obtaining consent **Signature of witness**

Declaration by researcher

I *(name)* ……………………………………………………… declare that:

- I explained the information in this document to …………………………………
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter.

Signed at (*place*) ………………………………………… on (*date*) ……………………… 20....

……………………………………………………… ………………………………………………………
Signature of researcher **Signature of witness**

APPENDIX E

GENERAL INFORMATION, ANTHROPOMETRIC, PHYSICAL AND PSYCHOLOGICAL DATA COLLECTION FORMS

PhASRec PHYSICAL ACTIVITY, SPORT & RECREATION | NORTH-WEST UNIVERSITY YUNIBESITI YA BOKONE-BOPHIRIMA NOORDWES-UNIVERSITEIT POTCHEFSTROOM CAMPUS ®

Demographic Questionnaire: Soccer Project **Date:____________**

PLAYER PERSONAL INFORMATION					
PLAYER NAME & SURNAME					
DATE OF BIRTH	year / month / day				
AGE (YEARS)					
CONTACT NUMBER					
NEXT OF KIN					
NEXT OF KIN CONTACT NUMBER					
POSITION	ATTACKER	MIDFIELDER	DEFENDER	GOALKEEPER	
NAME OF CLUB					
JERSEY NUMBER					
YEARS OF PLAYING EXPERIENCE					
YEARS OF PLAYING AT UNIVERSITY/CLUB LEVEL					
RACE	BLACK AFRICAN	INDIAN	COLOURED	CAUCASIAN	OTHER
DATE OF LAST MENARCH					
PRESCRIBED MEDICATION					

PhASRec PHYSICAL ACTIVITY, SPORT & RECREATION | NORTH-WEST UNIVERSITY YUNIBESITI YA BOKONE-BOPHIRIMA NOORDWES-UNIVERSITEIT POTCHEFSTROOM CAMPUS

SOCCER PROTOCOL

NAME AND SURNAME				
DATE OF BIRTH				
POSITION	DEFEND	MIDFIELD	ATTACK	GOAL

STATURE (cm)	
WEIGHT (kg)	

BODY COMPOSITION

TEST COMPONENT	1ST READING	2ND READING	MEAN
TRICEPS SKINFOLD (MM)			
SUBSCAPULAR SKINFOLD (MM)			
SUPRASPINALE SKINFOLD (MM)			
ABDOMINAL SKINFOLD (MM)			
FRONT THIGH SKINFOLD (MM)			
MEDIAL CALF SKINFOLD (MM)			
TEST COMPONENT	**1ST READING**	**2ND READING**	**MEAN**
HUMERUS BREADTH (CM)			
WRIST BREADTH (CM)			
FEMUR BREADTH (CM)			
ANKLE BREADTH (CM)			
TEST COMPONENT	**1ST READING**	**2ND READING**	**MEAN**
RELAXED ARM GIRTH (CM)			

FLEXED ARM GIRTH (CM)			
WAIST (MINIMUM) GIRTH (CM)			
GLUTEAL (HIP) GIRTH (CM)			
MID THIGH GIRTH (CM)			
TEST COMPONENT	**1ST READING**	**2ND READING**	**MEAN**
ACROMIALE-RADIALE LENGTH (CM)			
RADIALE-STYLION RADIALE LENGTH (CM)			
MIDSTYLION-DACTYLION (CM)			
FOOT LENGTH (CM)			

FLEXIBILITY

ILIOPSOAS FLEXIBILITY (˚)	R	L
QUADRICEPS FLEXIBILITY (˚)	R	L
HAMSTRING FLEXIBILITY (˚)	R	L
ANKLE FLEXIBILITY (˚) (PLANTAR)	R	L
ANKLE FLEXIBILITY (˚) (DORSI)	R	L
ANKLE FLEXIBILITY (˚) (INVERSION)	R	L
ANKLE FLEXIBILITY (˚) (INVERSION)	R	L

EXPLOSIVE POWER

	PAAL	PAAL	PAAL
VERTICAL JUMP (cm)	REACH	1	2
	TOTAL		
TENDO PEAK POWER (W)	1	2	
TENDO SPEED (M/SEC)	1	2	

LOWER LIMB STABILITY

CENTRE OF PRESSURE TEST (EYES OPEN)	R	L
CENTRE OF PRESSURE TEST (EYES CLOSED)	R	L
LIMITS OF STABILITY TEST	R	L

SPEED

10m (sec)	1	2
30m (sec)	1	2
40m (sec)	1	2

ANAEROBIC POWER

RAST TEST (sec)		**1.**	
		2.	
MAXIMUM POWER:		**3.**	
MINIMUM POWER:		**4.**	
AVERAGE POWER:		**5.**	
FATIGUE INDEX (%):		**6.**	
HR_{MAX} (BPM)			
LACTATE (MMOL)			

AEROBIC POWER

YO-YO LEVEL	LEVEL
DISTANCE (m)	m
HR_{MAX} (BPM)	
LACTATE (MMOL)	

Speed level	Repetitions							
5	1 (40)							
9	1 (80)							
11	1 (120)	2 (160)						
12	1 (200)	2 (240)	3 (280)					
13	1 (320)	2 (360)	3 (400)	4 (440)				
14	1 (480)	2 (520)	3 (560)	4 (600)	5 (640)	6 (680)	7 (720)	8 (760)
15	1 (800)	2 (840)	3 (880)	4 (920)	5 (960)	6.(1000)	7.(1040)	8.(1080)
16	1(1120)	2(1160)	3(1200)	4(1240)	5(1280)	6 (1320)	7 (1360)	8 (1400)
17	1(1440)	2(1480)	3(1520)	4(1560)	5(1600)	6 (1640)	7 (1680)	8 (1720)
18	1(1760)	2(1800)	3(1840)	4(1880)	5(1920)	6 (1960)	7 (2000)	8 (2040)
19	1(2080)	2(2120)	3(2160)	4(2200)	5(2240)	6 (2280)	7 (2320)	8 (2360)
20	1(2400)	2(2440)	3(2480)	4(2520)	5(2560)	6 (2600)	7 (2640)	8 (2680)
21	1(2720)	2(2760)	3(2800)	4(2840)	5(2880)	6 (2920)	7 (2960)	8 (3000)
22	1(3040)	2(3080)	3(3120)	4(3160)	5(3200)	6 (3240)	7 (3280)	8 (3320)
23	1(3360)	2(3400)	3(3440)	4(3480)	5(3520)	6 (3560)	7 (3600)	8 (3640)

SLEEP

1. How many hours did you sleep during the previous night?

2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours	9 hours	10 hours

2. Rate the quality of sleep of the previous night:

Very poor	Poor	Average	Good	Very good

3. How many times did you awaken during the previous night?

1	2	3	4	5 and more

PROFILE OF MOOD STATES (ISP)

PLEASE COMPLETE ALL STATEMENTS BY PLACING A CIRCLE AROUND THE NUMBER TO INDICATE LEVEL OF AGREEMENT WHERE:
1 = STRONGLY DISAGREE, 2 =DISAGREE, 3 = NEUTRAL, 4= AGREE AND 5 = STRONGLY AGREE

	STRONGLY DISAGREE	DISAGREE	NEUTRAL	AGREE	STRONGLY AGREE
1. I feel anxious…………………………………………………………….…………..	**1**	**2**	**3**	**4**	**5**
2. I feel sad………………………………………………………………………….	**1**	**2**	**3**	**4**	**5**
3. I am confused…………………………………………………………………….	**1**	**2**	**3**	**4**	**5**
4. I feel angry….…………………………………………………………………….	**1**	**2**	**3**	**4**	**5**
5. I feel energetic…………………………………………………………..............	**1**	**2**	**3**	**4**	**5**
6. I feel tired.………………………………………………………………………..	**1**	**2**	**3**	**4**	**5**

<u>TO BE COMPLETED PRIOR, AS WELL AS BEFORE AND AFTER THE TESTS / MATCHES</u>

SELF-EVALUATION QUESTIONNAIRE (STAI-1)

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel ***right now***, that is, ***at this moment***. There is no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your ***present*** feelings best.

1 = Not at all; 2 = Somewhat; 3 = Moderately so; 4 = Very much so.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

ONLY TO BE COMPLETED PRIOR TO TEST 1

SELF-EVALUATION QUESTIONNAIRE (STAI-2)

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you ***generally*** feel. There is no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your ***present*** feelings best.

1 = Not at all; 2 = Somewhat; 3 = Moderately so; 4 = Very much so.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel pleasant…………………………………………………………………………………………	1	2	3	4
2. I feel nervous and restless……………………………………………………………………	1	2	3	4
3. I feel satisfied with myself……………………………………………………………………	1	2	3	4
4. I wish I could be as happy as others seem to be…………………………………………	1	2	3	4
5. I feel like a failure………………………………………………………………………………	1	2	3	4
6. I feel rested………………………………………………………………………………………	1	2	3	4
7. I am "calm, cool and collected…………………………………………………………………	1	2	3	4
8. I feel that difficulties are piling up so that I cannot overcome them………………	1	2	3	4
9. I worry too much over something that really doesn't matter…………………………	1	2	3	4
10. I am happy………………………………………………………………………………………	1	2	3	4
11. I have disturbing thoughts……………………………………………………………………	1	2	3	4
12. I lack self-confidence…………………………………………………………………………	1	2	3	4
13. I feel secure……………………………………………………………………………………	1	2	3	4
14. I make decisions easily………………………………………………………………………	1	2	3	4
15. I feel inadequate………………………………………………………………………………	1	2	3	4
16. I am content……………………………………………………………………………………	1	2	3	4
17. Some unimportant thought runs through my mind and bothers me………………	1	2	3	4
18. I take disappointments so keenly that I can't put them out of my mind…………	1	2	3	4
19. I am a steady person…………………………………………………………………………	1	2	3	4
20. I get in a state of tension or turmoil as I think over my recent concerns and interests………………………………………………………………………………………	1	2	3	4

APPENDIX F LANGUAGE EDITOR LETTER

M.B. BRADLEY

P.O. Box 37326
Faerie Glen
Pretoria 0043

072 369 5149

Student:	**Date**
Ms A. Broodryk	2017/11/2

Document submitted for editing
The psycho-hormonal influence of fatigue on amateur female soccer players

The above thesis was submitted to me for language editing, which was completed on 2 November 2017.

MBradley

M.B. BRADLEY (MA) - Language editor

Cell no 072 3695 149

APPENDIX G

INSTRUCTIONS TO AUTHORS:

PHYSIOLOGY & BEHAVIOR JOURNAL

PHYSIOLOGY & BEHAVIOR
Official journal of the International Behavioral Neuroscience Society

ELSEVIER

AUTHOR INFORMATION PACK

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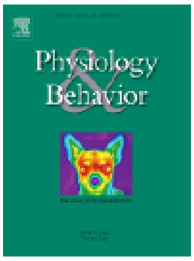

ISSN: 0031-9384

DESCRIPTION

Physiology & Behavior is aimed at the causal **physiological mechanisms** of **behavior** and its modulation by **environmental factors.** The journal invites original reports in the broad area of **behavioral** and **cognitive neuroscience**, in which at least one variable is physiological and the primary emphasis and theoretical context are behavioral. The range of subjects includes behavioral neuroendocrinology, psychoneuroimmunology, learning and memory, ingestion, social behavior, and studies related to the mechanisms of psychopathology. Contemporary reviews and theoretical articles are welcomed and the Editors invite such proposals from interested authors.
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AUDIENCE

Physiologists, neurologists, psychologists, pharmacologists.

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The confidence interval represents a plausible range of values within which the true (but unknown) population value lies (Cumming, G. [2012]. *Understanding the new statistics*. New York: Routledge). The greatest likelihood will arise from effects with narrow confidence intervals and therefore high precision.

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APPENDIX G

INSTRUCTIONS TO AUTHORS: *JOURNAL OF STRENGTH AND CONDITIONING RESEARCH*

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Chapter in an edited book
Yahara, ML. The shoulder. In: Clinical Orthopedic Physical Therapy. J.K. Richardson and Z.A. Iglarsh, eds. Philadelphia: Saunders, 1994. pp. 159–199.

Software
Howard, A. Moments ½software_. University of Queensland, 1992.

Proceedings
Viru, A, Viru, M, Harris, R, Oopik, V, Nurmekivi, A, Medijainen, L, and Timpmann, S. Performance capacity in middle-distance runners after enrichment of diet by creatine and creatine action on protein synthesis rate. In: Proceedings of the 2nd Maccabiah-Wingate International Congress of Sport and Coaching Sciences. G. Tenenbaum and T. Raz-Liebermann, eds. Netanya, Israel, Wingate Institute, 1993. pp. 22–30.

Dissertation/Thesis
Bartholmew, SA. Plyometric and vertical jump training.Master’s thesis, University of North Carolina, Chapel Hill, 1985.

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Article 2: The effects of aerobic fatigue on the psycho-hormonal state of amateur female soccer players

Journal of Sports Sciences

From: "Journal of Sports Sciences" <em@editorialmanager.com> 2017/07/26 12:50:55 P
Reply To: Journal of Sports Sciences
To: Adele Broodryk
Subject: Submission Confirmation for The effects of aerobic fatigue on the psycho-hormonal state of amateur fe

Jul 26, 2017

Dear Mrs Broodryk,

Your submission entitled "The effects of aerobic fatigue on the psycho-hormonal state of amateur female soccer players" has been received by journal Journal of Sports Sciences

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is http://rjsp.edmgr.com/.

Article 3: The effects of a soccer tournament on the psycho-hormonal states of collegiate female players

Journal of Strength and Conditioning Research

From: "Journal of Strength and Conditioning Research" <em@editorialmanager.com> 2017/11/03 7:56:20 PM
Reply To: Journal of Strength and Conditioning Research
To: Adele Broodryk
Subject: A manuscript number has been assigned to your JSCR submission

Nov 03, 2017

Dear Mrs Broodryk,

Your submission entitled "THE EFFECTS OF A SOCCER TOURNAMENT ON THE PSYCHO-HORMONAL STATES OF COLLEGIATE FEMALE PLAYERS" has been assigned the following manuscript number: JSCR-08-10202.

Our editors will review your manuscript within the next four to six weeks and will send their reviews back to our office. Reviewers do volunteer their time to complete the reviews of your article, and are very dedicated to getting reviews back as soon as possible. You will receive your reviews as quickly as possible. For updates, please check your status on this website, http://jscr.edmgr.com/.