The physiological impact of soccer on elite female players and the effects of active recovery training
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ABSTRACT

Female soccer is becoming more popular and professional in the world. There are, however, limited scientific data available on how elite female players respond to physical stress during soccer games. An effective recovery strategy following a game is important, because there are few recovery days between the games in international tournaments. The present thesis, which was designed to mirror a competitive situation, aimed to investigate changes in several physiological systems occurring in female elite players in response to two soccer games. It also aimed to investigate the effects of active recovery training on the recovery of several physiological systems.

METHODS:

Two elite female soccer teams played two 90-min games separated by 72 h active or passive recovery. The active recovery training (cycling at 60% HR peak, resistance training at <50% 1RM) lasted one hour and was performed 22 and 46 h after the first game. Countermovement jump (CMJ), 20-m sprint time and isokinetic knee strength were measured before, immediately, 5, 21, 45, 51, and 69 h after the first game, and immediately after the second game. The physical stress markers (CK, urea), oxidative stress markers (e.g., GSSG, lipid peroxidation), endogenous (e.g., UA, thiols) and dietary antioxidants (e.g., tocopherols, carotenoids) and a large battery of cytokines (e.g., IL-6, TNF-β) were analysed in blood.

RESULTS:

No significant differences were observed in the performance parameters, oxidative stress and antioxidant levels or inflammatory response between the active and passive recovery groups. Sprint and isokinetic knee strength were reduced by the same extent after both games. CMJ decreased after the first game and remained reduced throughout the study period. Blood physical stress markers, GSSG and endogenous antioxidants increased with similar amplitude after both games together with unchanged lipid peroxidation. The dietary antioxidants showed either a rapid and persistent change (e.g., tocopherols) or a delayed rise (carotenoids) after the first game. A transient increase occurred in several pro-(e.g., IL-12, TNF-α, MCP-1), anti-inflammatory (e.g., IL-4, IL-10, INF-α) and mixed (IL-6) cytokines after the first game. Fewer cytokines increased in response to the second game.

CONCLUSION:

Two repeated elite female soccer games separated by 72 h induced similar acute changes in several physiological parameters. After the first game, differences in the recovery pattern of the neuromuscular parameters occurred. In particular, the slow recovery of CMJ indicates that special attention should be devoted to the training of explosive force. Furthermore, the recruitment of antioxidants in response to the transient increase in GSSG resulted in the maintenance of the redox-balance in female players. Similarly, a strong and balanced pro- and anti-inflammatory cytokine response occurred after one single female soccer game. The consequences of the dampened cytokine response during repeated soccer games are, however, unknown. In general, the majority of the parameters had recovered prior to the second game and the physiological alterations induced by the first game did not affect the performance of players in the second game. Finally, active recovery training conducted after a soccer game does not accelerate the recovery time for neuromuscular, oxidative stress, antioxidant and inflammatory responses in elite female players.

KEY WORDS:

Football, Training, Recovery, Intermittent exercise

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Female soccer is becoming more popular and professional in the world. There are, however, limited scientific data available on how elite female players respond to physical stress during soccer games. An effective recovery strategy following a game is important, because there are few recovery days between the games in international tournaments. The present thesis, which was designed to mirror a competitive situation, aimed to investigate changes in several physiological systems occurring in female elite players in response to two soccer games. It also aimed to investigate the effects of active recovery training on the recovery of several physiological systems. METHODS: Two elite female soccer teams played two 90-min games separated by 72 h active or passive recovery. The active recovery training (cycling at 60% HR
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# LIST OF ABBREVIATION

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AA</td>
<td>Ascorbic Acid</td>
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<tr>
<td>ANOVA</td>
<td>The Analysis of Variance</td>
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<tr>
<td>CHO</td>
<td>Carbohydrate</td>
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<tr>
<td>CK</td>
<td>Creatine Kinase</td>
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<tr>
<td>CMJ</td>
<td>Countermovement jump</td>
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<tr>
<td>CV</td>
<td>Coefficient of variance</td>
</tr>
<tr>
<td>d-ROMs</td>
<td>The Diacrons Reactive Oxygen Metabolites</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FIFA</td>
<td>Fédération Internationale de Football Association</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric Reducing/Antioxidant Power Assay</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granolyte/Macrophage Colony-Stimulating Factor</td>
</tr>
<tr>
<td>GSH</td>
<td>Reduced glutathione</td>
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<tr>
<td>GSSG</td>
<td>Oxidised glutathione</td>
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<tr>
<td>HIR</td>
<td>High Intensity Running</td>
</tr>
<tr>
<td>HPCL</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HR</td>
<td>Heart Rate</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IL-1 RA</td>
<td>IL-1 Receptor Antagonist</td>
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<tr>
<td>INF</td>
<td>Interferon</td>
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<tr>
<td>IP</td>
<td>ImmunoProtein</td>
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<tr>
<td>MCP-1</td>
<td>Monocyte Chemoattractant Protein-1</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MIG</td>
<td>The Monokine induced by Interferon-Gamma</td>
</tr>
<tr>
<td>MIP</td>
<td>Macrophage Inflammatory Protein</td>
</tr>
<tr>
<td>NIST 970 SRM</td>
<td>National Institute of Standards and Technology, Standardized Reference Material nr 970</td>
</tr>
<tr>
<td>RANTES</td>
<td>Regulated upon Activation Normal T-cell Expressed and Secreted</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive Nitrogen Species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thioburbiuric acid-reactive substances</td>
</tr>
<tr>
<td>TGSH</td>
<td>Total Glutathione</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>UA</td>
<td>Uric Acid</td>
</tr>
<tr>
<td>UEFA</td>
<td>Union of European Football Associations</td>
</tr>
<tr>
<td>( \text{VO}_{2} \text{max} )</td>
<td>Maximal oxygen uptake</td>
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</tbody>
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INTRODUCTION

1.1 BACKGROUND: FEMALE SOCCER

In the early eighteenth century, female soccer games were played as an annual ritual between married and single women in Scotland (Williamson 1991). Female soccer became increasingly popular during World War I when games were organised by factory workers in England to raise money for charity (Williamson 1991). In 1920, for example, a game was played with a crowd of 53,000 people in the stands (Newsham 1997). In 1921, however, the English Football Association first decided that permission was necessary for clubs to organise female soccer games and later forbade females from playing soccer stating that it was "quite unsuitable for females and should not be encouraged" (Williamson 1991). The ban on female soccer was not lifted until 1971.

Today, female soccer is one of the fastest growing sports and has 26 million participants around the world (FIFA 2007). For example, Germany has over one million registered female soccer players (Deutscher Fussball-Bund 2009), while both Sweden and Denmark have approximately 60,000 registered players (Dansk Boldspil-Union 2009; Svenska Fotbollförbundet 2008). In 2006, 448 female international games were played in 134 countries (FIFA 2007). Moreover, several countries have leagues with full-time professional players. For national teams, the FIFA Women’s World Cup, the Olympic Games and the UEFA Women’s Championship are the most prestigious tournaments. At the club level, in addition to the domestic national leagues, the most prestigious competition is the UEFA Women’s Champions League.

During international female soccer tournaments, such as the FIFA Women’s World Cup and Olympic games, only two days of recovery are allowed between games compared to four to five days of recovery in male soccer tournaments. There are no reports, as far as we know, investigating whether two days of recovery after a game is sufficient recovery time for female players. Due to the short period of time separating two soccer games, it is important to optimise the recovery of players. It is therefore important to study the impact of a soccer game on several physiological systems in order to design effective recovery strategies. Such information about elite female soccer players is scarce, however.
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1.2 PHYSIOLOGICAL ASPECTS OF FEMALE SOCCER

1.2.1 Characteristics of female soccer players

The general characteristics of female soccer players have been extensively described in the literature (Davis & Brewer 1993; Jensen & Larsson 1992; Rhodes & Mosher 1992; Stålen et al. 2005; Tamer et al. 1997; Todd et al. 2002; Tumilty & Darby 1992). These studies showed that average range in height (158-170 cm), weight (55-65 kg), \( VO_2 \) max (47-58 mL.min\(^{-1}\).kg\(^{-1}\)), vertical jump performance (31-44 cm) and 20-m sprint time (3.00-3.31 s) vary among players in the various levels of competition and the different positions of players in the field (Krustrup et al. 2005; Mohr et al. 2008; Polman et al. 2004; Siegler et al. 2003; Stålen et al. 2005; Tumilty & Darby 1992).

1.2.2 Workload during games

Heart rate and blood lactate levels. The workload during a female soccer game is relatively high, but includes variations between players. The evaluation of heart rate during games shows that the mean heart rate represents ~85% of \( HR_{peak} \) (average range 161-177 bpm) and that the players may reach near maximum values (~97% \( HR_{peak} \); average range 171-205 bpm) several times during a game (Andersson et al. 2010; Davis & Brewer 1993; Krustrup et al. 2005). A blood lactate value of approximately 5 mmol.L\(^{-1}\) is usually reported by the end of a game (Davis & Brewer 1993; Krustrup et al. 2010). However, somewhat higher lactate values can be found after an intense work period during games (Krustrup et al. 2006). Both the mean heart rate and blood lactate levels reported in female players are within the same ranges of values reported in males (Bangsbo 1994).

Movement pattern analysis during games. The first reports on the movement pattern during female soccer games showed that players covered an average distance of 8.5±2.2 km (Davis & Brewer 1993). More recent studies report total distances around 10 km per game (Gabbett & Mulvey 2008; Hewitt et al. 2007; Krustrup et al. 2005; Mohr et al. 2008) which is similar to data on male players (Krustrup et al. 2005; Mohr et al. 2008; Mohr et al. 2003b). The total distance includes a large amount of walking and jogging (>50%). The distance covered in high intensity
running (HIR), which includes running speeds over 15 km/h and sprinting (>25 km/h), has therefore been suggested to be a better indicator of the physical stress during a game. In a study by Mohr et al. (2008) it was shown that top international female players covered an average of 1.7 km of HIR during a game. This differs from elite male players who covered approximately 2-3 km in HIR during a game (Mohr et al. 2003b). The amount of HIR performed by female soccer players is related to the competition level and may range between 0.7-2.0 km during a game (Krustrup et al. 2005; Mohr et al. 2008). It has also been reported that the same female player covered a longer distance of HIR when playing an international game than when playing a domestic league game (Andersson et al. 2010; Gabbett & Mulvey 2008). Moreover, several studies show that the performance of players decreases towards the end of a game (Gabbett & Mulvey 2008; Mohr et al. 2008; Mohr et al. 2003b). For example, the distance in HIR during the second half is shorter compared to the first half and this reduction in HIR occurs during the last 30 or 15 min of the game (Andersson et al. 2010; Gabbett & Mulvey 2008; Mohr et al. 2003a; Mohr et al. 2008). Altogether, data on heart rate and movement pattern indicate the relatively high workload of a female soccer game and the occurrence of fatigue by the end of the games.

1.2.3 The effects of a soccer game on performance and neuromuscular fatigue markers

Intensive game-activities affect the force-generating capacity of the neuromuscular system. The evaluation of sprint capacity, jump ability and isokinetic knee strength has been used to investigate neuromuscular fatigue (Raastad & Hallén 2000). There is little information on neuromuscular fatigue and recovery following a soccer game, especially in female soccer players. In males, it has been shown that sprint performance, countermovement jump (CMJ), and isokinetic peak torque knee extension and flexion are reduced after a single game and that these changes last up to several days following a game (Ascensão et al. 2008; Fatouros et al. 2009; Ispirlidis et al. 2008; Magalhães et al. 2010; Mohr et al. 2004; Raastad et al. 2002). In female players, one report showed a decline in 30-m repeated sprint performance but unchanged CMJ performance immediately after a single soccer game (Krustrup et al. 2010). However, a second study on female players showed that CMJ performance was reduced at 24 h but not immediately after a single soccer game (Hoffman et al. 2003). Thus, few and inconsistent findings are available on changes...
in neuromuscular parameters following a female soccer game. Additionally, knowledge of the effects of repeated soccer games on neuromuscular parameters is also limited.

1.2.4 The effects of a soccer game on blood markers of physical stress

Creatine kinase (CK), urea and uric acid (UA) are biochemical makers used for the evaluation of the physiological stress imposed by exercise. Increases in serum creatine kinase levels in response to strenuous exercise may be a consequence of both metabolic and mechanical stress (Brancaccio et al. 2007). Uric acid and urea are markers of both enhanced nucleotide cycle turnover and the breakdown of amino acids (Viru & Viru 2001). It has recently been shown that CK, urea and uric acid increased and remained elevated several days after a single soccer game in male players (Ascensão et al. 2008; Bangsbo 1994; Ispirlidis et al. 2008; Rowsell et al. 2009). Such information is not available for female players.

1.3 EXERCISE, OXIDATIVE STRESS AND ANTIOXIDANTS

1.3.1 Oxidative stress

During high-intensity exercise the production of free radicals is enhanced (Davies et al. 1982). Disturbances in the balance of free-radical production and antioxidant defences in favour of free-radical production may lead to so-called oxidative stress (Nikolaides et al. 2008). Under conditions of oxidative stress, free radicals may damage various tissues including muscle cells (Davies et al. 1982). Free radicals do, however, participate in many biological processes and their presence is essential for normal cell function. For example, it has been shown that in unfatigued skeletal muscle, free radicals have positive effects on the excitation-contraction coupling and are essential for optimal contractile function (Reid 2001). Exercise-induced increases in free radicals have also been suggested to act as signals to enhance the production of enzymes relevant to cell defence and the adaptation to exercise (Gomez-Cabrera et al. 2008b; Jackson 2008).

A free radical is a molecule or just a single atom with an unpaired electron which makes it highly reactive. Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Oxygen-centred free radicals contain two unpaired electrons in their outer shell. As the free radicals in biological systems are derived from or associated with the presence of molecular oxygen, free radicals in biological systems are mostly ROS. Examples of ROS are the superoxide anion (O$_2^-$), the peroxyl radical (ROO$^-$) and the hydroxyl (·OH) (Asmus & Bonifacic 2000). The free radicals may also derive from reactive nitrogen species (RNS) that include nitric oxide (NO$^-$) and nitrogen dioxide (NO$_2^-$). ROS and RNS have a strong tendency to extract electrons to reach a chemically more stable state and may cause damage to cellular components (Ji 2000). The oxidative damage to cellular targets is characterised by a progressive change or degradation of biomolecules as lipids, proteins and DNA (Blomhoff 2005). Lipid peroxidation can thus be used as a marker for the occurrence of oxidative stress.
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1.3.2 Antioxidant compounds

In normal physiological conditions, the production of ROS and RNS is in a finely-tuned equilibrium with the antioxidant system. This makes it possible for the body to sustain a balanced redox status (Djordjević 2004). The antioxidant system includes enzymatic and non-enzymatic endogenous and dietary antioxidant compounds. The enzymatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are major parts of the endogenous antioxidant system (Ji 2000). The non-enzymatic endogenous antioxidants such as uric acid and thiols (including total glutathione, cysteine, homocysteine and cysteine-glycine) are recognised as key physiological antioxidants that directly scavenge ROS and RNS and also enhance the functional ability of other antioxidants (Hellsten et al. 1997; Sen & Packer 2000). In addition, there are several potent antioxidants available in the diet. Tocopherols (also known as vitamin E), for example, are efficient radical scavengers that can convert superoxide, hydroxyl and lipid peroxyl radicals to less reactive molecules (Powers et al. 2004). Ascorbic acid (AA) (also known as vitamin C) is also an efficient antioxidant as it can directly scavenge ROS (Packer et al. 1979). Some carotenoid compounds are also effective dietary antioxidants. The antioxidant function of a carotenoid is dependent on its structure and on the specific chemical formation of the oxidizing species (Young & Lowe 2001). For example, lycopene is one of the most potent antioxidants of the carotenoid family as it can directly quench singlet oxygen (Di Mascio et al. 1989), while β-carotene is very reactive to peroxyl radicals (Burton & Ingold 1984) and zeaxanthin can limit
the diffusion of oxygen into membranes (Subczynski et al. 1991). Together, the endogenous and dietary antioxidant compounds act in concert in the antioxidant system against ROS and RNS (Powers & Sen 2000).

### 1.3.3 The effects of a soccer game on oxidative stress and antioxidant compounds

During high intensity exercise, whole-body oxygen consumption may rise up to 20-fold (Saltin & Åstrand 1967), while oxygen consumption in active muscles may reach 100 times the resting level (Davies et al. 1982). About 95-98% of the total oxygen consumption is used to produce ATP while 2-5% may undergo one electron reduction with the production of ROS and RNS. It has been shown that exhaustive exercise produces an excessive amount of ROS and RNS leading to oxidative stress (Sastre et al. 1992). It is also suggested that in well-trained athletes only limited oxidative stress occurs (Bloomer et al. 2006) which could be explained by a well-adapted antioxidant defence system in trained athletes (Bloomer et al. 2006; Brites et al. 1999; Cazzola et al. 2003).

Most studies on oxidative stress and antioxidant status following exercise have used endurance exercise protocols (Aguiló et al. 2005; Cases et al. 2006; Tauler et al. 2005). Because the physiological load of intermittent exercise, such as soccer, differs from continuous steady-state exercise, an extrapolation of data from continuous steady-state exercise to intermittent exercise should be made with caution (Nieman & Bishop 2006). Information on oxidative stress and antioxidants in soccer is, however, limited. Recently, four reports showed the occurrence of increased oxidative stress (increased lipid peroxidation) together with increased blood antioxidant compounds following a single game in male players (Ascensão et al. 2008; Fatouros et al. 2009; Ispirlidis et al. 2008, Magalhães et al. 2010). These studies indicate that elevations in antioxidant compounds following the games were not able to quench an excessive increase in ROS production, thereby causing oxidative stress. In these studies, however, only a limited number of antioxidant compounds was analysed (uric acid and total antioxidant capacity). In two studies, lipid peroxidation increased immediately after the game but a significant increase in uric acid occurred only at 24 h after the game (Fatouros et al. 2009; Ispirlidis et al. 2008). In the third and forth study, total antioxidant capacity and uric acid increased immediately after the game in parallel with increased lipid peroxidation (Ascensão et al. 2008; Magalhães et al. 2010). The normalisation of total antioxidant capacity...
occurred within 24 h, while uric acid remained elevated for more than 72 h (Ascensão et al. 2008). It is suggested that one single soccer game is associated with increases in the level of oxidative stress and deterioration of muscle performance throughout a 72 h recovery period (Ascensão et al. 2008; Fatouros et al. 2009).

Knowledge on the impact of a soccer game on the antioxidant system, including several members of the endogenous and dietary defence systems remains, however, limited. Moreover, available data include measurements conducted in response to one single soccer game. There is no data on the effects of repeated soccer games on oxidative stress markers and antioxidant levels.

It is also important to note that studies on soccer-associated changes in oxidative stress markers and antioxidant levels have been performed on male players (Ascensão et al. 2008; Fatouros et al. 2009; Ispirlidis et al. 2008, Magalhães et al. 2010). As oestrogens may have a protective function against ROS during exercise in untrained females (Akova et al. 2001), the existence of sex differences in the soccer-associated oxidative stress and antioxidant responses cannot be excluded.

1.4 THE INFLAMMATORY RESPONSE TO EXERCISE

Exercise initiates an inflammatory response that is similar to that observed after trauma or sepsis. This response includes both a systemic and a local immune response (Pedersen 2000). The systemic response, also known as the acute phase response, involves mobilisation of leukocytes, particularly neutrophil cells, into the circulation (Malm et al. 2004; Ostrowski et al. 1999; Peake et al. 2005a). In addition, lymphocytes (including natural killer cells) are also mobilised, and the production of cytokines is increased after exercise (Cox et al. 2007; Peake et al. 2005a; Peake et al. 2005b).

1.4.1 Cytokines and chemokines

Cytokines are a group of proteins produced by immune and non-immune cells. Many cytokines may be broadly classified as either pro-inflammatory (e.g., interleukin (IL)-12, INF-γ and TNF-α) or anti-inflammatory (e.g., INF-α, IL-10, IL-4, IL-1β), while some cytokines are suggested to have both pro-and anti-inflammatory functions (e.g., IL-6) (Moldoveanu et al. 2001). The majority of studies investigating the cytokine response during exercise have been conducted...
using continuous steady-state endurance exercise (Nieman et al. 2001; Ostrowski et al. 1998b) or resistance exercise (Chan et al. 2003; Paulsen et al. 2005).

Chemokines (e.g., IL-8, MCP-1, GM-CSF, and MIG) have also been shown to be up-regulated following endurance exercise (Åkerstrom et al. 2005; Ostrowski et al. 2001). Chemokines are chemotactic cytokines as they have the ability to induce directed chemotaxis (Warren et al. 2004). The release of chemokines causes leukocytes to adhere to vascular endothelium and subsequently to migrate into the tissue spaces. Chemokines may also have broader functions including a role in angiogenesis, collagen production and proliferation of hematopoietic precursor cells (Kunkel 1999; Mantovani 1999). Some chemokines are considered pro-inflammatory (e.g., IL-8) and induce the migration of leukocytes to an injured or infected site (Laing & Secombes 2004).

1.4.2 The effects of a soccer game on the inflammatory cell response

Few studies are available on the inflammatory cell response in soccer. Increases in circulatory leukocyte cell count, mainly caused by increases in neutrophil cells, have been reported in male players following soccer games (Ispirlidis et al. 2008; Malm et al. 2004; Rowsell et al. 2009; Magalhães et al. 2010). There are currently three studies available on the cytokine response in male players after soccer games. In one study, IL-6 and IL-1b increased immediately after a single soccer game (Ispirlidis et al. 2008). The authors did, however, report that IL-1b was below detection levels at all other time points during the study period (Ispirlidis et al. 2008). A second study reported increased levels of IL-6 and TNF-α following a soccer specific intermittent exercise protocol (Bishop et al. 2002). A third study revealed unchanged levels of IL-6, IL-1b and IL-10 following four consecutive soccer games in youth players (Rowsell et al. 2009). The cytokines in this study were measured more than 20 h after the end of each game. Since alterations in cytokine levels can be brief and rapidly normalised (Shephard 2002), the lack of soccer-associated changes in cytokines in the study of Rowsell et al., (2009) can be due to the timing of blood sample collection.

To our knowledge there is no data on the response of a large number of circulating pro- and anti-inflammatory cytokines following elite soccer, especially in female players.
1.5 ACTIVE RECOVERY STRATEGIES IN SOCCER

The use of post-exercise recovery methods has gained more attention within sport science research (Gill et al. 2006; King & Duffield 2009; Kinugasa & Kilding 2006; Vaile et al. 2008a). Methods include contrast-water therapy (Cochrane 2004; Kinugasa & Kilding 2006), active recovery training (Dawson et al. 2005; Gill et al. 2006) and cold-water immersion (Vaile et al. 2008b).

The scientific evidence supporting the effectiveness of such recovery strategies after a soccer game is limited. In one study an active cool-down program performed immediately after a single game had a positive effect on the recovery time for jump and sprint performance as well as subjective muscle soreness (Reilly & Rigby 1999). In male junior soccer players cold-water immersion performed immediately after soccer games reduced the perception of general fatigue and leg soreness but had no effect on a battery of physical performance tests, indices of muscle damage or inflammatory markers (cytokines IL-1b, IL-6 and IL-10) (Rowsell et al. 2009).

Active recovery training performed one day after a soccer game is recommended in order to achieve a faster return to a normal physical state (Barnett 2006; Reilly & Ekblom 2005). To our knowledge, there are no studies evaluating the effectiveness of active recovery training one day after a soccer game. The theoretical advantage of active recovery training would be to accelerate the recovery time of neuromuscular parameters and blood markers of physical stress and a quicker normalisation of the redox status and immunological systems. It is hypothesized that this strategy is necessary for optimal competitive performance and will help the players to cope with high training and game loads (Barnett 2006; Reilly & Ekblom 2005). The effectiveness of low-intensity training performed after a soccer game has not previously been evaluated in elite players.
The overall aim of this thesis was to investigate physiological and biochemical changes occurring in response to elite soccer games and to establish the time course of recovery for the neuromuscular and immunological systems, and the redox status.

The physiological effects of active recovery training conducted during the period separating two repeated soccer games were investigated in a population of elite female players.

The specific aims were:

i.) to study the acute changes imposed by one soccer game on neuromuscular fatigue parameters, blood markers of physical stress, the redox status and the inflammatory response in elite female players (Study I, II and III)

ii.) to study the recovery pattern of neuromuscular fatigue parameters, blood markers of physical stress, the redox status and the inflammatory response in elite female players during a period of 72 h following the first soccer game (study I, III, and IV)

iii.) to investigate the effects of low-intensity recovery training on the recovery pattern of neuromuscular fatigue parameters, blood markers of physical stress, the redox status and the inflammatory response in elite female players (study I, III, and IV)

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2 AIMS OF THE THESIS

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2 METHODS & MATERIALS

3.1 SUBJECTS

Twenty-two elite female soccer players (age 22±3 years; height 167±5 cm; weight 64±5 kg; VO₂ peak 55±3 ml.kg⁻¹·min⁻¹; HR peak 198±6 beats·min⁻¹) from two teams from the highest divisions in Sweden and Norway played two international 90-min friendly games separated by 72 h of active or passive recovery. Two defenders and one midfielder were unable to take part in the testing sessions during the period between the two games and were therefore not included in the data analyses. Because the physical loading of goalkeepers differs from that of other players they were not included in the analyses. Study I, on the neuromuscular fatigue and physical stress changes associated with the soccer games included 17 players. Study II and IV, on changes in oxidative stress markers and antioxidant levels included 16 players. Study III, on inflammatory changes included 10 players due to financial restrictions.

3.2 STUDY DESIGN

The two games were played during a period of four days and were separated by two days of either active or passive recovery. The same players in both teams participated in both games and occupied the same field position. A randomised blocked design was used to assign the players into an active recovery group (n=8) or a passive recovery group (n=9). The players were matched for age, height, weight, maximal oxygen consumption and field playing position. The active recovery training consisted of a low-intensity training program (sub-maximal cycling at 60% of HR peak and low-intensity resistance training <50% 1 repetition max (RM)) performed at 22 h and 46 h after the first game. On game day, baseline values for the performance parameters were obtained 3 h prior to the game. Subsequent performance tests were carried out immediately, 5 h, 21 h, 27 h, 45 h, 51 h and 69 h after the first game and immediately after the second game. Blood samples were taken 3 h before, immediately after (within 15-20 min), 21 h, 45 h, 69 h after the first game and immediately after the second game (Fig 1). Two days before the commencement of the study, all subjects performed a maximal oxygen consumption
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running test on a treadmill. One day before the start of the study, the players were familiarised with all testing procedures.

3.3 ACTIVE RECOVERY TRAINING

The active recovery training consisted of a low-intensity training program lasting one hour. The training consisted of 20 min sub-maximal cycling (60% of HFpeak, approximately 45% of VO_{2peak}) and 30 min low-intensity resistance training (<50% 1 RM), and 10 min sub-maximal cycling (60% of HFpeak) (Fig. 2). The low-intensity resistance training included exercises for both the upper and lower body. The exercise intensity during the cycling was monitored using a heart-rate monitor.
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The study was designed to mirror an actual competitive situation for female players participating in an international tournament, where two days of recovery between the games are allowed. The total time for the study period was seven days including travelling. In order to standardise the physical activity and nutritional intake during the study period the players stayed in the same hotel and ate every meal together. The games were conducted in the middle of the season (during the summer break) when the players were accustomed to high training and game loads. The first game in the study was played four days after the last game in the domestic leagues. Therefore, all players had rested at least three days from games and two days from soccer training prior to the first game.

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The active recovery training consisted of a low-intensity training program lasting one hour. The training consisted of 20 min sub-maximal cycling (60% of HF peak, approximately 45% of \(2 \cdot \text{VO}_{\text{peak}}\)) and 30 min low-intensity resistance training (<50% 1 RM), and 10 min sub-maximal cycling (60% of HF peak) (Fig. 2). The low-intensity resistance training included exercises for both the upper and lower body. The exercise intensity during the cycling was monitored using a heart-rate monitor (S610i, Polar Electro OY, Kempele, Finland). The rationale for using cycling as the low-aerobic training was that it increases blood flow in the leg muscles whilst minimising the load on the muscles which is proposed to be beneficial for recovery (Reilly & Ekblom 2005). The performance of low-intensity resistance weight training may enhance the protein metabolism in the exercised muscle, which would also be beneficial for recovery. The session was designed to mirror the recovery training used by many Nordic soccer teams. During the active recovery sessions the subjects drank 1 L of a sports drink that provided a carbohydrate load of 30-60 g per hour. During the one-hour period when the active recovery group performed the low-intensity exercise, the control group was instructed to rest.

Figure 2. Participants performing the active recovery training.

3.4 DIET

The food intake was standardised for all players during the study period, starting on the evening before the first game. All players were given a meal plan formulated by a nutritionist and the players ate each meal together. The composition of the meals was developed using a national food database ("Food on data" 4.3 LKH, Norway). Intake of carbohydrate (CHO), protein and fat were adjusted to each player’s body weight (55/60/65/70 kg respectively) to meet the recommendations for daily recovery in players participating in moderate training (intake of \(\geq 6\) g/kg body mass CHO, and \(\geq 1.2\) g/kg body mass protein) (Maughan et al. 2004). The food was chosen to replicate the player’s normal diet as much as possible and did not contain any food items with known high-antioxidant levels. The meal plan included a variation of bread, cereals, milk/yoghurts, meat, pasta/rice, fruit and vegetables to ensure...
adequate intake of macro- and micronutrients. In addition, the players were instructed to drink a sports drink during the games providing approximately 30-60g CHO/h (Maxim Energy™, Ishøj, Denmark). The sports drink did not contain vitamin C. After the blood sample was taken at the end of each game, each player consumed a CHO intake of 1 g/kg body weight within 30 min to ensure optimal recovery (banana, yoghurt, and sports drink) (Maughan et al. 2004).

3.5 EVALUATION OF WORKLOAD DURING THE GAMES

In order to compare the workload of the games, each player’s heart rate was recorded during the games. The players wore heart-rate monitors around their chests (Polar Team System, Polar Electro OY, Kempele, Finland) and data were continuously collected every 5 s. In addition to heart-rate measurements the players’ movement pattern and fluid loss were also recorded during the games. The movement pattern, including total distance covered and distance covered in high-intensity running (HIR) was collected from a total of ten players who played in different field positions (four defenders, three midfielders and three forwards). These ten players gave a representation of the movement pattern during the games. Each of these ten players was filmed in close-up by a digital camera (Canon DM-MV 600, Canon Inc., Tokyo, Japan) during the entire first and second games. The cameras were positioned at the side of the field, at the level of the midfield line, at a height of about 15 m above the field and at a distance of 30-40 m from the side line. The videotapes were later replayed on a monitor for computerized coding of the activity pattern. The locomotor speed ranges for high-intensity running were chosen according to Krustrup et al., (2005). These speeds were: moderate-speed running (15-17 km·h⁻¹), high-speed running (18-24 km·h⁻¹) and sprinting (> 25 km·h⁻¹). The distance covered for each activity within each interval was determined as the product of the total time and mean speed for that activity. All game recordings were analysed by the same experienced observer with a coefficient of variation (CV) for test-retest analysis of <3% for total distance covered and <5% for distance covered in high-intensity running.

The players' body mass was measured before and immediately after the games using a digital scale (Seca 708, Seca Ltd, Birmingham, United Kingdom). The
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The players’ body mass was measured before and immediately after the games using a digital scale (Seca 708, Seca Ltd, Birmingham, United Kingdom). The players' fluid intake was recorded during the games and the total fluid loss was calculated by the formula: Fluid loss = Δ Body Weight + fluid intake.

3.6 BLOOD SAMPLING

First in the testing procedure, blood was collected from an antecubital vein into 4-ml EDTA vacutainers and a 9 ml vacutainer tube. The blood in the 9-ml vacutainer tube coagulated at room temperature for 30-45 min. Serum was pipetted off and stored in Eppendorf tubes at -80 °C until analysis. One of the EDTA tubes was centrifuged at 1500 g at 4 °C for 15 min to get the required plasma. Plasma was further centrifuged for 5 min at 11 000 g at 4 °C; the supernatant was stored at – 80 °C until analysis.

3.7 NEUROMUSCULAR FATIGUE AND BLOOD MARKERS OF PHYSICAL STRESS

3.7.1 Sprint, CMJ and isokinetic strength tests

Prior to the sprint, CMJ and isokinetic knee strength tests, the players performed a standardised warm-up consisting of five min low-intensity running (using the YOYO intermittent endurance test level 1) (Krustrup et al. 2003). After the warm-up the players performed three maximal 20-m sprints: the best result was used in the data analysis. The players were instructed to stand with one foot on a marker and the time was started when the subjects touched a mechanical switch (placed 88 cm from the marker) in the first step. Sprint time was thereafter measured with photocells every 10-m (CV<1%). The photocells were placed at a height of 133 cm.

CMJ was performed on a force plate (SG 9, Advanced Mechanical Technology Inc., Newton, MA, USA) and low-pass filtered at 1050 Hz. The CMJ started from a standing position with the hands fixed to the hips. Jump-height was calculated from the vertical reaction force impulse during take-off. At each test the players performed three jumps with the best used in the data analysis (CV<5%).

Isokinetic concentric knee extensions and flexions (Fig. 3) were performed using two instruments, the Cybex 6000 dynamometer (Lumex, Ronkonfoma, NY, USA)
and the Technogym REV 9000 (Gambettola, Italy). This combination of equipment was chosen in order to enable testing of all subjects to be completed in the shortest possible period of time. Although controlled studies revealed that there were no differences in performance measured using the two instruments, the same subject was always tested on the same machine. The range of motion was set from a knee angle of 90° to 20° from full extension and the angular velocity of contraction of the isokinetic dynamometer rotating lever arm was set at 60°·s⁻¹. The subjects performed four warm-up contractions followed by three maximal contractions. Peak torque was analysed (CV<5%) and one leg was tested (dominant leg). The tight testing time schedule prevented isokinetic maximal strength at 45 h and sprint performance at 51 h after the first game from being measured.

Figure 3. A player performing the isokinetic knee-strength test.

3.7.2 Perceived muscle soreness

Perceived muscle soreness was assessed using a 7-point Likert scale (Morgan et al. 1988). It is designed to measure the level of muscle soreness in the lower body. The scale consisted of the following verbal anchors: 1 = very, very good; 2 = very good; 3 = good; 4 = tender but not sore; 5 = sore; 6 = very sore; and 7 = very, very sore. Scores were recorded to the nearest 0.5 decimal point.
3.7.3 Blood markers of physical stress; CK, urea, uric acid and glucose

CK, urea, uric acid and glucose were measured in plasma and were analysed with standard routine measurements in a Modular P® Analyzer (Hitachi, Tokyo, Japan). CVs for all variables were <5%.

3.8 OXIDATIVE STRESS MARKERS AND ANTIOXIDANT COMPOUNDS

3.8.1 Oxidative stress markers

The Diacrons reactive oxygen metabolites (d-ROMs) test was used to assess lipid peroxidation and was performed according to the manufacturer’s instructions (Diacron International, Grosseto, Italy). The analysis was fully automated, using a Technicon RA 1000 system (Technicon Instruments Corporation, NY, USA). The d-ROMs test monitors a rather persistent radical cation formed in the reaction of alkoxy and peroxy radicals derived from the hydroperoxides with a suitable additive N, N, diethyl-para-phenylenediamine (DEPPD). Thus, the d-ROMs level is proportional to the serum hydroperoxide concentration in plasma (Alberti et al. 2000). Hydroperoxides are products of the peroxidation of proteins, peptides, amino acids, lipids, and fatty acids (Nakayama et al. 2007). The test was carried out in the kinetic mode. Results are expressed in arbitrary units (CARR Units) (Cesarone et al. 1999) with a CV <10%. According to the manufacturer’s instructions a normal value for d-ROMs is between 250 and 300 CARR U in healthy individuals. In trained individuals lower basal values have been reported (< 250 CARR U) (Banfi et al. 2006). A value between 301 and 320 CARR U indicates a borderline condition of oxidative stress status, while a value above 320 CARR U indicates oxidative stress (Diacron International, Grosseto, Italy).

Quantification of oxidized glutathione (GSSG) and reduced glutathione (GSH) was performed using a two-dimensional chromatographic system with parallel Hypercarb columns coupled with dual fluorescence detectors (FLD). After sample preparation as previously described (Sakhi et al. 2006), 10 μL of the supernatant was injected into the chromatographic system. Derivatisation of GSSG was performed using monobromobimane (MBB) and ortho-phthalaldehyde (OPA). The CV for the method was below 7%. The ratio of GSH:GSSG was calculated by dividing GSH with GSSG. We analysed glutathione in plasma. In comparison to whole blood...
glutathione, plasma contains less than 1% glutathione (Ji 2000). Plasma glutathione can, however, be used as a model for intracellular glutathione, as it is assumed that plasma glutathione reflects intracellular whole blood glutathione. Reduced plasma glutathione (GSH) constitutes about 50-70% of total plasma glutathione while GSSG constitutes about 5-10% of GSH. Moreover, the fraction of TGSH in our study includes other components other than pure glutathione, such as adducts of plasma proteins, while GSH and GSSG are free plasma glutathione. Hence, the observed value of TGSH is higher compared to GSH and GSSG.

### 3.8.2 Antioxidant compounds

For the determination of the dietary antioxidant ascorbic acid, heparin plasma was immediately acidified using an equal volume of 10% meta-phosphoric acid (MPA) and stored at -70 °C until analysis within 3 months. Samples were analysed by high performance liquid chromatography (HPLC) (Karlsen et al. 2005, 2007) with a CV below 5%. Plasma calibrators quantified against the NIST 970 SRM served as standards.

For the determination of the tocopherols by HPLC, proteins were precipitated by the addition of 3 volumes of isopropanol, followed by centrifugation at 3000 g at 4 °C for 15 min. The internal standard tocol was added with the isopropanol and 5 µL of the clear supernatant were used for analysis (Richheimer et al. 1994). A fluorescence detector operated at 295 nm (ex) and 330 nm (em) was used for detection with a CV below 5%. Standards prepared in 1% bovine serum albumin in phosphate buffered saline were used for quantification. Total tocopherols is the sum of α-, β-, γ-, and Δ-tocopherols.

For analysis of the dietary antioxidants total polyphenols, 50 µL heparin plasma were mixed with 150 µL ethanol for 2 min, before centrifugation at 3000 g at 4 °C for 15 min. Fifty microliters of the clear supernatant were used for the Folin-Ciocalteu method as previously described (Maskarinec et al. 1999). Quercetin prepared in ethanol served as standard solution, and the results are given as µmol/L quercetin equivalents (QE) with a CV below 10%.

The total carotenoids is the sum of lutein, zeaxanthin, β-krytopoxanthin, α-carotene, β-carotene and lycopene. These dietary antioxidants were determined in plasma by HPLC. Proteins were precipitated and removed by the addition of a 4.5 volume of isopropanol followed by centrifugation at 3000 g at 4 °C for 15 min. The internal standard astaxanthin was added in the isopropanol. Twenty-five microliters...
of the clear supernatant were used for analysis. The mobile phases consisted of A: 20% water and 24% acetone in ethanol and B: acetone. The gradient conditions were as follows: from 2 to 100% B within 20 min, followed by 100% B for 15 min. Detection was performed at 453 nm using a variable wavelength detector. Plasma calibrators quantified against the NIST 968c SRM were used as standards and the CV for the method was below 5%.

The non-enzymatic endogenous antioxidants thiols (including total glutathione (TGSH), cysteine, homocysteine and cysteine-glycine) were analysed in plasma with the use of chemical reduction and according the “homocysteine by HPLC” kit provided by Biorad Laboratories GmbH (Munich, Germany). Standard solutions prepared in PBS served as calibrators with a CV <5%. Ferric reducing/antioxidant power assay (FRAP) was determined in plasma as described elsewhere (Benzie & Strain 1999).

3.9 INFLAMMATORY CELLS AND CYTOKINE RESPONSE

3.9.1 Leukocyte cell count

Leukocyte cell numbers were analysed with a Sysmex K-1000 (TOA Medical Electronics Co., Ltd., Kobe, Japan). Coefficient of variation for neutrophil and lymphocyte cell counts are <4%. Leukocytes in EDTA whole blood were counted as total number of leukocytes (white blood cells), neutrophils, lymphocytes and mixed cells. Mixed cells are monocytes, basophils, eosinophils and granulocytes.

3.9.2 Cytokine analysis

IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, INF-γ, INF-α, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, TNF-α, GM-CSF, IP-10, MIP-1α, MCP-1, MIG, Eotaxin and RANTES were measured in plasma by a validated sandwich immunoassay based protein array system commercial kit (Biosource International, kit no. LHC009, Camarillo, CA, USA). Cytokine detection was performed according to the manufacturer’s instruction, with assay diluents as blank. Calibration standards were prepared in the assay buffer. In brief: antibody-coupled microspheres specific for the different cytokines were incubated with plasma. Antigen binding was then detected after incubation with biotinylated detector antibodies. Detection was performed with
the use of a flow-based dual laser detector with real-time digital signal. The Luminex 100 IS instrument (Biosource, Nivelles, Belgium) with the Star Station acquisition program (v2 Applied Cytometry Systems, Sheffield, UK) were used to process the data. According to the manufacturer, both intra-assay variations and inter-assay variations for all antigens measured by the multiplex system are below 10%. Furthermore, the sensitivity of multiplex Bead Array assays for the detection of soluble cytokines from various manufacturers has been compared (Khan et al. 2004). First, Bead Array and ELISA values appeared to be comparable between the manufacturers (Khan et al. 2004). Second, the minimal detection range for the kit used in our study (Biosource kit) was comparable and even several-fold higher than that of other kits (Khan et al. 2004). Third, the sensitivity limits are equivalent to those of most ELISA’s (Martins et al. 2002). All samples were run in double samples according to the manufacturer’s recommendations.

3.10  STATISTICAL ANALYSES

Data are presented as means and standard deviation (SD), or standard error of the mean (SE). All data were tested for their normal distribution prior to statistical calculations. The statistical significance of the differences between groups and time points was determined using a two-way (group x time) repeated-measures ANOVA using absolute values. Where appropriate, Dunnett’s post hoc test and Tukey’s post hoc test were applied. In addition to describing the changes that occurred from baseline values, we also analysed the gradient of change from immediately before and after the first and second games (Study I, III, IV). In study II, the d-ROMs data were not normally distributed and were log-10 transformed prior to ANOVA. In study III, not normally distributed data were analysed using Friedman’s non-parametric related samples test with a Dunn’s post hoc test. Selected bivariate relationships were examined with the Pearson’s product-moment correlation coefficient test for normally distributed data, whereas the Spearman rank correlation coefficient test was used to analyse data that did not meet the assumption of normality. P values below 0.05 were considered statistically significant. The Statistical Package for the Social Sciences (SPSS Inc, version 12.0) and Statistica (StatSoft Inc, version 7.0) were used for the analyses.
3.11 ETHICAL APPROVAL

The players were informed of the experimental procedures and possible discomforts associated with the study and gave their written informed consent to participate. The study was conducted according to the policy statement set forth in the Declaration of Helsinki and approved by the Regional Ethics Committee of Uppsala, Sweden (Dnr 2004: M-364).
RESULTS AND DISCUSSION

4.1 WORKLOAD DURING THE GAMES

4.1.1 Main findings

Heart rate.

No significant differences were observed in heart-rate values between the active recovery and passive groups during the games. The mean heart rate was relatively high in both games (> 160 bpm). The HR ranged between 144–173 bpm in the first game and 152-180 bpm in the second game, showing individual differences. The mean heart rate and time spent above 85% and 90% of HR peak were slightly, but significantly, higher during the second game (Table 1).

Table 1. Heart rate in beats.min⁻¹, % HRpeak and time spent above 85% and 90% of HRpeak during the games for the active recovery and passive groups.

<table>
<thead>
<tr>
<th></th>
<th>Game 1</th>
<th>Game 2</th>
<th>Total game</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1st half</td>
<td>2nd half</td>
<td>Total game</td>
</tr>
<tr>
<td>HR (beats.min⁻¹)</td>
<td>165±3</td>
<td>162±3</td>
<td>163±3</td>
</tr>
<tr>
<td>% of HRpeak (%)</td>
<td>83±1</td>
<td>82±1</td>
<td>82±1</td>
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<tr>
<td>&gt; 85% HRpeak (min)</td>
<td>23±3</td>
<td>18±3</td>
<td>41±6</td>
</tr>
<tr>
<td>&gt; 90% HRpeak (min)</td>
<td>9±3</td>
<td>6±2</td>
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Passive group (n=9)

<table>
<thead>
<tr>
<th></th>
<th>Game 2</th>
<th>Total game</th>
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</thead>
<tbody>
<tr>
<td>HR (beats.min⁻¹)</td>
<td>162±2</td>
<td>165±2</td>
</tr>
<tr>
<td>% of HRpeak (%)</td>
<td>82±1</td>
<td>83±1</td>
</tr>
<tr>
<td>&gt; 85% HRpeak (min)</td>
<td>22±2</td>
<td>23±3</td>
</tr>
<tr>
<td>&gt; 90% HRpeak (min)</td>
<td>9±2</td>
<td>8±2*</td>
</tr>
</tbody>
</table>

Values are in means ± SE. * denotes significantly higher (p<0.05) compared to the first game.
4 RESULTS AND DISCUSSION

4.1 WORKLOAD DURING THE GAMES

4.1.1 Main findings

Heart rate. No significant differences were observed in heart-rate values between the active recovery and passive groups during the games. The mean heart rate was relatively high in both games (> 160 bpm). The HR ranged between 144–173 bpm in the first game and 152-180 bpm in the second game, showing individual differences. The mean heart rate and time spent above 85% and 90% of HRpeak were slightly, but significantly, higher during the second game (Table 1).

Table 1. Heart rate in beats.min⁻¹, % HRpeak and time spent above 85% and 90% of HRpeak during the games for the active recovery and passive groups.

<table>
<thead>
<tr>
<th>Active group (n=8)</th>
<th>Game 1</th>
<th>Game 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st half</td>
<td>2nd half</td>
</tr>
<tr>
<td>HR (beats.min⁻¹)</td>
<td>165±3</td>
<td>162±3</td>
</tr>
<tr>
<td>% of HRpeak (%)</td>
<td>83±1</td>
<td>82±1</td>
</tr>
<tr>
<td>&gt; 85% HRpeak (min)</td>
<td>23±3</td>
<td>18±3</td>
</tr>
<tr>
<td>&gt; 90% HRpeak (min)</td>
<td>9±3</td>
<td>6±2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Passive group (n=9)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st half</td>
<td>2nd half</td>
<td>Total game</td>
</tr>
<tr>
<td>HR (beats.min⁻¹)</td>
<td>162±2</td>
<td>160±2</td>
<td>161±2</td>
</tr>
<tr>
<td>% of HRpeak (%)</td>
<td>82±1</td>
<td>81±1</td>
<td>81±1</td>
</tr>
<tr>
<td>&gt; 85% HRpeak (min)</td>
<td>22±2</td>
<td>17±3</td>
<td>38±5</td>
</tr>
<tr>
<td>&gt; 90% HRpeak (min)</td>
<td>9±2</td>
<td>5±2</td>
<td>14±4</td>
</tr>
</tbody>
</table>

Values are in means ± SE. * denotes significantly higher (p<0.05) compared to the first game.

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Movement pattern analysis. The game duration was similar (90±0 min) in both games. The ten players analysed in the movement pattern analysis during games covered similar total distance (9.4 ± 0.8 km vs 9.9 ± 0.6 km respectively; NS) and high intensity running distances (1.09 ± 0.47 km vs 1.10 ± 0.43 km respectively; NS) in both games. The HIR distance ranged between 0.42 -1.98 km in the first game and 0.61-1.75 km in the second game, showing individual differences in the amount of work performed during the games. When analysing the HIR distance in 15-min periods during the games, the distances in the last 30 min of both games were significantly lower (~24%, p<0.05) compared to the first 15-min period of the games. The distance covered in HIR in the last 30 min of both games was also ~17% lower compared to the last 15-min period of the first half (30-45 min) (Fig. 4).

Figure 4. High-intensity running distance in each 15-min period during the games (n=10). ■ = game 1, □ = game 2. * denotes significantly lower compared with period 0-15 for both games. # denotes significantly lower compared with period 30-45 min for both games.

4.1.2 Discussion.

Based on the mean heart-rate values it can be concluded that the aerobic system was highly taxed during both games. The mean heart rate in both games was similar to that previously reported in competitive elite female soccer games (Andersson et al. 2010; Davis & Brewer 1993; Krustrup et al. 2005). The mean heart rate differed between the first and second game. Such small inter-match variability (~ 4%) could be expected between games. The high mean heart rate, time spent > 85% of HR peak and HIR distance imply that the players were able to work at a high intensity in both games. Importantly, the work intensity (measured as HR) in the second game did not differ between the active and passive recovery groups. This finding indicates that the active recovery training did not influence the game performance of players in the second game. The distance covered in HIR during both games was also similar to what has been previously reported in high-level competitive female soccer players (Andersson et al. 2010; Gabbett & Mulvey 2008; Krustrup et al. 2005; Mohr et al. 2008), which confirms that the work performed during the games was high. The reduction of HIR towards the end of the games may indicate the occurrence of fatigue (Andersson et al. 2010; Mohr et al. 2008). The facts that the distance in HIR was reduced by the same extent at the end of both games and that the reduction occurred at a similar time point (Fig. 4) during the games imply that the players’ performance in the second game was not affected by the physical stress imposed by the first game.

4.2 NEUROMUSCULAR FATIGUE AND BLOOD MARKERS OF PHYSICAL STRESS AFTER ELITE FEMALE SOCCER GAMES (PAPER I)

4.2.1 Main findings

The effects of active recovery training. At baseline, there were no significant differences in absolute values for sprint, CMJ, isokinetic strength, blood CK, urea and uric acid and perceived muscle soreness between the active and passive recovery groups. At all time points, we also found no significant differences between the two groups in the recovery pattern of any of the neuromuscular fatigue and blood physical stress markers. There was, however, a significant time effect on the neuromuscular fatigue and blood physical stress markers. As a consequence changes in these parameters are presented as a mean of both groups.

Neuromuscular fatigue. Sprint performance and knee extensor or flexor strength decreased immediately after both games with the amplitude of change being comparable after both games. Sprint performance returned to baseline 5 h after the first game while peak torque in knee extension and knee flexion returned to baseline...
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Movement pattern analysis. The game duration was similar (90±0 min) in both games. The ten players analysed in the movement pattern analysis during games covered similar total distance (9.4 ± 0.8 km vs 9.9 ± 0.6 km respectively; NS) and high intensity running distances (1.09 ± 0.47 km vs 1.10 ± 0.43 km respectively; NS) in both games. The HIR distance ranged between 0.42 - 1.98 km in the first game and 0.61-1.75 km in the second game, showing individual differences in the amount of work performed during the games. When analysing the HIR distance in 15-min periods during the games, the distances in the last 30 min of both games were significantly lower (~24%, p<0.05) compared to the first 15-min period of the games. The distance covered in HIR in the last 30-min of both games was also ~17% lower compared to the last 15-min period of the first half (30-45 min) (Fig. 4).

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Neuromuscular fatigue. Sprint performance and knee extensor or flexor strength decreased immediately after both games with the amplitude of change being comparable after both games. Sprint performance returned to baseline 5 h after the first game while peak torque in knee extension and knee flexion returned to baseline.
at 27 h and 51 h after the first game, respectively. CMJ performance decreased immediately after the first game and remained significantly reduced throughout the study period. The pre-second game levels for sprint and isokinetic strength, but not CMJ, had therefore returned to baseline levels before the start of the second game. As a consequence of the already reduced CMJ levels at the start of the second game, CMJ did not decline further immediately after the second game.

**Blood markers of physical stress.** Creatine Kinase, urea and uric acid significantly increased with similar magnitude after both the first and second games. The peak value for CK (451±59 U·L⁻¹) was observed 21 h after the game and returned to baseline levels 69 h after the first game. The peak values for urea and uric acid were observed immediately after the games and returned to baseline levels 21 h after the first game. Additionally, changes in perceived muscle soreness followed the same patterns as CK.

**Relationship between blood markers of physical stress and game performance parameters.** The increase in CK levels after the games was significantly related to the distance covered in HIR during both games \( (R^2 = 0.58, r = 0.76, p<0.05) \) (Fig. 5). Figure 5 also shows the occurrence of inter-individual differences in the amplitude of changes in blood physical stress and game performance markers.

![Figure 5. Relationship between the distance covered in HIR and acute change in CK following the first (◊) and second (■) games.](image-url)
4.2.3 Discussion

The acute reductions in the performance parameters (sprint, isokinetic knee strength and CMJ) observed in our study are of similar magnitude to data reported after a single soccer game or soccer specific running protocol in male players (Ascensão et al. 2008; Balsom et al. 1993; Krustrup et al. 2006; Oliver et al. 2008). The acute reductions in CMJ and sprint performance together with the decline in isokinetic strength indicate that different aspects of the force-generating capacity are compromised in response to a female soccer game. The reduction in the force-generating capacity may be caused by structural disruptions within myofibers (Gibala et al. 1995; Raastad & Hallén 2000), increased inorganic phosphate (Pi), alterations in the excitation-contraction process (involving alteration in Ca²⁺ release and low muscle pH due to lactic acid accumulation) (Warren et al. 2001; Westerblad et al. 2002), selective glycogen depletion (Krustrup et al. 2006) and impairment of the stretch-shortening cycle (SSC) (Avela & Komi 1998).

We also observed differences in the recovery time after the game for the different neuromuscular parameters. Sprint performance was the first physical capacity to return to baseline after the game (5 h) and was followed by knee extension peak torque (27 h) and flexion (51 h). CMJ did not recover throughout the remaining time points of the study. Although strong correlations have been shown between maximal strength and the performance in sprint and vertical jump (Wisloff et al. 2004) our results suggest differences in the recovery pattern between these physical qualities. The differences in recovery pattern can be explained by the existence of differences in the amount of muscle work and in the inter-muscular coordination between sprint, jump and isokinetic knee flexion and extension (Iossifidou et al. 2005; Jacobs et al. 1996; Jacobs & Schenau 1992).

The long-lasting reductions in jump performance (>72 h) and isokinetic strength (>45 h) indicate some slow recovering processes within the muscle. Myofibrillar disruptions have been observed after heavy-strength training and downhill running (Friden et al. 1981; Gibala et al. 1995). This may have contributed to the long-lasting reductions in the force-generating capacity though these ideas have not been directly examined after soccer games.

The increase in blood CK indicates an increased permeability in muscle cell membranes during the games. The observed increases in uric acid and urea suggest that soccer games also result in an increase in AMP levels with a subsequent increase in the formation of ammonia (NH₃) as well as the enhancement of protein
breakdown. The moderate increase in CK and urea indicates alterations in muscle membranes and in protein metabolism.

The existence of a relationship between changes in CK and distance in HIR indicates that the work performed in high speeds during a game is a good indicator of the stress put on leg muscles during a soccer game (Fig. 5). Moreover, the inter-individual variability in the amplitude of changes in performance parameters and physical stress markers after the games may be related to the amount of work performed by each player in the field and also to the physical fitness status of the player.

The active recovery training used in this study is widely used by teams in many countries and is believed to accelerate the recovery after a soccer game. Our results clearly show that the time course of the recovery of performance parameters and the blood physical stress markers were not significantly improved by the active recovery training. Our results are in favour of previous studies in elite athletes suggesting the lack of evidence supporting the use of active recovery strategies (Barnett 2006; Gill et al. 2006). Nevertheless, it should be emphasised that low-intensity training did not have any detrimental effects on recovery.

In conclusion, our data clearly demonstrate the existence of differences in the recovery pattern of various neuromuscular parameters and physical stress markers in response to female soccer games. We can conclude that the majority of the performance parameters (with the exception of CMJ) and physical stress markers had recovered before the start of the second game. Our data also show that the recovery time for neuromuscular fatigue and physical stress markers are not accelerated by active recovery training.

4.3 OXIDATIVE STRESS MARKERS AND ANTIOXIDANT COMPOUNDS AFTER ELITE FEMALE SOCCER GAMES (PAPER II AND IV).

4.3.1 Main findings

The effects of active recovery training. The active recovery training did not have an effect on the oxidative stress markers and antioxidant compounds at any time points during the study. There was, however, a significant time effect in the oxidative stress markers and antioxidant compounds. For this reason, changes in these parameters are presented as a mean for both groups.
Oxidative stress markers. The analyses of acute changes occurring immediately after the first and second games revealed a significant increase in GSSG whereas GSH remained unchanged. As a result of the increased GSSG and unchanged GSH the GSH:GSSG ratio decreased immediately after both games. The decrease in the GSH:GSSG ratio was statistically significant only after the first game, however. The increase in GSSG after the first game was brief as GSSG levels returned to baseline within 21 h. During the period separating the two games, GSH significantly decreased at 45 h and 69 h after the first game and thus the pre-second game GSH levels were lower than the levels prior to the first game. Lipid peroxidation measured by d-ROMs was not acutely changed after both games and remained unchanged at all time points except at 69 h when it slightly, but significantly, decreased from baseline levels.

Endogenous (non-enzymatic) antioxidant compounds. The general response pattern of the endogenous antioxidants was characterised by a robust increase in blood immediately after both games. More specifically, both TGSH, UA and FRAP increased with a similar magnitude after both games. The endogenous antioxidants cysteine, cysteine-glycine and total thiols also increased after both games. Although these compounds increased following both games, changes in cysteine, cysteine-glycine and total thiols were statistically significant only in response to the second game.

Dietary antioxidant compounds. The response patterns in the dietary antioxidants showed either a rapid and persistent increase (α-tocopherol, total tocopherols and AA) or a delayed rise (total carotenoids) in blood after the first game (Fig. 6a-d). Alpha-tocopherol, total tocopherols and AA increased immediately after the first game and remained elevated at all time points except for a temporary normalisation of AA at 21 h (Fig. 6c). Consequently, the pre-second game dietary antioxidant levels of α-tocopherol, total tocopherols and AA were higher than the levels prior to the first game. Immediately after the second game, there were no significant increases in total tocopherols (Fig. 6a) and AA, whereas α-tocopherol slightly decreased. There were no immediate changes in carotenoids after both the first and the second games (Fig. 6b). The carotenoids did, however, slowly increase after the first game (starting at 21 h) and stayed elevated at all further time points. Consequently, the pre-second game levels of several carotenoids (lutein, β-crytopxanthin, α-carotene, lycopene, and total carotenoids) were higher than the levels prior to the first game. Finally, total polyphenols significantly decreased immediately after the first game, remained
reduced until 69 h after the first game and were then unchanged after the second game (Fig. 6d). Finally, as shown in figure 6 a-d, the amplitude of changes in oxidative stress markers and antioxidant compounds after the games varied between players.

**Figure 6 a-d. Changes in dietary antioxidant compounds in female elite soccer players during two games separated by 72-h recovery. Values are relative changes (%) from baseline and each curve represents an individual player: a) total tocopherols b) total carotenoids c) AA and d) total polyphenols. IP = immediately post games. * denotes significant changes compared to baseline (P<0.05).**

### 4.3.2 Discussion

The significant increase in blood GSSG observed immediately after the games indicates increased ROS production. Considering that 2-5% of total VO\(_2\) results in
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the formation of ROS (Jackson 1998), and that the players worked at an intensity level of ~85% of HRpeak (approximately 67% of VO2peak) (Table 1), an increased production of ROS during the soccer game was expected. The increase in GSSG led to a decreased GSH:GSSG ratio after both games (statistically significant only after the first game). Prior to the second game the GSH:GSSG ratio was already reduced compared to baseline. This may explain why the decrease in GSH:GSSG ratio was statistically significant only after the first game and not after the second game. Interestingly, our study showed no significant changes in the level of d-ROMs after both games. Previous studies in male players showed increased lipid peroxidation (measured by malondialdehyde, MDA and thioburbiuric acid-reactive substances, TBARS) in plasma following a single soccer game (Ascensão et al. 2008; Ispirlidis et al. 2008). The opposite results between our study performed on elite female players and those from Ascensão et al. (2008) and Ispirlidis et al. (2008) performed on non-elite male players may be explained by an improved protection against oxidative insults in well-trained athletes (Brites et al. 1999; Cazzola et al. 2003) or by the protective effects of oestrogens (Kendall & Eston 2002). It has been shown that trained women have lower resting MDA levels that those of to trained men (Bloomer & Fisher-Wellman 2008). This indicates that sex differences in the response of oxidative stress markers and antioxidant levels may exist in soccer players.

Increases in ROS may modulate a variety of cellular processes that may cause structural changes in the muscle fibres and reduction of muscle contractile function (Peake et al. 2007). For example, increased ROS can affect sarcolemmal function (Dudley et al. 2006), calcium regulation (Abramson & Salama 1989), myofilament interaction (Callahan et al. 2001), and mitochondrial metabolism (Hauser et al. 1995; Pruijn & Schoonen 1992). It is therefore possible that the increased GSSG observed after the games may have contributed to the decline in force-generating capacities (reduced performance parameters). However, no significant correlations were observed between the neuromuscular fatigue parameters and GSSG levels after the games.

Our study showed for the first time the occurrence of a strong soccer-game-induced endogenous antioxidant response in female players. TGSH, UA and FRAP increased with similar amplitude after both games. Although the endogenous antioxidants cysteine, cysteine-glycine and total thiols also increased after both games, changes in the level of these antioxidants reached a statistically significant level only after the second game. Our data therefore indicate that endogenous
antioxidants may play an important role in the antioxidant defence during the acute phase following intermittent exercise. Generally, the workload induced by a soccer game is able to stimulate a strong acute endogenous antioxidant response which seems to be maintained during repeated soccer games.

The acute increase in blood levels of the dietary antioxidants tocopherols and AA and decrease of polyphenols after the first game indicate a role of some dietary antioxidants in the early line of defence against increased ROS production. Contrary to the rapid normalisation of the endogenous antioxidants, however, there was a persistent elevation of AA and tocopherols and reduction of polyphenols after the first game. The long-lasting changes of these dietary antioxidant compounds suggest that they are involved in the stabilisation and maintenance of homeostasis in the redox-balance several days following the first soccer game. As the response of these dietary antioxidants was long-lasting, they did not further increase immediately after the second game, thereby suggesting sufficient levels of available dietary antioxidants in the circulation during the second soccer game.

There were no acute changes in the carotenoids following the games suggesting that carotenoids are not involved in the acute response to increased ROS after exercise. We did, however, observe a delayed and persistent increase of carotenoids starting 21 h after the first game. This recruitment pattern suggests that carotenoids are slowly mobilised from their storage sites (such as the adipose tissue) possibly to strengthen the blood antioxidant capacity in response to the soccer game.

The active recovery training had no influence on the restoration of oxidative stress markers and antioxidant levels during repeated soccer games in elite female players. Furthermore, increases in GSSG are associated with exercise intensity (Ji et al. 1992) and the low-intensity recovery training in the time period after the first game did not cause increased GSSG.

Similarly to the game-performance parameters (e.g., HR and HIR), inter-individual differences were observed in the amplitude of changes in oxidative stress markers and antioxidant levels after the games. The increase in free-radical production and antioxidants is related to the increase in oxygen consumption that occurs during exercise. Therefore, the inter-individual variability in oxidative stress markers and antioxidants can be related to the amount of aerobic work performed by the players during the games. However, we failed to show a relationship between the workload (measured as HR) during the games and increases in GSSG or antioxidants after the games.
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In conclusion, our results suggest a model in which the concomitant increase in endogenous and dietary antioxidants in response to the transient increase in GSSG prevented the occurrence of lipid peroxidation measured by d-ROMs in well-trained female players (Fig. 7). The robust but brief increases in the endogenous antioxidants after the games indicate their involvement in the acute response to increased free-radical production. The dietary antioxidant response seems to be related to both the acute defence against increased free-radical production and to the maintenance of a prolonged balanced redox after a female soccer game. Finally, the use of active recovery training in the period between two elite female soccer games had no impact on the oxidative stress and antioxidant systems.

Figure 7. Interaction between oxidative stress markers and endogenous and dietary antioxidants following a soccer game in well-trained female soccer players.
4.4 INFLAMMATORY RESPONSE AFTER ELITE FEMALE SOCCER GAMES (PAPER III)

4.4.1 Main findings

The effects of active recovery training. There were no differences in the cytokine response between the passive and the active recovery groups at any of the time points. Importantly, there was a significant time effect on the inflammatory cytokine response. For this reason, changes in plasma cytokines and leukocyte cell count at different time points in the experiments are presented as a mean for both groups.

Leukocyte cell count. Total leukocyte and neutrophil cell counts increased similarly following both games and returned to baseline levels within 21 h after the first game.

Cytokine response. Immediately after the first game significant elevations in plasma concentration of twelve cytokines (IL-12, TNF-α, INF-γ, IL-6, IL-2R, IL-4, IL-5, IL-7, IL-10, IL-13, IL-17, and INF-α) and three chemokines (IL-8, MIG, and MCP-1) were observed (Fig. 8a-d). All cytokines and chemokines returned to baseline levels within 21 h after the first game and remained at baseline levels until the beginning of the second game. The cytokine response following the second game differed from that of the first game. Only two cytokines, IL-12 and IL-6, and the same chemokines IL-8, MIG and MCP-1 increased significantly after the second game. The amplitude of changes of these cytokines and chemokines after the second game was of similar amplitude to that seen after the first game (Fig. 8b-d).
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Figure 8. Plasma inflammatory cytokine response in female elite soccer players during two games separated by 72-h recovery. Values are absolute changes (mean ± SE) from baseline: a) anti-inflammatory cytokines, b) IL-6, c) pro-inflammatory cytokines and d) chemokines: IP = immediately post games: * denotes significantly higher than baseline (P<0.05).

4.4.2 Discussion

It has been shown that neutrophil cell counts increase progressively with exercise intensity (Sureda et al. 2009). Here we show that neutrophil cell counts increased similarly following both games. This is in accordance with the fact that both games in the present study were of a comparable intensity. The amplitude of the increase of cell counts and the time to return to baseline levels are in agreement with data reported in male players (Ascensão et al. 2008; Ispirlidis et al. 2008). This would suggest the absence of sex differences in the leukocyte response after soccer games. It
can also be concluded that leukocyte mobilisation is similar during repeated soccer games that are separated by 72 h.

Our study clearly showed a robust, but transient, increase in several pro- and anti-inflammatory cytokines and chemokines after the first game. It can be hypothesized that the cytokine release following the games might have been triggered by increases in catecholamines and cortisol, changes in metabolic activity, the occurrence of membrane disruptions in muscle cells, and increased free-radical production (Nieman et al. 2005; Steensberg et al. 2003; Suzuki et al. 2000). The large cytokine response after the first soccer game may also be explained by interactions between cytokines. All pro-and anti-inflammatory cytokines and chemokines had returned to baseline within 21 h and thus were within normal baseline ranges at the start of the second game.

Another main finding was the unexpectedly dampened cytokine response after the second game. There are several possible hypotheses behind this phenomenon. First, it has been shown that the cytokine response during exercise is dependent on the exercise intensity and duration (Pedersen 2000). Our results indicate that the intensity in both games was comparable or even slightly higher in the second game. The dampened cytokine response can therefore not be explained by a lower game intensity in the second game. It has also been reported that carbohydrate (CHO) loading attenuates the cytokine response following exercise (Bishop et al. 2002; Chan et al. 2003) and that CHO availability may influence the magnitude of the post-exercise disturbance in markers of immune function (Cox et al. 2008). In the present study, diet was carefully supervised several days before the second game but for only two meals before the first game. Thus, there is a possibility that the muscle CHO levels before the second game were higher than those before the first game. A third possible explanation is that a second bout of exercise would cause a dampening of the inflammatory response (Pizza et al. 2001). This concept is called the “repeated bout effect” (McHugh 2003). This phenomenon is, however, reported to occur mostly in untrained subjects (Nikolaidis et al. 2007; Pizza et al. 2001). As the players in our study were well-trained, it seems unlikely that the dampened cytokine response was due to the repeated bout effect. The dampened cytokine response may also be the result of a fast suppressive counter-regulation (Weinstock et al. 1997). The second game was played 72 h after the first game and whether a fast systemic counter-regulation following the first game might occur and influence the cytokine response in a second game played 72 h later is unknown. Finally, it has been
suggested that high levels of antioxidants (using antioxidant supplements) attenuates cytokine production (Fischer et al. 2004; Vassilakopoulos et al. 2003). In our study, several dietary antioxidants were significantly elevated both prior to and directly after the second game. The dampened cytokine response may therefore be connected to the high blood antioxidant levels observed before and after the second game.

The active recovery training performed 22 and 46 h after the first game did not affect the inflammatory mediators in elite female players. This finding is similar to data reported in previous studies where strategies as active recovery or cold-water immersion did not accelerate the recovery of several neuromuscular or physical stress markers (Gill et al. 2006; Kinugasa & Kilding 2009; Tessitore; 2008) and cytokine response (Rowsell et al. 2009) following intermittent exercise.

It is suggested that the structural changes occurring in the muscle fibre during exercise elicit a repair response when the mechanical disruption of myofibers initiates local and systemic production of cytokines (Pedersen & Hoffman-Goetz 2000). Consequently, increased cytokine levels have mainly been associated to eccentric muscle actions and it has been suggested that muscle damage (indicated by elevated CK) is associated with increases in cytokines, such as IL-6 (Bruunsgaard et al. 1997; Nieman et al. 2005). However, other studies have failed to find a correlation between IL-6 and muscle damage (Croisier et al. 1999; Ostrowski et al. 1998a; Ostrowski et al. 2000). It has therefore been suggested that exercise-induced muscle damage is not the major cause of increased plasma IL-6 concentrations following eccentric exercise (Peake et al. 2005c). In the present study we did not find any correlation between changes in CK and IL-6 or any of the other cytokines. Our results do not support the existence of a relationship between muscle damage markers and changes in cytokines.

The production of cytokines following exercise has also been closely associated with increases in neutrophil cell counts (Suzuki et al. 1999). We did not however find a relationship between the amplitude of plasma cytokine changes and the amplitude of neutrophil cell count changes.

In conclusion, this study shows that one single elite female soccer game leads to an immediate mobilisation of immune cells and a robust, but transient, increase in plasma concentration of both pro- and anti-inflammatory cytokines. Our data show that when a second game is played 72 h after the first game a dampened cytokine response occurs. The mechanisms and the practical implications of the dampened cytokine response are unknown. Our findings indicate that active recovery training
does not influence the recovery pattern of inflammatory parameters in elite female soccer players. This is in accordance with the lack of effects of this recovery strategy on neuromuscular and physical stress parameters, oxidative stress markers and antioxidant levels.
5 CONCLUSIONS

Female soccer is one of the fastest growing sports in the world. There are, however, limited scientific data on how elite female players respond to the physical stress imposed by a soccer game. Additionally, knowledge about the recovery processes following a game is also limited. Efficient recovery following a game in international tournaments is important since there are few recovery days between games. The present thesis was designed to mirror an actual competitive situation for elite female players in order to study the physiological impact of soccer on female players and to better understand the recovery of several physiological systems during two repeated soccer games.

5.1 MAIN FINDINGS OF THE THESIS

i) Immediately after one soccer game in elite female players a reduction in performance parameters was observed indicating the occurrence of neuromuscular fatigue. Increases in blood markers of physical stress and perceived muscle soreness also occurred immediately after a game. The first game resulted in the enhancement of free-radical production and the concomitant recruitment of endogenous and some dietary antioxidant compounds, which maintained the redox balance. A balanced recruitment of several pro-and anti-inflammatory cytokines also occurred.

ii) During the 72-h recovery following the first game, marked differences occurred in the recovery pattern of the various performance and blood physical stress markers. In general, the majority of the neuromuscular fatigue parameters (with the exception of CMJ) and physical stress markers had returned to baseline levels before the start of the second game. The changes that occurred in blood oxidative stress markers and endogenous antioxidants after the first game were brief and had returned to baseline levels within 21 h. The changes in blood levels of dietary antioxidants were, however, more long-lasting. The soccer-game-induced increases in the inflammatory cytokines were brief and had returned to baseline levels within 21 h after the first game.
iii) The low-intensity recovery training performed between two games did not influence the recovery time of the performance parameters, blood physical stress markers, the restoration of the redox balance or the inflammatory parameters in elite female players.

iv) Two repeated elite female soccer games induced similar acute changes in the neuromuscular fatigue parameters (with the exception of CMJ), elevation in blood physical stress markers and perceived muscle soreness. Similar acute oxidative stress reactions and recruitment of endogenous antioxidants occurred after both games. The dietary antioxidants (with the exception of carotenoids), however, had dissimilar acute recruitment patterns after both games. Dissimilar inflammatory responses also occurred after the two games, with a marked damped cytokine response after the second game.

5.2 IMPLICATIONS

In general, the recovery patterns of the soccer-induced changes in neuromuscular fatigue parameters, physical stress markers, the redox status and inflammatory parameters indicate that 72 h between two games is an adequate recovery period in elite female soccer players. An inter-individual variability in the amplitude of soccer-induced changes in the physiological variables occurred and may be related to the amount of work performed by each player in the field. It is important to highlight the fact that the game performance (HIR distance and HR) in the second game was also not affected by the physiological changes imposed by the first game. However, the fact that CMJ did not recover during the 72-h period after the first game indicates that a particular attention should be devoted to the training of explosive force.

Since the amount of HIR distance during the game was correlated to changes in CK, HIR may be a useful indicator of the stress put on leg muscles during a game and may help to individualise the recovery period in soccer players.

Low-intensive recovery training performed at 22 and 45 h after the first game did not accelerate the recovery of neuromuscular fatigue and physical stress markers, the redox-balance or the inflammatory response in elite female players. The potential benefits behind the use of this post-game recovery strategy are not supported by our
data. Nevertheless, because of the lack of both positive and negative effects on recovery, it is suggested that low-intensity exercise can be performed in the recovery period between soccer games without risks of deteriorating performance in upcoming games.

This thesis demonstrated the effectiveness of the antioxidant systems in elite female soccer players. Based on our findings, the regular use of antioxidant supplements in well-trained female athletes may be questionable. It has been shown that supplementation with vitamin C may inhibit cellular adaptations to exercise because it prevents the expression of key transcription factors involved in mitochondrial biogenesis (Gomez-Cabrera et al. 2008a). The use of supplementation in elite female players may interfere with soccer-induced training adaptations and should not be recommended.

The strong pro- and anti-inflammatory cytokine response observed after one single soccer game in the female players might be an important factor involved in the adaptation to regular training. The use of anti-inflammatory drugs may therefore interfere with training adaptations. In this respect, it has been suggested that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) attenuates the exercise-induced increase in satellite cell numbers and thereby hampers skeletal muscle adaptation to exercise (Mackey et al. 2007). Interestingly, the second game played 72 h after the first one was associated with a dampened inflammatory reaction and a more pronounced pro-inflammatory cytokine response. The implications of this phenomenon during repeated soccer games deserve further attention.

5.3 STUDY STRENGTHS AND LIMITATIONS

The originality of this thesis is the characterisation of physiological changes that occur in elite female players following two 90-min soccer games conducted as competitive games. The study was performed during the game season and both games included well-trained players who had a demanding training and game schedule. The strength of this study was that the experiment was conducted in an actual competitive situation mirroring the real physiological and environmental factors associated with elite female soccer. Additionally, the ability to monitor the elite players during a period of five days and to standardise several important factors
during the study period further strengthens the conclusions associated with this work.

Paradoxically, the strength of this thesis may also be regarded as its weakness. Due to the limited number of players taking part in a soccer game (22 players), there were relatively few players per recovery group (active, passive). The scientific literature in the field does, however, highlight the need for data generated during experiments conducted in real competitive situations. Moreover, our findings are in line with data generated by other studies reporting the lack of beneficial effects of active recovery training (Barnett 2006; Gill et al. 2006; Rowsell et al. 2009). Furthermore, the game performance (measured as mean HR and time >85% \( HR_{peak} \)) was similar in both games and did not differ between the players in the active or passive recovery groups. Regardless of the recovery regime, the players were, thus, able to perform at a high intensity during the second game. This further supports the finding that active recovery training has no effect on various physiological systems. Nonetheless, the relative small sample size should be taken into account when interpreting the data on the effects of recovery training on the physiological variables measured in this thesis. Moreover, despite that the effects of active recovery training was evaluated using a large battery of physiological parameters, the possibility that this recovery strategy might have affected other parameters not evaluated in this study cannot be excluded.

It is also important to highlight the difficulty of measuring oxidative stress in plasma. It is rather difficult to detect reactive intermediates directly in vivo because of their short half-lives and therefore most studies evaluate various stress markers in plasma, blood or urine (Urso & Clarkson 2003). In general, every assay has its advantages and disadvantages and no single measurement can adequately describe oxidative damage (Nikolaidis et al. 2008). It is suggested, therefore, that the use of a battery of measurements is important to reliably monitor changes in the redox system (Halliwell & Whiteman 2004). In the present study, the use of several biomarkers allowed us to highlight the fact that despite lack of change in lipid peroxidation measured by d-ROMs, the increased in GSSG, the decreased GSSG:GSH ratio and increased in antioxidant levels all occurred in response to exercise.
5.4 FUTURE DIRECTIONS

In the present thesis several aspects related to changes in neuromuscular fatigue markers, the redox-status and inflammatory parameters were revealed for the first time in elite female soccer players. This provides a platform for future experiments aimed at further understanding the physiological changes associated with elite female soccer. International soccer tournaments may include up to six consecutive games. The implications of our findings in such a situation, where several consecutive games are separated by only short recovery periods, remain to be elucidated. The context of international tournaments may also include other important parameters that may affect the recovery process after a game, including warm and humid climates, nutritional aspects and psychological factors. The influence of these aspects on recovery deserves further attention.

Interestingly, CMJ did not recover during the 72-h recovery period after the first game. There are only a few studies that have investigated the recovery pattern of performance parameters following soccer games. Furthermore, there are conflicting results regarding the recovery of CMJ. The understanding of the mechanisms behind the slow recovery of CMJ in elite female players merits further investigations.

The dampened cytokine response observed following the second game also merits further attention. It has previously been shown that several immunological parameters were suppressed for several days after two consecutive games in young male players (Malm et al. 2004). The implications of the dampened cytokine response when athletes play multiple soccer games in tournaments are unknown.

Future investigations should also determine whether other forms of recovery strategies are able to accelerate the recovery of neuromuscular fatigue and blood physical stress markers, the redox-status and the inflammatory response in elite soccer players.
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I wish to express my warmest gratitude to all those who contributed to the studies and made this thesis possible. I would especially like to thank:

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SVENSK SAMMANFATTNING

INTRODUKTION: Damfotboll har blivit mer populär och mer professionell runtom i världen. Det finns emellertid lite vetenskaplig data om hur matchbelastningen påverkar fysiologiska parametrar för elitdamfotbollspelare. I internationella turneringar är det dessutom få dagar mellan matcher och effektiv återhämtning är viktig för spelarna. Syftet med denna avhandling var därför att studera fysiologiska förändringar som sker efter matcher i en så verklig situation som möjligt för elitspelare. Akuta och efterföljande fysiologiska förändringar undersöktes i samband med två fotbollsmatcher, liksom effekten av aktiv återhämtningsträning eller passiv vila på återhämtningsförmågan i perioden mellan matcherna.

METOD: Två elitdamfotbollslag spelade två 90-min matcher med 72 tim mellanrum och som innehöll aktiv återhämtningsträning eller passiv vila. Den aktiva återhämtningsträningen (cykling 60% av HRpeak och lågintensiv styrketräning på <50% 1RM) varade i en tim och utfördes dagarna mellan matcherna. De fysiska mätningarna, countermovementhopp (CMJ), 20–m sprint, maximal isokinetisk knäflexion och flexion, mättes före, direkt efter och 5, 21, 45, 51 och 69 tim efter den första matchen och direkt efter den andra matchen. I blodet mättes fysiska stressmarkörer (t ex. CK, urea), oxidativa stressmarkörer (t ex. GSSG, lipidperoxidation), endogena (t ex. urinsyra, thiols) och dietära antioxidanter (t ex. tocoferoler, karotenoider) samt ett stort batteri av cytokiner (inklusive IL-6 och TNF-α).

RESULTAT: Inga signifikanta skillnader observerades mellan den aktiva och passiva återhämtningsgruppen i de olika parametrarna. Direkt efter båda matcherna observerades försämringer med liknande amplitud i sprintförmåga, maximal isokinetisk knäextension och flexion. CMJ försämrades direkt efter den första matchen och var reducerad under hela undersökningsperioden. Direkt efter båda matcherna observerades ökningar med liknande amplitud i plasma CK, urea, uric syra, FRAP, GSSG, TGSH, leukocyter och neutrophiler samt oförändrad lipid peroxidation. De dietära antioxidanterna visade antingen en akut och långvarig förändring (tocadoferoler, AA och polyphenoler) eller en fördröjd ökning (karotenoider) efter den första matchen. En signifikant, men snabbt övergående ökning av flera pro-inflammatoriska (IL-12, INF-γ, IL-17, MCP-1, IL-8 och MIG), anti-inflammatoryiska (IL-2R, IL-4, IL-5, IL-7, IL-10, IL-13, INF-α) cytokiner och IL-
Physiological impact of soccer on elite women. A clear decreased cytokine response was observed after the first match. During the second match, a clear increased cytokine response was observed.


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