Performance, Fatigue and Recovery in Soccer

Functional and biochemical analysis of professional players during a soccer season

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**KEYWORDS:** SOCCER SEASON, TRAINING STATUS, SOCCER PHYSIOLOGY, PROFESSIONAL PLAYERS, TIME-MOTION ANALYSIS, HORMONAL STATUS, OXIDATIVE STRESS, MUSCLE DAMAGE.
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**R-I.**
Silva, JR., Magalhães, J., Ascensão, A., Rebelo, A. Changes in soccer players’ physiological and physical characteristics throughout the season - A Review.

**R-II.**
Silva, JR., Magalhães, J, Ascensão, A., Rebelo, A. Strength and Muscle Power training in Soccer - A Review.

**S-I.**

**S-II.**
Silva, JR., Ascensão, A., Marques, F., Rebelo, A., Magalhães, J.(2012) Neuromuscular function, hormonal and redox status, muscle damage of high-level soccer players throughout a season competition. Under review

**S-III.**

**S-IV.**
Silva, JR., Ascensão, A., Marques, F., André Seabra., Rebelo, A., Magalhães, J. Neuromuscular function, hormonal and redox status, muscle damage of professional soccer players during the 72-h recovery after a high-level competitive match. Under review.
Abstract

Training and matches, in particular, represent a major source of stress during the soccer competitive season. Soccer physiology-related factors show that players need a well-developed physical fitness in order to cope with the soccer-specific demands. However, little is known regarding the alterations and putative relationships between soccer players’ physical fitness, hormonal environment and oxidative status, muscle damage and inflammatory markers under demanding soccer competitive stress conditions.

In this dissertation, a series of studies (5) comprising distinct experimental designs involving semi-professional and professional players under scenarios of acute and chronic exposure was used to analyse the impact of soccer competition and training on several distinct physical fitness parameters, hormonal and redox status, muscle damage and inflammation markers.

In studies I, II and III we investigated, (i) the effects of an entire soccer season on physical fitness, game physical performance, hormonal and oxidative status, muscle damage and inflammatory parameters in a team of professional male soccer players; (ii) the association between these parameters and the influence of individual match playing time (IMPT); (iii) changes in match activity and fatigue development during official soccer games in different moments of a season; (iv) and the influence of training status on match activity and fatigue. Performance in 5m and 30m sprint (T5 and T30), countermovement jump (CMJ), change of direction speed (COD; T-test), knee extensors (KE) and flexors (KF) isokinetic strength, hamstrings/quadriceps strength ratio (H/Q) and bilateral differences (BD), and Yo-Yo intermittent endurance test 2 (YYIE2) were evaluated throughout the season. IMPT was also quantified. Plasma testosterone (T), cortisol (C), creatine kinase (CK), superoxide dismutase (SOD), glutathione peroxidase (GPX) and reductase (GR) activities, myoglobin (Mb), C-reactive protein (CRP), uric acid (UA), protein sulfhydryls (-SH), malondialdehyde (MDA) contents and total antioxidant status (TAS) were measured.

In studies IV and V the aims were to investigate (i) the impact of Loughborough Intermittent Shuttle Test (LIST) versus soccer match on heart rate (HR), muscle damage (CK and Mb), redox status (TAS, UA, MDA and -SH), blood leukocytes counts and neuromuscular function (20 meters sprint, CMJ, KE and KF) throughout recovery, and (ii) the impact of an official high-level competitive match on hormonal (T, C), redox status (TAS, UA, SOD, GPX, GR, MDA and -SH), muscle damage (CK and Mb) and inflammatory (CRP) parameters, and on neuromuscular function (T5, T30, COD, CMJ, KE and KF).

In general, data collected present an interesting scenario regarding players’ physical fitness and match activity (MA) throughout the season. Professional players showed an improved MA and expressed low levels of fatigue during the match towards the end-of-season. The interrelationship between neuromuscular parameters (NP) and between NP and game-physical performance highlighted the relevance of muscle strength and power in soccer. Moreover, IMPT influenced physical, hormonal and oxidative-related markers. Despite the increase in certain stress bio-markers during season and post-match recovery periods, higher standards players seem to cope with soccer demands
throughout the season and show lower post-match performance impairments and faster recovery kinetics than lower level players.

Keywords: soccer season, training status, soccer physiology, professional players, time-motion analysis, hormonal status, oxidative stress, muscle damage.
Resumo

Os treinos e os jogos, em particular, representam um elevado fator de estresse fisiológico nos jogadores de alto nível. Como tal, os jogadores de futebol necessitam de um elevado nível de condição física para responder às exigências que lhe são colocadas durante um exigente período competitivo. Contudo, pouco se sabe sobre as alterações e as relações que se estabelecem entre a condição física (CF), a atividade física do jogador no jogo (AFJ), o ambiente hormonal e oxidativo, marcadores de lesão/agressão muscular esquelética (LAME) e inflamatórios em jogadores profissionais de futebol durante exigentes condições competitivas.

Nesta dissertação, 5 estudos com distintos desenhos experimentais, envolvendo jogadores semi-profissionais e profissionais em cenários de exposição aguda e crónica, foram realizados para analisar o impacto da época desportiva em distintos parâmetros da CF, na resposta hormonal e oxidativa e em marcadores inflamatórios e de LAME.

Nos estudos I e II e III foram estudadas, (i) alterações de performance de testes de CF e no padrão de AFJ, na resposta hormonal e oxidativa e em marcadores inflamatórios e de LAME; (ii) correlações entre as alterações funcionais e bioquímicas e o tempo individual de jogo ao longo da época (TIJ); (iii) alterações no padrão AFJ e no desenvolvimento de fadiga (DF) durante o jogo e em diferentes momentos da época desportiva; (iv) a influência do estado de treino no padrão AFJ e no DF durante o jogo. A performance em sprint de 5 e 30 metros (T5 e T30), salto contra-movimento (SCM), sprint com mudança de direção (SMD; T-test), momento máximo de força dos músculos extensores e flexores do joelho (MMFE e MMFF) e no YoYo Intermittent Endurance Test Level 2 (YYIE2) foram avaliados durante a época desportiva. O TIJ foi quantificado. Os níveis plasmáticos de testosterona (T) e cortisol (C), a atividade das enzimas creatina quinase, (CK), superóxido dismutase (SOD), glutatonia peroxidase (GPX) e reductase (GR), o conteúdo de mioglobina (Mb), proteína C-reativa (CRP), ácido úrico (UA), grupos sulfídricos proteicos (-SH), malondialdeído (MDA) e estado antioxidante total (TAS) foram determinados.

Os estudos IV e V tiveram como principais objetivos investigar, (i) o impacto do Loughborough Shuttle Intermitente Test (LIST) versus jogo amigável de futebol ao nível da frequência cardíaca (FC) marcadores de LAME, estado redox, contagem de leucócitos e função neuromuscular (T20, SCM, MMFE e MMFF) durante o período de recuperação, (ii) o impacto de um jogo oficial de futebol profissional na resposta hormonal (T e C) e redox (TAS, UA, SOD, GPX, GR, MDA, -SH), em marcadores de LAME (CK, Mb) e inflamação (CRP) e na função neuromuscular (T5 e T30, SCM, SMD, MMFE, MMFF).

Em geral, os resultados sugerem um cenário interessante no que respeita ao nível do estado de treino e da AFJ ao longo da época. Foi observada uma melhoria da AFJ e menor DF durante os jogos realizados perto do final da época. A relação entre diferentes parâmetros da função neuromuscular e entre estes e a AFJ, observadas nos estudos efetuados, realçam a importância da função neuromuscular no futebol de alta competição. O TIJ influencia alguns parâmetros físicos, marcadores hormonais e de estresse oxidativo. Os resultados observados sugerem que apesar do aumento de indicadores de
stresse durante períodos de treino, competição e após o jogo, os futebolistas profissionais demonstram possuir uma capacidade efetiva para responder e tolerar as exigências colocadas pela época desportiva. Da mesma forma, aparentam evidenciar uma menor deterioração da performance e uma recuperação mais rápida após o jogo comparativamente à que tem sido descrita em futebolistas de menor nível competitivo.

Palavras-chave: época desportiva, estado de treino, fisiologia do futebol, alto rendimento, análise tempo-movimento, resposta hormonal, stresse oxidativo, lesão muscular.
Table of Contents

Acknowledgments
List of Publications
Abstract
Resumo
General Introduction
Theoretical Background

Review I
Changes in soccer players' physiological and physical characteristics throughout the season.

Review II
Strength and muscle power training in soccer.

Experimental Work

Study I
Individual match playing time during the season affects fitness-related parameters of male professional soccer players.

Study II
Neuromuscular function, hormonal and redox status, muscle damage of high-level soccer players throughout a season competition. Under Review 189

Study III
Training status and match activity of professional soccer players throughout a season. J Strength Cond Res [Epub Ahead of Print] 229

Study IV

Study V
Neuromuscular function, hormonal and redox status, muscle damage of professional soccer players after a high-level competitive match. Under Review 309

General Discussion 345

Conclusions 381

References 385

Appendix 411
General Introduction
General Introduction

Soccer has been a topic of intense research in the last decade. The activity of top level soccer players during the competitive season entails one week cycles of training, taper, competition and recovery (Reilly & Ekblom, 2005). However, this cycle is altered by several irregularities in the competitive fixture list, match day being not necessarily consistent during in season (Reilly & Ekblom, 2005). Moreover, professional players in the top clubs may have additional commitments, such as cup and other knock-out matches, playing for their club in continental leagues or representing their country in international matches (Reilly & Ekblom, 2005).

Activities specific to matches are characterized by periods of high-intensity interspersed with periods of low-intensity exercise. The activity periods vary in intensity and duration and are punctuated by recovery pauses when activity is light or the player is static (Drust et al., 2007). Superimposed on this irregular and unpredictable activity profile are actions directly related to play, e.g., physical challenges with opponents in contesting possession of the ball and jumps to head the ball (Carling et al., 2005).

Data concerning physiological factors shows that players might possess a well-developed physical fitness in order to cope with the soccer’s physiological demands (e.g., aerobic and anaerobic power, muscle strength and agility) (Bangsbo, 1994; Kraemer et al., 2004; Reilly et al., 2008; Reilly & Ekblom, 2005; Svensson & Drust, 2005). Although the majority of reports on players’ physical and physiological characteristics are essentially descriptive, they provide a logical framework to gain insight into factors determining success in the game (Drust et al., 2007; Reilly & Gilbourne, 2003). Data reveal that in spite of evidence of anthropometric predispositions for the different playing positions within soccer (Carling & Orhant, 2010b), the game demands sufficient skill that substantial deviations from this profile remain compatible with performance of a high standard (Shephard, 1999). Different studies suggest that elite soccer players cover 8 to 13 km during matches (Bangsbo et al., 2006; Bangsbo et al., 1991) at a mean intensity close to the anaerobic threshold (AT) (Stolen et al.,
Moreover, energy expenditure during a match averages 70-75% of maximal oxygen consumption (VO$_2$max) (Bangsbo et al., 2006; Reilly & Ekblom, 2005), which suggests that performance at the elite level may, in part, be determined by aerobic fitness (Reilly & Ekblom, 2005). The component of the aerobic fitness has been extensively assessed in a laboratory setup applying valid and reliable protocols (Dittrich et al., 2011). In this regard VO$_2$max and anaerobic threshold (AT) assessed by ventilatory threshold (VT) and/or lactate threshold (LT) are two of the most frequently used parameters in monitoring aerobic fitness of soccer players in laboratory. The determination of soccer players’ VO$_2$max is important, as the oxygen transport system strengthens the ability to exercise during the 90-min (Bangsbo, 1994) and to recover between the short bouts of high-intensity exercise of the game (Dupont et al., 2005; Hoff, 2005; Tomlin & Wenger, 2001). Research on this topic has shown that professional soccer players have VO$_2$max values ranging from 52 to 66 ml.kg$^{-1}$.min$^{-1}$, depending on the point in the soccer season when the analysis were performed (Casajus, 2001; Clark et al., 2008; Edwards et al., 2003a; Haritodinis et al., 2004; Heller et al., 1992; Kalapotharakos et al., 2011; Mercer et al., 1997; Metaxas et al., 2006; Mohr et al., 2002). The AT is defined as the highest exercise intensity, at which the production and clearance of lactate promotes an equilibrium in lactate concentration (Stolen et al., 2005). Several methods have been implemented to determine AT, including blood lactate and ventilatory measurements. LT and VT have been advocated as physiological parameters more sensible to detect changes in players’ fitness than VO$_2$max (Clark et al., 2008; Edwards et al., 2003a; Helgerud et al., 2001). In this regard, different physiological parameters during sub-maximal exercise in laboratory (e.g., speed at LT, heart rate or percentage of VO$_2$max at fixed blood lactate concentrations) have been determined in professional players throughout the soccer season. In this regard, the percentage of VO$_2$max at 4mmol lactate concentration was shown to be equivalent to 77 to 78% of VO$_2$max during in-season and at 75% of VO$_2$max at the start of season (Brady et al., 1997; Clark et al., 2008; Edwards et al., 2003a; Kalapotharakos et al., 2011; Mohr et al., 2002). In addition, maximal aerobic speed, which reflects the maximum aerobic
capacity, and combines VO_{2\text{max}} and running economy into a single factor (Billat & Koralsztein, 1996), along with time to exhaustion during maximal incremental tests (Arent et al., 2010; Edwards et al., 2003a; Haritodinis et al., 2004; Metaxas et al., 2006; Mohr et al., 2002) have also been used as physical fitness parameters in players motorization. The energy cost of a run (running economy) is usually expressed as oxygen cost per meter, or minute at a defined intensity and is measured at sub-maximal work-rates (Stolen et al., 2005). Although not frequently, assessment of running economy has also been used in soccer physiology research and training control, with significant improvements been reported during pre-season training (Bogdanis et al., 2009; Bogdanis et al., 2011; Helgerud et al., 2001; Impellizzeri et al., 2006). Nevertheless, the previous laboratory assessments in addition to require expensive equipment and being time consuming, involved exercise modes that are not truly team-sport relevant limiting test specificity (Dittrich et al., 2011). With this concern, field tests have been used in order to provide results that are specific to the sport and so, may be more valid than laboratory tests (Svensson & Drust, 2005). Research using specific endurance field tests (e.g., Yo-Yo tests) has shown that soccer players possess high levels of aerobic fitness (Edwards et al., 2003b; Oliveira, 2000; Rampinini et al., 2009a; Rampinini et al., 2009b; Randers et al., 2007) with higher performances been reported at higher standards of competition (Edwards et al., 2003b; Rampinini et al., 2009a; Rampinini et al., 2009b; Randers et al., 2007).

As previously stated, professional players need a well-developed physical fitness in order to cope with soccer-specific physiological demands (Bangsbo, 1994; Kraemer et al., 2004; Reilly et al., 2008; Reilly & Ekblom, 2005; Svensson & Drust, 2005). The importance of possessing a well-developed neuromuscular system in order to deal with the soccer-specific activities in high-level competition is supported by the individual levels of neuromuscular performance (e.g., sprint and jump abilities) as well by the activity profile of players in the match (Bloomfield et al., 2007; Cometti et al., 2001; Dauty & Potiron Josse, 2004; Little & Williams, 2005; Mujika et al., 2008; Reilly et al., 2000). In fact, despite low to medium intensity running is the predominant activity patterns of
soccer players, muscle power-based efforts such as sprints, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power of the neuromuscular system (Cometti et al., 2001), are essential factors to successfully perform in soccer. Values ranging from 38 to 55-cm and from 37 to 40-cm have been observed in professional players' performance on jumping exercises such as the countermovement and squat jump, respectively (Bogdanis et al., 2011; Casajus, 2001; Clark et al., 2008; Kotzamanidis et al., 2005; Malliou et al., 2003; Mercer et al., 1997; Ronnestad et al., 2008; Sedano et al., 2011; Thomas & Reilly, 1979). Among other neuromuscular qualities, good in-line and shuttle sprint ability has also been reported as important requirements for high-level soccer performance (Aziz et al., 2005; Cometti et al., 2001; Dauty & Potiron Josse, 2004; Little & Williams, 2005; Lopez-Segovia et al., 2010; Ostojic, 2003; Wisloff et al., 2004; Wong et al., 2010). Therefore, literature consistently asserts that soccer players' performance is closely related to the efficiency of different energy-related systems (Stolen et al., 2005). The importance of soccer players' fitness has been highlighted since early investigations into soccer performance (Jacobs et al., 1982; Karlsson, 1969; McMaster & Walter, 1978; Raven et al., 1976; Reilly & Thomas, 1976; Thomas & Reilly, 1979; Withers et al., 1982). This was highlighted in review publications focusing on the different soccer determinants, such as the physiological characteristics of soccer players (Reilly & Gilbourne, 2003; Shephard, 1999; Stolen et al., 2005; Svensson & Drust, 2005), soccer players' biomechanics (Lees & Nolan, 1998), determinants of soccer players' performance (Bangsbo et al., 2007; Bangsbo et al., 2006; Reilly et al., 2008), and soccer specific training-induced effects (Hill-Haas et al., 2011; Hoff, 2005; Hoff & Helgerud, 2004; Iaia et al., 2009a). Nevertheless, despite the fact that a considerable number of review papers have analysed distinct aspects of soccer physiology, reviews concerning the knowledge provided by investigations regarding the time-line of the alterations in soccer specific and no-specific endurance and neuromuscular parameters throughout the season have not yet been composed (review I and II). Although the information of players' physiological and functional characteristics is a matter of undeniable interest, the understanding of how
these characteristics change throughout the season is important for coaches, medical departments and researchers since can be valuable information for soccer training periodization and monitoring.

Studies on soccer physiology showed that during training and competition, players undergo a great deal of stress (Bangsbo et al., 2006). Both preseason and in-season practices and soccer matches generate strains to various physiological systems of the player (e.g., musculoskeletal, nervous and cardiovascular) (Bangsbo et al., 2006; Brites et al., 1999; Filaire et al., 2003; Kraemer et al., 2004; Malm et al., 2004a, 2004b; Rebelo et al., 1998a). These high demands are confirmed by players’ activity pattern analysis during the matches (Bloomfield et al., 2007; Mohr et al., 2003; Rampinini et al., 2007b; Randers et al., 2010; Rienzi et al., 2000), as well as by the examination of the performance and/or biochemical responses to friendly matches or specific protocols performed in laboratory and field conditions, and the subsequent recovery period (Ascensao et al., 2008; Ispirlidis et al., 2008; Krstrup et al., 2006b; Nicholas et al., 2000; Small et al., 2008; Small et al., 2009). Therefore, it is important that an adequate balance between training, competition and recovery is achieved so that the desired training-adaptations might occur. In fact, high volumes of training and/or competition interspersed by insufficient recovery may induce fatigue (Filaire et al., 2003), and ultimately an overreaching state (Kraemer & Ratamess, 2005; Petibois et al., 2002). In this way, to prevent performance declines and to ensure that training programs are effective is necessary to include regular performance tests as a component of training control (Filaire et al., 2001). Results from field tests provide information on specific performance changes related to the sport (Svensson & Drust, 2005). Moreover, fitness tests in association with physiological data should be used to monitor changes in players’ fitness and to guide their training prescription (Svensson & Drust, 2005). Thus, the regular evaluation of players’ physical performance and recovery capacity in order to detect or avoid fatigue can be a matter of special importance, as soccer players should be able to successfully perform in competitive seasons of around 10-11 months duration.
In order to achieve a higher efficiency of the training monitoring, research protocols aimed to study physical performance should include specific performance variables (e.g., game physical performance), biochemical and muscle-status markers (Bishop et al., 2008) and analyze fatigue and recovery state. Thus, in addition to sport-specific performance parameters, hormonal, oxidative and muscle damage markers have been suggested as important biomarkers for training monitoring in different athletic populations (Bishop et al., 2008; Kraemer & Ratamess, 2005; Margonis et al., 2007; Rebelo, 1999). Therefore, to better understand the recovery pattern of the player under such demanding competitive stress conditions, a longitudinal coverage of physical performance and biochemical profile at several time points throughout the season may be useful (SH-I and SH-II). However, there is a lack of research concerning the effects of an entire season on physical fitness, physiological and biochemical status of professional players engaged in professional soccer. Regarding physical fitness evaluated by physical tests, most of the studies relied on particular functional or physiological data such as anthropometrics (Casajus, 2001; Clark et al., 2008; Kraemer et al., 2004; Mercer et al., 1997), \( \text{VO}_{2}\text{max} \) and anaerobic threshold measurements (Casajus, 2001; Clark et al., 2008; Mercer et al., 1997; Metaxas et al., 2006), specific intermittent endurance test (Bangsbo et al., 2008; Rebelo, 1999), exercise performance in incremental treadmill tests until exhaustion (Casajus, 2001), muscle power through jump and sprint tests (Aziz et al., 2005; Casajus, 2001; Malliou et al., 2003) and lower limb strength evaluated by isokinetic devices (Malliou et al., 2003; Mercer et al., 1997). However, inconsistency has been reported in the literature regarding the impact of training and competition in the variation of certain fitness parameters throughout the season (Aziz et al., 2005; Caldwell & Peters, 2009; Casajus, 2001; Clark et al., 2008; Edwards et al., 2003a; Faude et al., 2011; Kraemer et al., 2004; Malliou et al., 2003; Mercer et al., 1997; Metaxas et al., 2006).

As previously emphasized, different studies reported evidence that the demands of competitive soccer may impose strains at physiological and psychological levels (Faude et al., 2011; Kraemer et al., 2004; Rebelo et al., 1998; Reilly & Ekblom, 2005). In fact, disturbances in mood state (e.g.,
increases tension, and anger) during competition periods have been described (Filaire et al., 2003; Faude et al., 2011; Rebelo, 1999). Also, data reported the occurrence of inadequate immune function associated with periods of high physical stress (Malm et al., 2004a; Rebelo et al., 1998a; Reinke et al., 2009). Moreover, alterations in anabolic-catabolic hormonal environment (Handziski et al., 2006; Kraemer et al., 2004) and increased levels of oxidative stress were reported after high-intensity periods of soccer training (pre-season) (Arent et al., 2010). These reports highlight that during the soccer season, players’ physical performance is not only determined by appropriate conditioning but also by the ability to recover and regenerate following multiple stress stimuli (i.e., training and competition) (Kraemer et al., 2004). However, there are some contradictions regarding the idea that players may accumulate “fatigue” as the season progresses, leading to performance impairments (Reilly & Ekblom, 2005). In fact, even though soccer players should be able to successfully perform throughout a long competitive period, the integrative analysis of the longitudinal changes in players’ physical fitness, in addition to changes in stress bio-markers during the season and recovery period, are still scarce. In fact, little is known regarding the putative relationships between soccer players’ physical fitness, hormonal environment and oxidative status under demanding soccer competitive stress conditions (soccer season; S-II). On the other hand, one of the less understood and more scarcely studied phases of the soccer season is the off-season period. Therefore, studies investigating if off-season allows players to recover their organic homeostasis are needed (S-II). Moreover, despite the growing number of match commitments in national and international contexts, the influence of the individual match playing time during season on the referred parameters has never been reported (S-I and S-II). Knowledge clarifying this gap in literature may allow improving the management of training periodization, competition scheduling, and, eventually, of specific ergogenic interventions (e.g., antioxidants supplementation).

Players’ game-physical performance has been investigated through match analysis systems. This technology is widely used in professional soccer to study tactical and physical performance of players and referees (Abt & Lovell, 2009).
Research with some of the most up-to-date technologies such as the multi-camera methods (Abt & Lovell, 2009; Rampinini et al., 2007b; Randers et al., 2010), global position systems (Randers et al., 2010) and video based time-motion analysis (Mohr et al., 2003; Randers et al., 2010) revealed detailed information about players’ movement patterns during games (Bloomfield et al., 2007; Krstrup et al., 2005; Mohr et al., 2003). Moreover, these methods allow the identification of performance decrements during soccer games, and thereby the study of game-induced fatigue (Randers et al., 2010). Regarding physical fitness alterations during the season, these methodological interventions were able to detect that seasonal variations in game-physical performance occur during the season (Mohr et al., 2003; Rampinini et al., 2007b). However, the number of studies focusing on seasonal variations in game-physical performance is scarce and most of the studies only considered three time points of the season (beginning, middle and end of season) (Mohr et al., 2003; Rampinini et al., 2007b). Therefore, studies dealing with a higher number of time points for data collection throughout the season are needed to better comprehend the seasonal variations in game-related physical parameters. Moreover, research of seasonal alterations in fatigue patterns (e.g., temporary fatigue during the match) of soccer players has never been reported (S-III). The official match represents a major source of physical demand and physiological stress during the competitive season (Impellizzeri et al., 2005). A study performed by Impellizzeri et al. (2005), in which rating of perceived exertion (RPE) was used to quantify the internal training load (RPE-Tl,d), showed that in weeks comprising two official matches, the RPE-Tl,d can represent about 50% of total weekly training load decreasing to 25% in weeks with only one match performed. During a 90-minute game, elite-level players may cover approximately 10-13 km (Abt & Lovell, 2009; Bangsbo et al., 2006; Bangsbo et al., 1991; Di Salvo et al., 2007; Mohr et al., 2003; Odetoynbo et al., 2009; Rampinini et al., 2007b) with a sprint bout occurring approximately every 90 seconds, each lasting an average of 2–4 seconds (Bangsbo et al., 2006; Bangsbo et al., 1991; Mohr et al., 2005; Vigne et al., 2010). The previous data concerning player match activity, in addition to the already mentioned evidence
that match is performed at an average intensity close to the anaerobic threshold (80–90% of maximal heart rate) (Stolen et al., 2005) with an energy expenditure averaging 70-75% of maximal oxygen consumption (VO$_2$max) (Bangsbo et al., 2006; Reilly & Ekblom, 2005), suggest that performance at the elite level may, in part, be determined by aerobic fitness (Reilly & Ekblom, 2005). Within this endurance context, numerous explosive bursts of activity are required, including jumping, kicking, tackling, turning, sprinting, changing pace, and sustaining forceful contractions to maintain balance and control of the ball against defensive pressure (Stolen et al., 2005). Data revealed that several signs of fatigue can be manifested temporally during (Bangsbo et al., 2006; Mohr et al., 2003, 2005) and toward the end of the game (Bangsbo et al., 2006; Mohr et al., 2003, 2005), and persist afterwards (Ascensao et al., 2008; Mohr et al., 2004; Rebelo et al., 1998b) with a time-dependency for the fitness parameter evaluated. Moreover, biochemical analyses have shown increases in muscle damage markers (Ascensao et al., 2011; Ascensao et al., 2008; Ispirlidis et al., 2008), a robust pro- and anti-inflammatory cytokine response (Andersson et al., 2010a; Ispirlidis et al., 2008), increased catabolic (Ispirlidis et al., 2008), and pro-oxidant states (Ascensao et al., 2008) immediately after “friendly” soccer matches and throughout the post-match recovery period. A considerable amount of studies used friendly matches and soccer specific protocols performed in laboratory as a model to reproduce the overall physical demands of the game. Data emerging from high-level competitive games are scarce. Moreover, analysis concerning the impact of distinct models of soccer performance (friendly match vs. soccer specific protocol) in functional, physiological and biochemical parameters is needed (S-IV). In addition, the analysis of high-level official soccer matches is interesting for both coaches and researchers as these truly represent the real context of physiological stress. Moreover, although associations between pro-oxidant redox status, inflammation, muscle damage and physical performance have been established in high-level male (Ispirlidis et al., 2008; Rampinini et al., 2011) and female players (Andersson et al., 2010a; Andersson et al., 2010c) and low-level male soccer players (Ascensao et al., 2011; Ascensao et al., 2008; Fatouros et al.,
2010) after friendly matches, they have not been yet examined in the same cohort of male professional players during the post-match recovery period that follows a high-level competitive official match (S-V). In addition, there is some evidence that players of higher standards may possess a higher ability to cope with the physiological stress of the match (Andersson et al., 2008; Ascensao et al., 2008; Fatouros et al., 2010; Odetooyinbo et al., 2009; Rampinini et al., 2011) and/or periods of intensified training (Michalczyk et al., 2008; Nakagami et al., 2009).

It has been observed through time-motion analysis that performance during the match is dependent on multiple factors, such as standard and type of competition, playing position and player physical capacity (Bangsbo et al., 1991; Krustrup et al., 2003a; Krustrup et al., 2005; Mohr et al., 2003; Rampinini et al., 2007b). Recent studies observed that game-related physical performance changes throughout the season (Mohr et al., 2003; Rampinini et al., 2007b) and is related to player’s training status (Krustrup et al., 2003a; Krustrup et al., 2005; Rampinini et al., 2007a). However, it is important to highlight that even though data from laboratory and field tests provide a good indication of general and soccer-specific fitness, individual test results should not be used to conclusively predict performance in match-play because of the complex nature of performance in competition (Svensson & Drust, 2005). Nevertheless, some studies shown that soccer players’ performance in the Yo-Yo intermittent recovery test both in males (Krustrup et al., 2003a) and females (Krustrup et al., 2005), in incremental field tests (shorter version of the University Montreal Track Test) (Rampinini et al., 2007a) and in the repeated shuttle sprint ability test (Rampinini et al., 2007a) could be good indicators of game-related physical performance. However, it is important to note that data on non-locomotive activity, as well as unorthodox movements (e.g., shuffling, diving), soccer specific movements (e.g., heading, blocking), and accelerations and decelerations should be taken into account (Bloomfield et al., 2007). In fact, a massive metabolic load is imposed on players, not only during the more intense parts of the game, but every time acceleration occurs, even when speed is low (Osgnach et al., 2010). As so, it is not surprising that studies regarding soccer
players’ activity patterns and soccer’s physiological demands point toward the fact that a well-developed neuromuscular system may be of relevant importance in order to maintain or increase players’ performance during the game and particularly during the most intense periods of the match. Soccer players’ muscle strength (Arnason et al., 2004; Cometti et al., 2001) and performance in sport-specific muscle power efforts (Cometti et al., 2001; Mujika et al., 2008) were reported to be related to the competitive level of the player. Moreover, improvements in coordination specificities (Buchheit et al., 2010b) and a greater agility (Durandt et al., 2006) have been positively associated with performance and the ability to delay fatigue. In fact, rapid force production is considered essential for a wide range of athletes (Aagaard & Andersen, 2010; Hoff & Helgerud, 2004; Nummela et al., 2006), and high neuromuscular capacity may improve endurance performance (Aagaard & Andersen, 2010). In addition, some research (Bogdanis et al., 2011; Nunez et al., 2008; Wong et al., 2010) showed that training of the neuromuscular function and soccer specific endurance can be combined. This is advised since it can promote, among other factors, improvement in non-specific and soccer specific endurance and neuromuscular parameters. Despite these suggestions and the consensus amongst experts that high stress is imposed on the neuromuscular system during soccer games, until now, no studies have addressed whether or not muscle strength and power can be indicators of game-related physical performance. Therefore, studies aiming to respond to whether physical conditioning evaluated in field and laboratory tests is related with match performance and fatigue development are required (S-III).

Hence, although an extraordinary growth in the number of scientific investigations concerning soccer has been observed in the last decades, still more is needed in order to elucidate soccer player performance, fatigue and recovery-related factors during and after the match and throughout the season. Moreover, in the context of professional soccer this knowledge is even scarcer.

Taking into account the above mentioned scientific context concerning soccer physiology, the main purpose of this dissertation is to analyse performance,
fatigue and recovery of the soccer player during the season. This will be investigated through the analysis of functional and biochemical alterations induced by training and competition.

This goal is supported by all the specific aims of the papers produced on the compass of this dissertation that were, respectively:

To review (Review I and II):
(i) changes in soccer players’ physiological and physical characteristics throughout the season.
(ii) the effect of different training methodologies on soccer players’ physiological and functional characteristics.

To investigate (Paper I):
(i) the effects of an entire season on physical fitness parameters in a team of professional male soccer players.
(ii) the influence of match playing time during the season on physical parameters of professional male soccer players.

To investigate (Paper II):
(i) the impact of an entire soccer season on hormonal and redox status, muscle damage and inflammation and neuromuscular function of professional soccer players.
(ii) the association between individual match playing time of professional soccer players and neuromuscular function, hormonal and redox status, muscle damage and inflammation.

To analyse (Paper III):
(i) match activity and fatigue development during official soccer games in different moments of a season.
(ii) the influence of training status on match activity and fatigue development of professional soccer players.
To investigate (Paper IV):

(i) the impact of Loughborough Intermittent Shuttle Test (LIST) versus soccer match on heart rate (HR), muscle damage, redox status, blood leukocytes and neuromuscular function throughout 72 h recovery.

To examine (Paper V):

(i) the impact of an official match on hormonal and redox status, muscle damage and inflammation and neuromuscular function of professional soccer players.
THEORETICAL BACKGROUND
TITLE: Changes in soccer players’ physiological and physical characteristics throughout the season

RUNNING HEAD: Seasonal variations in physical fitness

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Contents

Abstract

1. Introduction

2. Changes in physiological and physical parameters throughout the competitive season
   2.1 Anthropometry
   2.2 Endurance capacity
      2.2.1 Maximal oxygen consumption
      2.2.2 Anaerobic Threshold
      2.2.3 Running economy
      2.2.4 Performance in maximal incremental tests
         2.2.4.1 High intensity intermittent exercise
         2.2.5 Sub-maximal intermittent exercise
   2.3. Neuromuscular function
      2.3.1 Jump ability
      2.3.2 Sprint ability
      2.3.3. Change of direction speed
      2.3.4. Strength
   2.4. Game-related physical parameters

3. Off-season induced changes in physiological and physical parameters
   3.1 Anthropometry
   3.2 Endurance-physiological parameters
   3.3 Soccer specific endurance
   3.4 Neuromuscular function

4. Conclusions

5. References
Abstract

This review focuses on the different antropometric, physiological and performance alterations that occur during the annual soccer training cycle (preseason, in-season and off season). From the considerable amount of research in this field, it has been observed that VO$_2$max increases during preseason and is maintained during in-season. Moreover, there is some evidence that a VO$_2$max threshold occurs in which no observations of significant increments were stated in soccer players. In addition, maximal aerobic speed (MAS) and different physiological parameters measured at submaximal exercise performance were shown to improve during preseason (3.1-9.1% and 4.5-21.6%, respectively). Moreover, although the majority of investigations did not report in-season VO$_2$max improvements, MAS and acute physiological adaptations during submaximal exercise performance may increase during this period. In addition, soccer specific endurance (SSE) follows a similar trend of adaptation. Faster and greater SSE improvements were observed during preseason. Also high average increments in SSE have been reported during in-season without significant modifications in VO$_2$max and smaller physiological adaptations in sub-maximal exercise. Moreover, game-related physical parameters in high-intensity categories showed great increases during in-season. Adaptations of players’ neuromuscular systems have been measured in sprints, COD and jump tests—with higher evidence in those jumps involving stretch-shortening cycle activities (SSC)—and all body movement interactions (jumps with arm swing). Data suggests that when early improvements are obtained (from PPS to BCP), they can be maintained and even further improved during in-season. This suggests that neuromuscular adaptations affecting SSC mechanisms may occur during in-season. This previous evidence highlights the high sensitivity and ability of field tests in the monitoring of players during training. Moreover, isoinertial assessment seems to be more effective evaluating players’ neuromuscular adaptations. In conclusion, probably other endurance physiological determinants (e.g., VO$_2$ kinetics and the ability to maintain an acid-base balance) can occur during the season and thus contribute to SSE increments. Moreover, among other factors, adaptation at the
neuromuscular level and the improvement in pacing strategies throughout the season cannot be excluded as mechanisms that could be responsible for the increase in-season game-physical performance. The off-season period appears to result in decrements of a variety of physiological and functional parameters.

1. Introduction

Soccer match-specific activity is characterized by periods of high-intensity exercise interspersed with periods of low-intensity exercise. The physiological demands of soccer require from players a high level of physical fitness (e.g., aerobic and anaerobic power, muscle strength and agility (Bangsbo, 1994; Kraemer et al., 2004; Reilly et al., 2008; Reilly & Ekblom, 2005; Svensson & Drust, 2005). Although reports of players’ physical and physiological characteristics are essentially descriptive, they nevertheless provide insights into factors determining success in the game (Reilly & Gilbourne, 2003). There are important reviews concerning physiological characteristics of soccer players (Reilly & Gilbourne, 2003; Shephard, 1999; Stolen et al., 2005; Svensson & Drust, 2005), soccer biomechanics (Lees & Nolan, 1998), determinants of players’ performance (Bangsbo et al., 2007; Bangsbo et al., 2006; Reilly et al., 2008) and specific training-induced effects (Hill-Haas et al., 2011; Hoff, 2005; Hoff & Helgerud, 2004; Iaia et al., 2009a). Preseason and in-season practices and soccer matches may impose strains on various physiological systems of the player (e.g., musculoskeletal, nervous and metabolic) (Bangsbo et al., 2006; Brites et al., 1999; Filaire et al., 2003; Kraemer et al., 2004; Malm et al., 2004a, 2004b; Rebelo et al., 1998). In fact, a high frequency and volume of training practices and a high number of competitive matches combined with insufficient time allowed to recover may induce fatigue (Filaire et al., 2003). To prevent performance decline and to ensure that training programme are effective, it is necessary to include regular performance tests as a component of training control (Filaire et al., 2001). This way, the knowledge of players’ physiological and functional characteristics is not only a matter of undeniable interest, as the understanding of how these characteristics altered throughout training is important for coaches, medical departments and researchers.
Therefore, in this paper we intend to analyse the alterations observed throughout the season (off season, preseason and in-season) along physiological parameters as well as in the physical performance of soccer players. Even though we do not intend to compare training methodologies, the experimental designs of the studies analysed will be referred to in order to allow the reader full access to research related to seasonal alterations in soccer players’ physiological status and physical fitness. The search for scientific literature relevant to this review was performed using US National Library of Medicine (PubMed), MEDLINE and SportDiscus databases. Literature searches were undertaken using several keywords including ‘soccer,’ ‘elite soccer,’ ‘seasonal alterations,’ ‘performance analysis,’ ‘soccer competition,’ ‘soccer physiology,’ ‘strength training,’ ‘isokinetic strength,’ ‘one repetition maximum,’ ‘neuromuscular performance,’ ‘fatigue,’ ‘recovery,’ ‘field tests,’ ‘intermittent endurance,’ ‘maximal power,’ ‘muscular power,’ ‘jump ability,’ ‘sprint ability,’ ‘agility,’ ‘change of direction,’ ‘training period,’ ‘detraining,’ ‘off season,’ ‘in season,’ ‘preseason,’ and ‘competition period.’ Relevant literature was also screened for additional articles arising from the reference list of included studies. Given the wide range of this review, other researchers have been included.

2. Changes in physiological and functional parameters throughout the season

2.1 Anthropometry

Although there are anthropometric predispositions for the different playing positions within soccer (Carling & Orhant, 2010), the game demands sufficient skill that substantial deviations from this profile remain compatible with performance of a high standard (Shephard, 1999). Changes in anthropometric variables such as Body Mass (BM), Body Fat (BF) and Lean Body Mass (LBM) throughout the season are presented in table 1. The majority of investigations did not report alterations in players’ BM during preseason and in-season periods (Aziz et al., 2005; Carling & Orhant, 2010;
Casajus, 2001; Kalapotharakos et al., 2011; Mercer et al., 1997; Metaxas et al., 2006; Silva et al., 2011). However, an increase in BM values from prior-preseason (PPS) (Edwards et al., 2003; Ostojic, 2003) and from the beginning of the competition period (BCP) (Ostojic, 2003) to end-of-competition period (ECP) were observed.

The high training volume normally administered during preseason period can be associated with alterations in players’ BF and LBM. In this matter, some investigations reported that both professional and semi-professional players experience decreases in BF during preseason (Caldwell & Peters, 2009; Kalapotharakos et al., 2011; Mercer et al., 1997). The observed preseason decrements in BF could be maintained towards the middle-competition period (MCP) or even further, with a decline in the ECP (Caldwell & Peters, 2009; Casajus, 2001; Kalapotharakos et al., 2011; Ostojic, 2003). Nevertheless, findings from studies with professional players did not corroborate this evidence (Aziz et al., 2005; Heller et al., 1992; Metaxas et al., 2006; Reinke et al., 2009; Silva et al., 2011). Curiously, a 6.9% increase in BF of professional players from MID to ECP was observed in a study by Carling and Orhant (2010).

Regarding LBM of professional soccer players, there are also contradictions regarding alterations of this parameter during preseason and in-season periods. Although certain studies did not observe any significant change in LBM between different time points of the season (Casajus, 2001; Metaxas et al., 2006; Ostojic, 2003), increases in LBM from PPS to BCP (Reinke et al., 2009), and from BCP to MCP (Heller et al., 1992) were observed.

Carling and Orhant (2010) examined elite players and confirmed the early observations of Casajus (2001) that observed no seasonal variations in BM of players with different positional roles. Nevertheless, the same was not observed in BF and LBM. Across all players, there were significant in-season variations in BF (between start- and mid-season and between mid- and end-season; 5.7% decrease and 6.9% increase, respectively) and in LBM (between start and mid-season and between start and end-of-season, 1.4% and 1.3% increase, respectively) (Carling & Orhant, 2010). Further analysis showed that variations differed across field positions. Defenders increased LBM in 2.4% from PPS to
ECP, and midfielders increased LBM in 5% from PPS to MCP. Also, midfielders’ BF decreased 8% from PPS to MCP and increased 9% from MCP to ECP (Carling & Orhant, 2010). These variations in body composition was not associated with players’ participation time (combined training and match exposure time), and did not differ across seasons (Carling & Orhant, 2010). The different investigations suggest that no unique and specific pattern of variation during preseason and in-season occurs. Factors related to training (e.g., type of strength training) and competition fixtures (e.g., extend of preseason and/or in-season period) and diet (e.g., Mediterranean diet) (Ostojic, 2003) may explain the different results observed in body composition of soccer players throughout the season.

2.2 Endurance capacity
Data from time-motion analysis showed that elite soccer players cover 8 to 13-km during a competitive match (Bangsbo et al., 1991; Bradley et al., 2009; Di Salvo et al., 2007; Di Salvo et al., 2009; Rampinini et al., 2007b) at a mean intensity close to the anaerobic threshold (AT) (Stolen et al., 2005). Moreover, energy expenditure during match play averages 70-75% of maximal oxygen consumption (VO\textsubscript{2}max), which suggests that a high level of physical performance in soccer may, in part, be determined by aerobic fitness (Bangsbo et al., 2006; Reilly & Ekblom, 2005). The determination of VO\textsubscript{2}max and anaerobic threshold (AT) assessed by ventilatory threshold (VT) and/or lactate threshold (LT) are two of the most frequent parameters of monitorization of aerobic fitness of soccer players in laboratory. For endurance sports, three main factors – VO\textsubscript{2}max, LT and efficiency – appear to play key roles in endurance performance (Joyner & Coyle, 2008). VO\textsubscript{2}max and LT interact to determine the VO\textsubscript{2} performance, which represents the maximal oxygen consumption that can be sustained for a given period of time (Joyner & Coyle, 2008). Efficiency interacts with the performance VO\textsubscript{2} to establish the speed or power that can be generated at this oxygen consumption (Joyner & Coyle, 2008). The energetic cost of a run (running economy), is usually expressed as oxygen cost per meter, or minute at a
defined intensity and is measured at sub-maximal work-rates (Stolen et al., 2005). The causes of variability in RE are not well understood, but it seems likely that anatomical traits, mechanical skill, neuromuscular skills, and storage of elastic energy are important factors [see refs. in (Hoff & Helgerud, 2004)]. Changes in soccer players' aerobic capacity has also been examined by maximal aerobic speed and time to exhaustion during maximal incremental tests performed in laboratory. In order to increase the ecological validity of the measurements, maximal and sub-maximal soccer specific field tests (e.g., Yo-Yo Intermittent Endurance and Recovery tests) have been widely used to monitor soccer player training status. These soccer-specific endurance tests have been advocated as tests that enhance the specificity of the evaluation (Svensson & Drust, 2005).

Although we do not intend to compare the effects of different training programs on physiological and physical characteristics of soccer players, the studies analysed in this review will be referred to in order to provide full coverage of seasonal alterations. High intensity training (HIT) comprises different modes of high-intensity exercise, namely high-intensity aerobic training (HIA), speed endurance training (SE) and repeated sprint ability training (RSA). Generally the common factor between these different modes relies in the high physiological stress and the sharing of some similar physiological and functional training-induced adaptations, imposed by the acute and chronic effects of the high-intensity exercise bouts.

### 2.2.1 Maximal oxygen consumption

Although no differences between professional and amateur players has been reported (Rampinini et al., 2009a), higher values of VO$_2$max have been positively associated with team level/success within the same league (Apor, 1988). The determination of soccer player's VO$_2$max is therefore important, as the oxygen transport system strengthens the ability to exercise during the 90-min (Bangsbo, 1994) and to recover between the short bouts of high-intensity exercise of the game (Hoff, 2005). As can be depicted in table 2, different studies verified VO$_2$max changes throughout the season. During preseason
training players appear to regain their oxygen capacity and maintain it throughout the season. Actually, studies with players from different competitive level showed improvements in VO$_2$max from PPS to BCP (Aziz et al., 2005; Caldwell & Peters, 2009; Kalapotharakos et al., 2011; Mercer et al., 1997; Metaxas et al., 2006). Also, higher values of VO$_2$max were observed in MCP (Aziz et al., 2005; Caldwell & Peters, 2009; Haritodinis et al., 2004; Kalapotharakos et al., 2011; Metaxas et al., 2006; Mohr et al., 2002b) and ECP (Aziz et al., 2005; Caldwell & Peters, 2009; Haritodinis et al., 2004; Magal et al., 2009; Metaxas et al., 2006) than in PPS. Moreover, increments in VO$_2$max (from PPS to BCP, MCP and ECP) seems to be independent of the position role (Metaxas et al., 2006). Nevertheless, seasonal variations in VO$_2$max were not entirely confirmed in other investigations (Clark et al., 2008; Edwards et al., 2003).

High-intensity aerobic training, both in general (interval running; HIA-IR) (Helgerud et al., 2001; Impellizzeri et al., 2006) and specific modes (small-sided games; HIA-SSG) (Impellizzeri et al., 2006), induces the increase in VO$_2$max throughout the preseason. Impellizzeri et al., (2006) observes that high-level Junior players performing HIA-IR or HIA-SSG, increased 7.4% and 6.4% the VO$_2$max, respectively. Helgerud et al., (2001) found in preseason a 10.7% increment in VO$_2$max after a HIA-IR program. Also, elite U-17 players, who performed a HIA program (2 sessions per week of a soccer specific dribbling circuit; HIA-SSDC), during 10-wks (6-wks preseason plus 4-wks in-season) had a 10.1% improvement in VO$_2$max (McMillan et al., 2005b). As already mentioned, the majority of longitudinal studies (Aziz et al., 2005; Casajus, 2001; Heller et al., 1992; Metaxas et al., 2006; Mohr et al., 2002a; Silvestre et al., 2006) and studies analysing the adaptations from specific training methodologies (Impellizzeri et al., 2006) did not detect significant further improvements after the initial increase in VO$_2$max found after preseason. In fact, Impellizzeri et al., (2006) observed that a 8-wk period of HIA starting at the BCP did not produce any further significant increase in VO$_2$max (HIA-IR group: 0.84%; HIA-SSG group: 0.65%) after the initial increment observed during preseason (8.2% and 7.1%, respectively). Nevertheless, Ferrari Bravo et al.,
(2008) trained in-season amateurs players for 7-wks with HIA-IR or RSA and observed an increase in VO$_2$max after the training program (6.6% and 5%, respectively). Also, Jensen et al. (2009) observed that U-20 elite players performing during the last 12wks of the competitive season a lower weekly volume of HIT (30-min session per week of HIA-SSG, with a ratio exercise/recovery of 2:1, e.g. 3-min play/1.5-min recovery) than reported in previous study results - a 5.4% increase in VO$_2$max. Nevertheless, Sporis et al., (2008b) observed that elite U-19 players performing HIA, 3 times a week, during 13-wks (6-wks of preseason and 7-wks of in-season) enhanced the VO$_2$max in 5.3%, with the greater part of increment being achieved (5.1%) after the first 6-wks of the preseason period (Sporis et al., 2008b).

In general, different studies showed improvements in VO$_2$max after a relatively short period of time (e.g., preseason training), while no further increments in this parameter were observed in-season. Moreover, no increase was observed in VO$_2$max when soccer players already possess VO$_2$max values around 61-62 ml.kg$^{-1}$.min. In fact, the referred increases in VO$_2$max found in different standard of players during in-season (Caldwell & Peters, 2009; Ferrari Bravo et al., 2008; Jensen et al., 2009; Magal et al., 2009) occurred under this threshold. Additionally, when professional players begin the competitive season with values above this threshold no improvements in VO$_2$max throughout the season were reported (Clark et al., 2008; Edwards et al., 2003). The general observation of no improvements in VO$_2$max after preseason suggests that there is a certain threshold of soccer players’ VO$_2$max above which it is very hard to induce VO$_2$max that may be related with soccer training-specific constrains, (e.g., insufficient training stimulus targeting VO$_2$max).

2.2.2 Anaerobic Threshold

The anaerobic threshold (AT) is defined as the highest exercise intensity, HR or VO$_2$, in which the production and clearance of lactate is equal (Stolen et al., 2005). Several methods exist to determine AT, including measurement of blood lactate and ventilatory measurements. Lactate threshold (LT) and ventilatory
threshold (VT) have been advocated as physiological parameters more sensible to detect changes in players’ fitness than VO$_2$max (Clark et al., 2008; Edwards et al., 2003; Helgerud et al., 2001). Moreover, LT might change without changes in VO$_2$max, and a higher LT means, theoretically, that players can maintain a higher average intensity in an activity without accumulation of lactate (Helgerud et al., 2001).

Studies examining changes in physiological parameters at sub-maximal intensities through different periods of a soccer season are presented in table 3. During preseason, the ability of high-level players’ to perform sub-maximal exercise improves (Brady et al., 1997; Clark et al., 2008; Edwards et al., 2003; Kalapotharakos et al., 2011; Mohr et al., 2003; Zoppi et al., 2006) and is maintained throughout the MCP (Brady et al., 1997; Clark et al., 2008; Mohr et al., 2002b) and ECP (Brady et al., 1997; Clark et al., 2008). Also, further improvements from BCP to MCP have been observed (Casajus, 2001). Nevertheless, increases in preseason (Dunbar, 2002) and between BCP and MCP were not corroborated by others (Brady et al., 1997; Dunbar, 2002; Heller et al., 1992; Kalapotharakos et al., 2011) and neither were decrements from MCP to ECP (Brady et al., 1997; Dunbar, 2002).

A large variety of physiological parameters can be motorized during sub-maximal exercise (table 3). Certain parameters were shown to be sensitive in one but not in other studies with players of similar standard. Improvements in BCP and MCP performances were detected by different parameters such as percentage of VO$_2$max and percentage of maximal heart rate at a lactate concentration of 4 mmol$^{-1}$ (%VO$_2$max$_{4mmol}$ and %HRmax$_{4mmol}$, respectively) (Kalapotharakos et al., 2011), velocity at a lactate concentration of 4 mmol$^{-1}$ (V$_{[La]4mmol}$) (Kalapotharakos et al., 2011), heart rate measures at different running speeds (Mohr et al., 2002) and speed at lactate threshold (LT$_{speed}$) (Brady et al., 1997; Zoppi et al., 2006).

McMillan et al., (2005a) observed that U-19 elite players increased the V$_{[La]4mmol}$ from PPS to early weeks of in-season (13.62 to 14.67 km.h$^{-1}$, respectively). Although improvements in speed at ventilator threshold (VT$_{speed}$) from BCP to
MCP were reported (Casajus, 2001) these were not confirmed in other studies of professional players (Heller et al., 1992). Moreover, the same evidence as been observed in \( LT_{\text{speed}} \) between PPS and MCP (Brady et al., 1997). Also, no improvement in preseason in the velocity at a lactate concentration of 2 and 3 mmol\(^{-1}\) during sub-maximal exercise was examined (Dunbar, 2002).

Regarding the effect of high-intensity aerobic training programs (HIA), it was reported that elite U-18 players performing HIA-IR (Helgerud et al., 2001; Impellizzeri et al., 2006) and HIA-SSG (Impellizzeri et al., 2006) had a 21.6% (Helgerud et al., 2001) and 4.5% improvements in \( LT_{\text{speed}} \) (Impellizzeri et al., 2006) during preseason, respectively. Also, improvements were observed of 15.9% (Helgerud et al., 2001) and 7.6% in \( LT_{\text{VO2}} \) (8% and 7.2% for HIA-IR and HIA-SSG group, respectively) (Impellizzeri et al., 2006) during preseason. Prolongation of the training program for more than 8-wks after BCP resulted in further increases in \( LT_{\text{speed}} \) (4.7%) and in \( LT_{\text{VO2}} \) (4%; combined groups) (Impellizzeri et al., 2006), which suggested that the further improvement in LT, but not in VO\(_2\)\(_{\text{max}}\), were probably related to central factors (i.e., VO2max), being rapidly restored in a relatively shorter time (4-wks), and peripheral factors (i.e., muscle oxidative enzymes) may require a longer time to improve (a further 8-wk period) (Impellizzeri et al., 2006).

### 2.2.3 Running economy (RE)

Changes in soccer players’ running economy (RE) have been analysed mainly in studies investigating the training-induced effects of high-intensity strength (HIS) and/or high-intensity aerobic training (HIA). HIA during preseason resulted in RE improvements (6.7%; (Helgerud et al., 2001); 1.4% with HIA-IR and HIA-SSG modes (Impellizzeri et al., 2006)). Also, preseason HIA and/or HIS performed by elite junior players’ have resulted in the increase of RE (4.7 %) both at the lactate threshold and at a fixed running velocity in a treadmill test (Helgerud et al., 2003; Hoff & Helgerud, 2003). Other groups of researchers (Bogdanis et al., 2011) examined the strength training effects of hypertrophy (H; 4 sets of 12 repetitions with 70% of 1RM, 3 times per week) or neural (N; 4 sets
of 5 repetitions with 90% of 1RM) adaptations programs during 6-week preseason of professional players. Nevertheless, the weekly cycle also involved a considerable amount of interval training and small group play that as previously mentioned are effective methodologies targeting endurance development. Although both groups increased RE (10.9 % and 4.2 %, for N and H, respectively), only N improved significantly. Although without statistical significance (p=0,07), Impellizerri et al., (2006) observed that a further 8-wk period of HIA-IR or HIA-SSG during in-season produced an additional improvement of 1.4% in RE at LT (Impellizzeri et al., 2006). In this matter, Macmillan et al., (2005b) did not found any change in RE after 10-wks of HIA (6-wks in preseason plus 4-wks in in-season). Nevertheless, given that RE has been assessed by treadmill running, and significant differences exist between this assessment mode and soccer specific activity during training and matches, there is some conviction that soccer specific work economy may improve somewhat during season, with relevant gains not detectable by conventional treadmill testing (Helgerud et al., 2001; McMillan et al., 2005b).

### 2.2.4 Performance during maximal incremental tests

Performance changes during maximal incremental tests performed in laboratory have been monitored throughout measures of time to exhaustion (table 4) and records of maximal aerobic speed. Time to exhaustion (T) has been observed to increase from PPS to BCP (Metaxas et al., 2006). Also, different studies observed improvements in T from PPS to MCP (Haritodinis et al., 2004; Metaxas et al., 2006; Mohr et al., 2002) and/or ECP (Edwards et al., 2003; Haritodinis et al., 2004; Metaxas et al., 2006). Even though former studies observed that these improvements occur in parallel to increments in VO$_2$max, Edwards et al., (2003) detected that professional soccer players increased T but not VO$_2$max from PPS to ECP. The researchers suggested that in a highly trained state, players may be able to supplement additional exercise performance time through enhanced anaerobic energy systems (Edwards et al., 2003).
Velocity at VO$_2$max (Maximal Aerobic Speed; MAS) reflects the maximum aerobic capacity and combines VO$_2$max and running economy into a single factor (Billat & Koralsztein, 1996). MAS is a good indicator of aerobic performance (Billat & Koralsztein, 1996), and its determination gives a practical assessment of aerobic demands during running performance (Kalapotharakos et al., 2011). Recent investigations observed that improvements in MAS after preseason training (9.1 %) remained until the MCP (Kalapotharakos et al., 2011). Dupont et al., (2004) observed that professional soccer players performing 2 weekly sessions of high-intensity training, during 10-wks of in-season, increased MAS in 8.8 %. Others professional players performing 2 sessions per week of HIS and SE during 8-wks of preseason obtained a 3.1% improvement in MAS (Wong et al., 2010).

2.2.4.1 High intensity intermittent exercise

Recent studies have shown that the level of competitiveness of the player is related with the intermittent exercise performance during games (Mohr et al., 2003), in soccer-specific endurance tests, as the Yo-Yo tests (Mohr et al., 2003; Rampinini et al., 2009a), and in repeated sprint ability tests with (RSSA) (Rampinini et al., 2009b) or without (RSA) (Aziz et al., 2008) changes of direction. Also a positive relationship was analysed between team success in the league and intermittent exercise performance in Yo-Yo Intermittent Endurance Test level 2 (YYI2) (Randers et al., 2007). Moreover, performance in Yo-Yo intermittent recovery test level 1 (YYIR1) (Krstrup et al., 2003), YYIE2 (Bradley et al., 2010) and in RSSA (Rampinini et al., 2007b) have shown to be good indicators of game-related physical performance.

A summary of studies examining changes in high intensity intermittent exercise tests through different periods of a soccer season is presented in table 5. It has been observed that professional players improve the performance in YYIR2 and YYIR1 tests from PPS to middle of preseason period (Krstrup et al., 2003; Krstrup et al., 2006) with further improvement in the BCP in YYIR2 (Iaia et al., 2009b; Krstrup et al., 2006), YYIR1 (Krstrup et al., 2003), YYIE2 (Bradley et al., 2009; Silva et al., 2011), and in the Intermittent Field Test (IFT (Rebelo &
Soares, 1997). Moreover, it was observed that improvements obtained in YYIR2 (Iaia et al., 2009b; Krstrup et al., 2006), in YYIE2 (Bradley et al., 2009) and in the Probst test (Nunez et al., 2008) during preseason are maintained in MCP. As may be expected, studies in the tracking of professional and semi-professional players found increased performance on YYIR1 (Krustrup et al., 2003), YYIE2 (Bradley et al., 2010; Silva et al., 2011) and Probst tests (Nunez et al., 2008) at ECP than in PPS. Similarly, Oliveira (Oliveira, 2000) observed that elite players improved YYIE2 performance 4-months and 7-months after PPS (21.9 and 30.1%, respectively). Also, further performance improvements in IFT could be observed from BCP to MCP (Rebelo & Soares, 1997). Nevertheless, Bradley et al., (2010) did not found any change in YYIE2 performance from PPS to MCP.

Some researchers observed that high intensity training (HIT) increases soccer specific endurance (SSE) during preseason. Wong et al., (2010) found a 19.7 % improvement in the performance of professional players in the YYIR1 test after a concurrent training program of speed-endurance and high-intensity strength (HIS). Bogdanis et al., (2011) observed that professional players increase performance in YYIE2 and Hoff’s dribbling track test (DTT) after preseason strength training-based in hypertrophy (H) and neural (N) methods. Interestingly, the authors report that although SSE evaluated by the DTT improved similarly in H and N groups (10 % and 9.6 %, respectively), there was a trend (p=0.067) to better performance in the YYIE2 in the N (29.4 %) than H (21.5 %) group. Moreover, improvement in fatigue resistance measured by a RSA test was only evident in N. There are reports that preseason HIT enhances performance and physiological improvements when evaluated by other forms of running exercises. Sporis et al., (2008a, 2008b) observed high-level junior players performing 6-wks of high-intensity aerobic training (HIA) in the form of a situational drill (HIA-SD) during preseason. This training intervention improves performance during in-line (200, 400, and 800, 1200 and 2400-m) and in shuttle running exercises (Sporis et al., 2008a, 2008b). Furthermore, the same group of researchers observed that professional players performing preseason (8-wks) HIA-SD were able to tolerate a higher maximal blood lactate concentration
during the 300y shuttle run test (14.1%) and improve test performance (2.2%) (Sporis et al., 2008a). Others report that high-level juniors players improve the time to perform 4 repetitions of the Ekblom’s circuit test after the application of a training program of HIA-IR or HIA-SSG (12.2% and 13%, respectively) (Impellizzeri et al., 2006). Moreover, further 8-wks of HIA-IR or HIA-SSG training during in-season enhanced Ekblom’s test performance (2.4% and 3.2%, respectively) (Impellizzeri et al., 2006). Similarly, Ferrari Bravo et al., (2008) observed that high-level junior and lower-level senior players improved YYIR1 performance after 7-wks of HIT during in-season. However, although the two different modes of HIT examined (HIA-IR and RSA) were effective in improving YYIR1 performance (12.5 vs. 28.1%, respectively), only the RSA group improved RSA performance (2.1%; Ferrari Bravo et al., 2008). According to the researchers, differences in the functional adaptations between the 2 modes of HIT (HIA-IR vs. RSA) may be related to enhancements in anaerobic metabolism and not with the ability to recover between sprints (Ferrari Bravo et al., 2008). Also, Jensen et al., (2009) observed that HIT (12-wks HIA-SSG) induced in-season 15.2% increases in YYIR2 and improves fatigue time (difference between fastest and slowest sprint time) during an RSA test. In this matter, better performance in the end than before the start of the HIA-SSG program and the beginning of the season (0.19-s vs. 0.24-s and 0.30-s) was analysed in the RSA test (Jensen et al., 2009).

Regarding repeated shuttle sprint ability (RSSA; 6 × 40-m, turns at 20-m, 20-s recovery) Impellizzeri et al., (2008) observed that elite players improved different parameters in an RSSA test performance throughout the season. Namely, best sprint (RSSA_best), mean time of the sprints (RSSA_mean) and fatigue index during the RSSA (RSSA_decrement) improved after preseason training, 1 %, 2.2 % and 22.7 %, respectively. Nevertheless, a small but likely 0.8 % worsening in RSSA_mean occurred from BCP to MCP and persisted until the ECP. Nevertheless, Mercer et al., (1997) did not observe any significant improvement in RSSA_best and RSSA_mean of professional players during a different version of RSSA (8x50-m shuttle sprints, turns at 25-m, 30-s recovery) after preseason period. Given that no information regarding preseason training were reported in
the previous study, we may hypothesise that differences between studies can be related with the physiological adaptations (e.g., neural adaptations) obtained during preseason. As already described (Bogdanis et al., 2009), different training methodologies (e.g., type of strength training) may influence RSA performance.

2.2.5 Sub-maximal intermittent exercise

Alterations in the ability to perform sub-maximal intermittent exercise have been examined. Precisely, soccer players $\%HR_{\text{max}}$ achieved at the 6-min point of the YYIR1, decreased from PPS to the middle of preseason, BCP and ECP (Krustrup et al., 2003). Also, a decrease in the $%HR_{\text{max}}$ and blood lactate concentrations at the end a sub-maximal version of the YYIR1 (5-min) were observed after 12 weeks of in-season HIA-SSG (87.3% to 81.3% $HR_{\text{max}}$ and from 5 to 2.5 mmol.l$^{-1}$ correspondingly) (Jensen et al., 2009).

2.3. Neuromuscular function

Muscle power-based efforts such as sprints, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power of the neuromuscular system, are essential factors to successfully performing in soccer (Cometti et al., 2001). During the match, players perform a wide range of soccer specific activities (e.g., sprints, jumps) that demand players to be able to sustain and produce forceful contractions (Stolen et al., 2005) that result in higher stresses on the neuromuscular system in order to cope with this essential efforts. This match-induced stresses in neuromuscular function has been verified by the post-match performance reductions in measures of strength and muscle-power efforts (Andersson et al., 2010a; Andersson et al., 2008; Ascensoe et al., 2008; Fatouros et al., 2009; Ispirlidis et al., 2008; Krustrup et al., 2011; Magalhaes et al., 2010; Rampinini et al., 2011).

Recent studies observed that agility (Mujika et al., 2008; Reilly et al., 2000), strength and short distance sprint speed (Cometti et al., 2001; Dauty & Potiron Josse, 2004) are related to player standard. Also, a positive relation with team success in the same league was reported for jump ability (Arnason et al., 2004).
Some contradictions regarding improvements in anaerobic and neuromuscular function during soccer season have been described (Aziz et al., 2005; Caldwell & Peters, 2009; Clark et al., 2008; Malliou et al., 2003; McMillan et al., 2005a; Mercer et al., 1997). The inconsistencies observed during longitudinal studies strengthen when analysing studies focusing training-effects of HIT (Dupont et al., 2004; Ferrari Bravo et al., 2008; Sporis et al., 2008a, 2008b), muscle strength training (ST) (Chelly et al., 2009; Gorostiaga et al., 2004; Kotzamanidis et al., 2005; Maio Alves et al., 2010; Mujika et al., 2009; Ronnestad et al., 2008; Ronnestad et al., 2011; Sedano et al., 2011; Thomas et al., 2009; Wong et al., 2010) and concurrent endurance and strength training (Lopez-Segovia et al., 2010; Nunez et al., 2008; Wong et al., 2010).

2.3.1 Jump ability

Concerning jump ability (table 6), there are reports of performance improvements in countermovement jump with arm-swing (CMJWAS) of professional players (Mercer et al., 1997) and in CMJWAS and squat jump with arm-swing (SJWAS) of semi-professional players (Caldwell & Peters, 2009) during preseason, that were maintained throughout in-season. Moreover, semi-professional (Caldwell & Peters, 2009) and professional players (Aziz et al., 2005) showed further performance improvements from BCP to MCP in CMJWAS and SJWAS, respectively. Also an improved CMJWAS (5%) was also observed in elite U-19 players after in-season ST (Sedano et al., 2011).

Regarding countermovement jump performance (CMJ), although Silva et al., (2011) observed a preseason enhancement followed by a decrement during in-season (from BCP to MCP), other studies do not observe alterations in jump ability exercise during different moments of the season (Casajus, 2001; Malliou et al., 2003; Mercer et al., 1997). Regarding variations resulting from strength training (ST), Bogdanis et al., (2009) examined preseason improvements (5.3-10%) that were not confirmed by other studies with senior (Ronnestad et al., 2008) and U-19 elite players (Maio Alves et al., 2010). Sedano et al., (2011) and Thomas et al., (2009) reported improvements in CMJ after ST performed
during in-season of elite U-19 (8%) and semi-professional players (5-7%), respectively.

Concerning the squat jump (SJ), longitudinal studies did not show any performance change throughout the season (Casajus, 2001; Malliou et al., 2003; Thomas & Reilly, 1979). This lack of preseason variation in SJ in professional players was also confirmed in a ST investigation (Ronnestad et al., 2008). Nevertheless, others (Maio Alves et al., 2010) observed that ST improved preseason SJ performance in elite U-19 players. Also, Thomas and Reilly (1979) analysed jump ability variations during horizontal jumps and observed increases in standing board jump during in-season, from BCP to MCP and ECP.

In a study of Clark et al. (2008), improvements in CMJ performance and in the average height during CMJ_{20-s(cm)} of professional players were only evident from PPS to MCP in the second of three seasons examined. Moreover, Casajus (2001) did not observe changes in anaerobic power from BCP to MID during the CMJ_{15-s} test. As such, it seems that the maximal mechanical power and ability to sustain fatigue during the repeatedly performance of CMJ did not significantly change throughout the season. However, different research examining jump ability performances changes, suggests that jumps involving stretch-shortening cycle activities (SSC) and all body movement interactions (use of arms) had more propensity to improve. This is not surprising, due to the greater specificity of this types of jumps (e.g., SSC, coordinate the different body segments).

### 2.3.2 Sprint ability

The change in sprint ability throughout the season has been analysed in a wide range of sprint distances (table 7). Preseason performance improvements in 15-m sprint of semi-professional (Caldwell & Peters, 2009), and in 50-m sprint of professional players (Ostojic, 2003) have been reported. Nevertheless, others did not observe improvements in professional players performance after this season period in 5-m (Aziz et al., 2005; Silva et al., 2011), 20-m (Aziz et al., 2005) and 30-m (Silva et al., 2011; Zoppi et al., 2006) sprints.
Studies examining preseason strength training programs (ST) observed that professional players improved 10-m and 40-m sprint (ranging from 1 to 2%) (Bogdanis et al., 2009). Nevertheless, others observed that professional players 10-m and 40-m sprint times improved in one but not in other group of the players involved in the investigation (Ronnestad et al., 2008). Another study performed during preseason, found that high-level junior players improved 5-m (7-9%) and 10-m sprint times (3.1-3-2%), independently of performing one or two weekly strength sessions per week (Maio Alves et al., 2010).

Data from longitudinal studies highlighted that there may be a tendency for players to improve their sprint performance throughout the season. Improvements were observed from BCP to MID in the 15-m and 30-m sprint performance of semi-professional (Caldwell & Peters, 2009) and professional players (Silva et al., 2011), respectively. Also, professional players improved sprint performance in 5-m and 20-m from PPS to ECP (Aziz et al., 2005) and in 50-m (Ostojic, 2003) from BCP to ECP, have been reported. An improvement in sprint performance was also observed in academic players in 10-m and 30-m distances (Magal et al., 2009) and in semi-professional players in 15-m (Caldwell & Peters, 2009) from BCP to ECP. Nevertheless, others did not find alterations in 36.5-m (Silvestre et al., 2006) and 40-m (Magal et al., 2009) sprint times of academic players during in-season (from BCP to ECP).

ST studies reported an in-season reduction in 10-m sprint time (0.32%) of elite U-19 players (Sedano et al., 2011). Nevertheless, this was not observed in semi-professional in a variety of sprint distances (5-m, 10-m, 15-m and 20-m sprints) (Thomas et al., 2009).

In spite of the wide range of sprint distances evaluated, data suggest that sprint ability may improve throughout the preseason and further in-season.

### 2.3.3. Change of direction speed

According Jones et al., (2009), tests of agility that do not include a perceptual or decision making component can be referred as measures of change of direction speed (COD) and can be defined as the ability to decelerate, reverse or change movement direction and accelerate again. These type of tests can also be
described as movements in which no immediate reaction to a stimulus is required, thus the direction change is pre-planned (Brughelli et al., 2008). In this matter, COD evaluated by Illinois agility run test (IART) was shown to improve in professional (Mercer et al., 1997) and semi-professional players during preseason (table8) (Caldwell & Peters, 2009). Nevertheless, this was not confirmed in professional players performing the t-test (Silva et al., 2011).

Strength training (ST) interventions during preseason induced a performance improvement in COD evaluated by Zig-Zag test (≈1.3-2.5%), T-test (≈1.2-2.1%), and IART (≈0.6-1%) (Bogdanis et al., 2009). Nevertheless, COD performance of elite junior players evaluated by the 505 Agility test did not improve (Maio Alves et al., 2010).

Regarding season alterations, certain reports suggested that professional (Mercer et al., 1997) and semi-professional players (Caldwell & Peters, 2009) may maintain increases in COD performance acquired during preseason during in-season. Also, when not improved during preseason, COD ability has been shown to develop during in-season (MCP and ECP) in regard to the beginning and end-of-preseason (Silva et al., 2011). Also, it was reported that semi-professional players performing in-season ST, show an increase in COD speed (5-10% 505 test) (Thomas et al., 2009). As such, it seems that generally, COD ability may improve during preseason period (Bogdanis et al., 2009; Caldwell & Peters, 2009; Mercer et al., 1997) and can be maintained or further improved throughout the competitive period (Caldwell & Peters, 2009; Silva et al., 2011; Thomas et al., 2009).

2.3.4. Strength

Isokinetic strength evaluation of soccer players lower limbs have been currently used in different studies despite the discrepancy in the angular velocities analysed. This methodology allows identifying eventual muscular and functional limitations (Ayalon et al., 2002; Croisier et al., 2008). Nevertheless, despite the popularity of isometric and isokinetic test in research, the literature indicates that they are inferior to isoentertial measures in predicting performance or tracking changes longitudinally (Harris et al., 2007). However, longitudinal
researches regarding seasonal alterations in professional players’ strength had relied exclusively on evaluations with isokinetic dynamometers (table 9). These investigations showed that no alterations occur during preseason in knee extensors (KE) and flexors (KF) strength at angular velocities of 60°/s\(^{-1}\) (Malliou et al., 2003; Mercer et al., 1997), 90°/s\(^{-1}\) (Silva et al., 2011) and in KE at 180°/s\(^{-1}\) (Malliou et al., 2003). Nevertheless, Kraemer et al., (2004) observed a significant decrease in KE from the BCP to MID in academic starter soccer players. Also, both starter and non-starter players had a decrease in KE from BCP to ECP. However, significant differences between the KF of starter and non-starter players were observed in beginning and in the middle of the competitive period.

Results regarding strength training (ST) interventions seem to highlight that isoenertial measures have a great sensitivity in tracking longitudinal changes. In fact, two studies examining the effects of strength training in preseason showed that professional players improved 1RM in Half-Squat ranging 11-17.3% (Bogdanis et al., 2009) and 23-26% (Ronnestad et al., 2008). Moreover, improvements in maximum strength (1RM) per lean leg volume (LLV; 1RM/LLV) (Bogdanis et al., 2009) and in the relative force (maximum force divided per body weight) (Manolopoulos et al., 2006) after high-intensity ST and complex ST interventions have been observed. In this respect, Bogdanis et al., (2009) found that the increases in 1RM/LLV were only obtained in players performing neural adaptations models, since no increments in 1RM/LLV were observed in players performing hypertrophy mode of ST. It was suggested that strength increments during preseason resulted in strength growths without increments in muscle volume (Bogdanis et al., 2009).

2.4. Game-related physical parameters

Match analysis is a widely used instrument in professional soccer to study tactical and physical performance of players (Abt & Lovell, 2009). This instrument allows careful analysis of performance during a game that is dependent of a large multiplicity of factors (e.g. training status) (Andersson et al., 2010b; Bangsbo et al., 1991; Bradley et al., 2009; Gregson et al., 2010;
Mohr et al., 2003; Rampinini et al., 2007a; Rampinini et al., 2007b) and that seasonal changes in game-related physical performance occur throughout the season (Mohr et al., 2003; Rampinini et al., 2007b). Mohr et al., (2003), using video-based time-motion analysis, observed that elite players covered a significantly greater total distance (TD) during games played in the ECP than in the MCP and BCP (5.8% and 3.7%). Rampinini et al., (2007b), using the multi-camera method, (ProZone [Leeds, England]) also observed that elite players covered a higher TD in games played at the ECP than at BCP (2.9%). Nevertheless, a significantly higher TD was also performed in MCP than at the BCP (2%) (Rampinini et al., 2007b).

The distance covered in high-intensity running (HI) during the match has been proposed to be of great importance for performance in elite soccer because this parameter clearly distinguishes players of different standards (Mohr et al., 2003). Studies analysing alterations in HI throughout the season observed that higher distances in HI are performed during games played in ECP that in the MCP and BCP (Mohr et al., 2003; Rampinini et al., 2007b). Mohr et al., (2003) observed an increase in HI (>15-km.h\(^{-1}\)), from ECP to MCP and BCP (29.4% and 19%). In the same order, Rampinini et al., (2007b) detected a change in HI (>14.4-km.h\(^{-1}\)) of 7.6% and 11.5%, respectively.

Although Morh et al., (2003) did not observe differences in sprinting (>30-km.h\(^{-1}\)) during the different time points of the season, Rampinini et al., (2007b) observed that a higher distance was covered in very high-intensity running (>19.8-km.h\(^{-1}\)) in the ECP than in the MCP and BCP (17.9% and 20.2%). Nevertheless, differences between studies are probably related to the pre-defined thresholds of the different intensity categories of each match analysis system. In fact, contradictions regarding the definitions used for high intensity categories can be observed in literature. Also, both discrepancies between systems in the accuracy of the determination of the distance covered at HI (Randers et al., 2010) and in the accuracy of the intensity of the pre-defined thresholds with players individualized thresholds of physiological stress (second ventilatory threshold) have been reported (Abt & Lovell, 2009).
3. Off-season induced changes in physiological and functional parameters

3.1. Anthropometry

Regarding off-season alterations in professional players BM (table 1), both increases (Sotiropoulos et al., 2009) and decreases in BM have been reported (Reinke et al., 2009). To comprehend alterations in BM, complementary analysis of players BF and LBM should be performed. Semi-professional (Caldwell & Peters, 2009) and professional players (Reinke et al., 2009) experience changes in %BF during the season. Also, a decrease in LBM of professional players usually occurs during off-season period (Reinke et al., 2009). Research regarding the effect of training programs in avoiding fitness decrements during this period has been matter of analysis. Sotiropoulos et al., (2009) observed that even though players who followed an oriented training program also had increases in BF, they had lower increases in weight and BF compared to the players that did not follow any organized training program.

3.2. Endurance-physiological parameters

Deconditioning during off-season resulted in VO$_2$max decrements in players of different standards (table 2) (Caldwell & Peters, 2009; Mohr et al., 2002; Sotiropoulos et al., 2009). Sotiropoulos et al., (2009) observed that the accomplishment of organized training program during a four-week off-season period did not prevent decreases in VO$_2$max, but did allow lower reductions in VO$_2$max than in those players that did not performed an organized activity (1.4% vs. 6.1% less, respectively). Moreover, Christensen et al., (2011) observed that only 2-wks of inactivity during off-season were reported to slower VO$_2$ kinetics (75% MAS) with a larger time constant (21.5±2.9 vs. 23.8±3.2-s, pre vs. post, respectively) and larger mean response time (45.0±1.8 vs. 46.8±2.2-s, pre vs. post, respectively). Also, a decrease in muscle oxidative capacity was evident by the reductions of muscle pyruvate dehydrogenase (17%) and maximal activity of citrate synthase (12%) and 3-hydroxyacyl-CoA (18%; Christensen et al., 2011). Also, evidence of off-season deconditioning is reflected in decreases in time to exhaustion during incremental tests.
(Sotiropoulos et al., 2009). Players’ ability to perform exercise of sub-maximal intensity also appears to be affected (table 3). Mohr et al., (2002) observed significant increases in elite players’ heart rate (bpm) at the different running speeds of 10, 14 and 17 km.h. These detraining effects may influence players’ preparation during preseason and, in a certain way, affect performance level especially in the first matches of competitive season (Kraemer et al., 2004).

3.3 Soccer Specific Endurance (SSE)

As observed in particular physiological endurance-related parameters, detraining impairs performance during SSE tests (table 5). In fact, Krstrup et al., (2006) report a 11% decrement in YYIR2 performance throughout off-season. The off-season inducing performance reductions in intermittent exercise was also confirmed in elite players YYIE2 (38% decrease) (Oliveira, 2000). Moreover, other investigations examined that after the first 2 weeks of inactivity during off-season significant performance reductions in YYIR2 test (29%) and in the repeated sprint performance (2%) occurred (Christensen et al., 2011).

3.4 Neuromuscular Function

Detraining effects are also evident in soccer players’ neuromuscular system. A performance decline in 15-m and COD ability (Illinois agility test) of semi-professional players (Caldwell & Peters, 2009) and in 50-m sprint time of professional players (Ostojic, 2003) have been described. Regarding impairments in the performance of jump ability there are some contradictions in literature. In fact, although no significant alterations in isokinetic knee extensors strength and in CMJ and SJ were examined after the offseason period of professional players (Malliou et al., 2003), a decrement was reported in CMJWAS of semi-professional players (Caldwell & Peters, 2009). Probably these differences in results regarding jump ability are be related to the length of the detraining period and/or with differences regarding jump kinetics and kinematics.
4. Conclusions

A distinct feature is presented in relation to the physiological and functional adaptations during preseason training. The majority of studies shows \( \text{VO}_2 \text{max} \) increases during preseason (average increments ranging 5 to 11%) that are maintained into in-season. Also, above a threshold of 62 ml.kg\(^{-1}\)min, no observations of significant increments were stated. MAS improves during preseason (3.1-9.1%), and improvements of different magnitude (4.5-21.6%) were also observed in sub-maximal exercise performance. Regarding in-season alterations, although the majority of investigations did not report in-season \( \text{VO}_2 \text{max} \) improvements, other endurance parameters, such as MAS and acute physiological adaptations during submaximal exercise performance, seem to increase during this period. In addition, soccer specific endurance (SSE) follows a similar trend of adaptation. Faster and greater SSE (e.g. endurance intermittent tests and game-physical performance) improvements were observed during preseason. Also great average increments in SSE (e.g. Yo-Yo tests) have been reported during in-season without significant modifications in \( \text{VO}_2 \text{max} \) and smaller physiological adaptations in sub-maximal exercise. This highlights the high sensitivity and ability of field measures in the training monitorization of players. Moreover, game-related physical parameters in high-intensity categories showed great increases during in-season. This may suggest that other endurance physiological determinants (e.g., \( \text{VO}_2 \) kinetics and ability to maintain acid-base balance) that have been demonstrated to be associated with the ability to increase performance (Christensen et al., 2011) and carry out high-intensity intermittent exercise (Rampinini et al., 2009a; Rampinini et al., 2009b) can occur during the season and so, contribute to SSE increments. Some studies have found correlations between \( \text{VO}_2 \text{max} \) and performance in RSA (Rampinini et al., 2009a) and YYIR tests (Rampinini et al., 2009b). Nevertheless, although the recently reported correlations between changes in SSE field tests (e.g., YYIET2) and changes in physical game performance (e.g., high-intensity running) during the season this was not evident for \( \text{VO}_2 \text{max} \) (Bradley et al., 2011).
Adaptations of players’ neuromuscular systems have been measured throughout sprint, COD and jump tests, with higher evidence in those jumps involving stretch-shortening cycle activities (SSC) and all body movement interactions (jumps with arm swing). Data provides evidence that when early improvements are obtained (from PPS to BCP), they can be maintained and even further improved during in-season. This suggests that neuromuscular adaptations affecting SSC mechanisms may occur during in-season.

Muscle-power base efforts and SSE have been shown to have a greater tendency to improve during in-season than the usually motorized physiological endurance parameters (e.g., VO$_2$max and AT). As such, investigations to clarify which adaptations (e.g., motor unit recruitment and synchronization; muscle morphology; soccer-activity efficiency) and interactions are related with players’ increments in SSE performance measured by field tests and game-related physical parameters (high-intensity running) are urgent. For example, although positive adaptations in running economy have mainly been reported and investigated during preseason, there are recent reports of increased RE (75% of MAS) in professional players after performing 2 weeks of intense HIT performed as soon the competitive season finishes (Christensen et al., 2011). Moreover, in addition to the fact that investigations on these matters is scarce, their lack of specificity in assessing RE is clear, which may suggest that soccer specific work economy may improve somewhat, with relevant gains not detected by less specific or ecological methods. Furthermore, recent reports highlight that enhancement in neuromuscular function can be a determinant in improving short and long-term endurance capacity (Aagaard & Andersen, 2010). Nevertheless, the increase in game-physical activity towards the end-of-season can result, at least in part, from an improvement in some form of pacing strategies by professional players. As such, the development and improvement of a generic (across all outfield positions) pacing strategy cannot be excluded (Edwards & Noakes, 2009). Nevertheless, there are contradictions regarding the concept of team sport players pacing their effort during matches (Aughey, 2010).
The off-season period appears to result in decrements of a variety of physiological and functional parameters. Moreover, it seems that such decrements are more evident in muscle power ability than in muscle strength. Furthermore, due to the lack of research conducted during off-season, it seems that the analysis of the efficiency of players performing training protocols in order allow them to recover from the stressful season, and in addition, avoiding a great magnitude of detraining effects is needed.

In conclusion, although an extraordinary growth in the number of scientific investigations concerning soccer has been observed in the last decades, there is yet much to elucidate regarding the physiological factors related with soccer players’ performance changes throughout the season.

5. References


Table 1. Summary of studies examining changes in soccer player's anthropometry characteristics throughout a soccer season.

<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country</th>
<th>n (years)</th>
<th>S Test</th>
<th>Training Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ECP1</td>
</tr>
<tr>
<td>Reinke et al. (2009)</td>
<td>Professional/German</td>
<td>10 (r:20-36)</td>
<td>T BM</td>
<td>90.1 ± 5.6</td>
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<tr>
<td>Sotiropoulos et al. (2009)</td>
<td>Professional/Greek</td>
<td>28 (23.2±2.55)</td>
<td>EG BM</td>
<td>78.14 ±4.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 (24.4±2.97)</td>
<td>CG BM</td>
<td>76.48 ± 2.65</td>
</tr>
<tr>
<td>Ostojic (2003)</td>
<td>Elite/Serbia</td>
<td>30 (23.5±3.1)</td>
<td>T BM</td>
<td>77.8±6.3</td>
</tr>
<tr>
<td>Aziz et al., (2005)</td>
<td>Elite/Singapore</td>
<td>41 (25.7±3.9)</td>
<td>T BM</td>
<td>70.6 ± 10.3</td>
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<tr>
<td>Metaxas et al. (2006)</td>
<td>Elite U-19/Greek</td>
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<td>70 ± 4.9</td>
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<td>12 (18.1±1)</td>
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<tr>
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<tr>
<td>Kalapotharakos et al. (2011)</td>
<td>Elite/Greek</td>
<td>12 (25±5)</td>
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<td>74.2 ± 6.5</td>
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<tr>
<td>Silva et al. (2011)</td>
<td>Elite/Portugal</td>
<td>18 (25.7±4.6)</td>
<td>T BM</td>
<td>76.5 ± 9.2</td>
</tr>
<tr>
<td>Reinke et al. (2009)</td>
<td>Professional/German</td>
<td>10 (r:20-36)</td>
<td>T BF</td>
<td>10.3±5.6</td>
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<tr>
<td>Edwards et al. (2003)</td>
<td>Professional/England</td>
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<td>T %BF</td>
<td>12.3±3.11</td>
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<td>7.77±1.79</td>
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<tr>
<td>Caldwell and Peters (2009)</td>
<td>Semi-professional/England</td>
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<td>%BF</td>
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<tr>
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<td>%BF</td>
</tr>
<tr>
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<td>T</td>
<td>%BF</td>
</tr>
<tr>
<td>Mercer et al. (1997)</td>
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<td>Heller et al. (1992)</td>
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</tbody>
</table>

Values are mean ± SD; **S** - sample; **ECP1** - end of competition period; **PPS** - Prior preseason; **EPS/BCP** - end preseason period/beginning competition period; **MCP** - Middle competition period; **ECP2** - end of competition period 2; **r** - ranging; **Czech** - czech republic; **T**- team; **EG** - experimental group; **CG** - control group; **CD** - central defender; **MF** - midfielder; **AT** - attacker; **BM**- Body mass (kg); **%BF**- percentage body fat; **LBM**- lean body mass; †sig. dif from ECP1; ‡ sig. dif from PPS; † sig. dif from EPS/BCP; ‡ sig. dif from MCP;
<table>
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<th>D/E</th>
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<th>PPS</th>
<th>EPS/BCP</th>
<th>MCP</th>
<th>ECP2</th>
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<td>Elite/Greek</td>
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<td>T</td>
<td>D</td>
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<td>122.9%</td>
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<td>12 (18.1±1)</td>
<td>MF</td>
<td>D</td>
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<td>54</td>
<td>119.5%</td>
<td>122.1%</td>
<td>117.7%</td>
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<td>10 (18.2±0.9)</td>
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<td>D</td>
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<td>127.2%</td>
<td>121%</td>
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<td>Mohr et al. (2002)</td>
<td>Elite/Denmark</td>
<td>11 (24.0±1.0)</td>
<td>T</td>
<td>D</td>
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<td>58.1±0.9</td>
<td>55.4±0.7*</td>
<td>59.0±0.8</td>
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<td>Elite/Spain</td>
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<td>D</td>
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<td>66.5</td>
<td>66.4</td>
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<td>D</td>
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<td>60.1±2.8</td>
<td>59.3±3.1</td>
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<td>Mercer et al. (1997)</td>
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<td>D</td>
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<td>56.8±4.9</td>
<td>62.6±3.8</td>
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<tr>
<td>Caldwell and Peters</td>
<td>Semi-professional</td>
<td>13 (24±4.4)</td>
<td>T</td>
<td>D</td>
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<td>58±1.9</td>
<td>58±1.2*</td>
<td>59±2.3†</td>
<td>58±2.2‡</td>
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</tr>
<tr>
<td>(2009)</td>
<td>/England</td>
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<tr>
<td>Aziz et al. (2005)</td>
<td>Elite/Singapore</td>
<td>41 (25.7±3.9)</td>
<td>T</td>
<td>E_BT</td>
<td></td>
<td>52.7±3.4</td>
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<td>Edwards et al. (2003)</td>
<td>Professional/England</td>
<td>12 (26.2±3.3)</td>
<td>T</td>
<td>D</td>
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<td>62.1±4.9</td>
<td>63.3±5.7</td>
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</table>

Table 2. Summary of studies examining changes in soccer players VO2 max (ml.kg⁻¹.min or in percentage variation) through different periods of a soccer season.
Table 2. Contd

<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country</th>
<th>n (years)</th>
<th>S</th>
<th>D/E</th>
<th>Training Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silvestre et al. (2006)</td>
<td>NCAAI/USA</td>
<td>25(19.9±1.3)</td>
<td>T</td>
<td>E_{Yo-Yo}</td>
<td>ECP1 59.8 ± 3.3</td>
</tr>
<tr>
<td>Magal et al. (2009)</td>
<td>NCAAIII/USA</td>
<td>12(20±0.9)</td>
<td>T</td>
<td>D</td>
<td>ECP1 51.05 ± 5.97</td>
</tr>
<tr>
<td>Sotiropoulos et al.</td>
<td>Professional/Greek</td>
<td>28 (23.2±2.55)</td>
<td>EG</td>
<td>D</td>
<td>ECP1 57.66 ± 2.56</td>
</tr>
<tr>
<td>(2009)</td>
<td></td>
<td>20 (24.4±2.97)</td>
<td>CG</td>
<td>D</td>
<td>ECP1 58.08 ± 2.60</td>
</tr>
</tbody>
</table>

Values are mean ± SD; S - sample; D/E – Direct (D) or estimated measurement (E) of VO$_2$max; ECP1 – end of competition period; PPS – Prior preseason; EPS/BCP - end preseason period/beginning competition period; MCP – Middle competition period; ECP2 – end of competition period 2; NCAA – National Collegiate Athletic Association Division; T - team; CD – central defender; MF – midfielder; AT – attacker; EG – experimental group; CG – control group; ↑ - increments in relation to PPS; E$_{20mMSRT}$ – estimated by performing the 20 meters Maximal Multistage shuttle run test (National Coaching Foundation, Leeds, UK); E$_{MSFT}$ – estimate by performing Multistage Fitness Test AA1CD; National Coaching Foundation, Leeds, E$_{BT}$ – estimated by performance of Beep* test (CD Australian Sports Commission); UK; E$_{Yo-Yo}$ – estimated by performance of Yo-Yo endurance tests; x - not specified; ≈ - approximately; † sig. dif from ECP1; ‡ sig. dif from PPS; † sig. dif from EPS/BCP; ‡ sig. dif from MCP; † sig. dif from ECP2
Table 3. Summary of studies examining changes in physiological parameters at sub maximal intensities through different periods of a soccer season

<table>
<thead>
<tr>
<th>Study</th>
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<th>n (years)</th>
<th>Parameter</th>
<th>Training Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohr et al. (2002)</td>
<td>Elite/Denmark</td>
<td>11(24.0±1.0)</td>
<td>HR_{opt} Speed 10 km.h</td>
<td>ECP1 132 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR_{opt} Speed 14 km.h</td>
<td>PPS 140 ± 5*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HR_{opt} Speed 17 km.h</td>
<td>EPS/BCP 160 ± 5</td>
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<td></td>
<td>MCP 128 ± 4.0</td>
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<td></td>
<td>ECP2 167 ± 3*</td>
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</tr>
<tr>
<td>Clark et al. (2008)</td>
<td>Elite/England</td>
<td>42(25±3.5)</td>
<td>AT% VO_{2max}</td>
<td>ECP1 80.5%</td>
</tr>
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<td>PPS 81.05%</td>
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<td>EPS/BCP 128 ± 4</td>
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<td>MCP 158 ± 4.0</td>
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<td>ECP2 175 ± 3</td>
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<td>Casajus (2001)</td>
<td>Elite/Spain</td>
<td>15(25.8±3.2)</td>
<td>VAT Speed (km.h)</td>
<td>ECP1 12.4 ± 1.5</td>
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<td>VAT_{HR} (bpm)</td>
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<td>VAT_O2</td>
<td>EPS/BCP 168 ± 6</td>
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<td>MCP 52.7 ± 8.5</td>
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<td>ECP2 13.9 ± 1.7</td>
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<tr>
<td>Dunbar (2002)</td>
<td>1st division/England</td>
<td>11 (ND)</td>
<td>V_{[La]2mmol-1}</td>
<td>ECP1 14.3 ± 1.4</td>
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<td>ECP2 15.0 ± 1.5</td>
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<td>Elite/Greek</td>
<td>12 (25±5)</td>
<td>V_{[La]4mmol-1}</td>
<td>ECP1 12.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PPS 13.6 ± 0.5</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>EPS/BCP 13.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MCP 13.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ECP2 13.6 ± 0.4</td>
</tr>
<tr>
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</tr>
<tr>
<td>Heller et al. (1992)</td>
<td>Elite/Czech</td>
<td>12(23.5±3.9)</td>
<td>VAT Speed (km.h)</td>
<td>ECP1 14.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VAT_{HR}max</td>
<td>PPS 92.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V_{[La]4mmol-1}</td>
<td>EPS/BCP 90.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MCP 79.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ECP2 81.1%</td>
</tr>
<tr>
<td>Brady et al. (1997)</td>
<td>Professional/Scotland</td>
<td>19</td>
<td>LT Speed (km.h)</td>
<td>ECP1 13.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PPS 13.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EPS/BCP 13.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MCP 13.4 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ECP2 13.4 ± 1.4</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Level/Country</td>
<td>n (years)</td>
<td>Parameter</td>
<td>Training Period</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------------</td>
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</tr>
<tr>
<td>Zoppi et al. (2006)</td>
<td>10</td>
<td>LT Speed (km.h)</td>
<td>10.5 ± 1.9</td>
<td>13.8 ± 1.3&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Edwards et al. (2003)</td>
<td>Professional/England</td>
<td>12(26.2±3.3)</td>
<td>LT VO2</td>
<td>51.47 ± 4.2</td>
</tr>
<tr>
<td>Magal et al. (2009)</td>
<td>NCAAIII/USA</td>
<td>12(20±0.9)</td>
<td>VAT%VO2max</td>
<td>78.25 ± 8.46</td>
</tr>
</tbody>
</table>

Values are mean ± SD; ECP1 – end of competition period; PPS – prior pre-season; EPS/BCP - end pre-season period/beginning competition period; MCP – middle competition period; ECP2 – end of competition period 2; Czech – Czech republic; HRbpms/Speed 10 km.h – heart rate beats per minute at speeds of 10/14/17 km.h; [La] speed 10-14-17 km.h – blood lactate concentration at different speeds of 10/14/17 km.h; AT%VO2 – percentage of VO2max at the lactate and ventilator threshold; VAT<sub>Speed</sub> (km.h) - velocity at ventilatory anaerobic threshold; VAT<sub>HR(bpm)</sub> - heart rate (bpm) at the ventilatory anaerobic threshold; VAT<sub>VO2</sub> - VO2 (ml.kg<sup>-1</sup> min<sup>-1</sup>) at the ventilatory anaerobic threshold; V<sub>[La] 2mol.L<sup>-1</sup></sub> - velocity at a blood lactate concentration of 2 mmol.L<sup>-1</sup>; V<sub>[La] 3mmol.L<sup>-1</sup></sub> - Velocity at a blood lactate concentration of 3 mmol.L<sup>-1</sup>; V<sub>[La] 4mmol.L<sup>-1</sup></sub> - Velocity at a blood lactate concentration of 4 mmol.L<sup>-1</sup>; VAT%HRmax - percentage of maximum Heart Rate at the ventilatory anaerobic threshold; VAT%VO2max - percentage of VO2max at the ventilatory anaerobic threshold; LT Speed (km.h) – speed at the lactate threshold; LT VO2 – oxygen consumption at the anaerobic threshold; ↑ - higher values than in PPS; ↓ - 6% lower values than in MCP; * sig. dif from ECP1; † sig. dif from PPS; ‡ sig. dif from EPS/BCP; ‡ sig. dif from MCP;
### Table 4. Summary of studies examining changes in time to exhaustion (min) during maximal incremental lab tests through different periods of a soccer season

<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country</th>
<th>n (years)</th>
<th>S</th>
<th>Training Period</th>
<th>ECP1</th>
<th>PPS</th>
<th>EPS/BCP</th>
<th>MCP</th>
<th>ECP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haritodinis et al. (2004)</td>
<td>Elite/Greek</td>
<td>12 (25±5.3)</td>
<td>T</td>
<td></td>
<td>14.6 ± 0.7</td>
<td>16.4 ± 0.6*</td>
<td>15.9 ± 0.8*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaxas et al. (2006)</td>
<td>Elite U-19/Greek</td>
<td>10(18.2±0.9)</td>
<td>CD</td>
<td></td>
<td>X</td>
<td>↑ Ω</td>
<td>↑ Ω</td>
<td>↑ Ω</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12(18.1±1)</td>
<td>MF</td>
<td></td>
<td>X</td>
<td>↑ Ω</td>
<td>↑ Ω</td>
<td>↑ Ω</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10(18.2±0.9)</td>
<td>AT</td>
<td></td>
<td>X</td>
<td>↑ Ω</td>
<td>↑ Ω</td>
<td>↑ Ω</td>
<td></td>
</tr>
<tr>
<td>Mohr et al. (2002)</td>
<td>Elite/Denmark</td>
<td>11(24.0±1.0)</td>
<td>T</td>
<td>5.67 ± 0.18</td>
<td>5.45 ± 0.17*</td>
<td>5.91 ± 0.12*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Edwards et al. (2003)</td>
<td>Professional/</td>
<td>12(26.2±3.3)</td>
<td>T</td>
<td>3.4 ± 0.54</td>
<td></td>
<td></td>
<td></td>
<td>3.8 ± 0.68*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>England</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Arent et al. (2010)</td>
<td>NCAAI /USA</td>
<td>12(19.5±1.5)</td>
<td>T</td>
<td>16.44 ± 1.9</td>
<td>17.32 ± 1.6*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD; **S** – sample; **ECP1** – end of competition period; **PPS** – Prior pre-season; **EPS/BCP** - end pre-season period/beginning competition period; **MCP** – Middle competition period; **NCAA** - National Collegiate Athletic Association Division; **ECP2** – end of competition period 2; **T**- team: **CD** – central defender; **MF** – midfielder; **AT** – attacker; **X**- not specified; ↑ - higher values than in PPS; *sig. dif from ECP1; † sig. dif from PPS;
Table 5. Summary of studies examining changes in high intensity intermittent exercise tests through different periods of a soccer season

<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country</th>
<th>n (years)</th>
<th>TEST (m)</th>
<th>Training Period</th>
<th>ECP1</th>
<th>PPS</th>
<th>MPS</th>
<th>EPS/BCP</th>
<th>MCP</th>
<th>ECP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rebelo and Soares (1997)</td>
<td>Elite/Portugal</td>
<td>11</td>
<td>IFT</td>
<td></td>
<td></td>
<td>1821</td>
<td>1904&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1992&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krstrup, Mohr et al. (2006)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; league/Denmark</td>
<td>15</td>
<td>YYIR2</td>
<td></td>
<td></td>
<td>600±8</td>
<td>928±21&lt;sup&gt;†&lt;/sup&gt;</td>
<td>1033±45&lt;sup&gt;**&lt;/sup&gt;</td>
<td>964±39&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt;/2&lt;sup&gt;nd&lt;/sup&gt; league/Denmark</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>873±43</td>
<td>780±35*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iaia et al. (2009)</td>
<td>Professional/Denmark</td>
<td>12 (22.4 ± 3.7)</td>
<td>YYIR2</td>
<td></td>
<td></td>
<td>742.4</td>
<td>1160±39&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1068±52&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silva et al. (2011)</td>
<td>Elite/Portugal</td>
<td>18 (25.7±4.6)</td>
<td>YYIE2</td>
<td></td>
<td></td>
<td>1120</td>
<td>2250&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1640&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
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</tr>
<tr>
<td>Bradley et al. (2010)</td>
<td>Elite/Danish</td>
<td>10 (ND- adult)</td>
<td></td>
<td></td>
<td></td>
<td>2732</td>
<td>2760</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Elite U-19/English</td>
<td>15 (under 19)</td>
<td></td>
<td></td>
<td></td>
<td>2171±519</td>
<td>2411±593&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2560±574&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2381±571&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Krstrup et al. (2003)</td>
<td>Elite/ ND</td>
<td>10</td>
<td>YYIR1</td>
<td></td>
<td></td>
<td>1760±59</td>
<td>≈2160&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2211±70&lt;sup&gt;**&lt;/sup&gt;</td>
<td>213±68&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Nunez et al. (2008)</td>
<td>Semi-professional/Spain</td>
<td>15 (28.2±3.4)</td>
<td>Probst</td>
<td></td>
<td></td>
<td>2006±207</td>
<td>3480±368&lt;sup&gt;**&lt;/sup&gt;</td>
<td>3620±454&lt;sup&gt;**&lt;/sup&gt;</td>
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<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD; **ECP1** - end of competition period; **PPS** – Prior preseason; **MPS** – Middle preparation period; **EPS/BCP** - end preseason period/beginning competition period; **MCP** –Middle competition period; **ECP2** – end of competition period; **ND** - not defined; **IFT** – intermittent field test (Bangsbo and Lindquist 1992); **YYIR2** – Yo-Yo intermittent recovery test level 2; **YYIR1** - Yo-Yo intermittent recovery test level 1; **YYIE2** - Yo-Yo intermittent endurance test level 2; ≈ - approximately; * sig. dif from ECP1; † sig. dif from PPS; ‡ sig. dif from EPS/BCP;
<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country</th>
<th>n (years)</th>
<th>Test(cm)</th>
<th>ECP1</th>
<th>PPS</th>
<th>EPS/BCP</th>
<th>MID</th>
<th>ECP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clark et al. (2008)</td>
<td>Elite/England</td>
<td>42(25±3.5)</td>
<td>CMJ</td>
<td>≈ 48</td>
<td>≈ 49</td>
<td>≈ 52.5</td>
<td>≈ 51</td>
<td>≈ 49.5</td>
</tr>
<tr>
<td>Silvestre et al. (2006)</td>
<td>NCAAI/USA</td>
<td>25(19.9±1.3)</td>
<td>CMJ</td>
<td>61.9±7.1</td>
<td>63.3±8.0</td>
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</tr>
<tr>
<td>Mercer et al. (1997)</td>
<td>Professional</td>
<td>15(24.7±3.8)</td>
<td>CMJ</td>
<td>44.1±7.4</td>
<td>44.8±6.8</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Casajus (2001)</td>
<td>Elite/Spain</td>
<td>15(25.8±3.2)</td>
<td>CMJ</td>
<td>41.4±2.7</td>
<td>40.8±2.7</td>
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</tr>
<tr>
<td>Malliou et al. (2003)</td>
<td>Professional/Greek</td>
<td>18(27.2±3.2)</td>
<td>CMJ</td>
<td>39.2±3.7</td>
<td>39.1±4.3</td>
<td>38.5±4.4</td>
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<td></td>
</tr>
<tr>
<td>Silva et al. (2011)</td>
<td>Elite/Portugal</td>
<td>18(25.7±4.6)</td>
<td>CMJ</td>
<td>42.4±4.04</td>
<td>44.8±4.5†</td>
<td>42.8±4.4†</td>
<td>42.23±4.38†</td>
<td></td>
</tr>
<tr>
<td>Caldwell and Peters (2009)</td>
<td>Semi-professional/England</td>
<td>13(24±4.4)</td>
<td>CMJWAS</td>
<td>57±4</td>
<td>54±3.2</td>
<td>57±3.4HT</td>
<td>57±3.4HT</td>
<td></td>
</tr>
<tr>
<td>Mercer et al. (1997)</td>
<td>Professional</td>
<td>15(24.7±3.8)</td>
<td>CMJWAS</td>
<td>54.6±8.5</td>
<td>52.7±8.2†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casajus (2001)</td>
<td>Elite/Spain</td>
<td>15(25.8±3.2)</td>
<td>SJ</td>
<td>39.0±3.3</td>
<td>39.2±3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malliou et al. (2003)</td>
<td>Professional/Greek</td>
<td>19(27.2±3.2)</td>
<td>SJ</td>
<td>37.8±3.7</td>
<td>38.2±4.2</td>
<td>37.4±4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas and Reilly (1979)</td>
<td>Professional/England</td>
<td>31 (r: 18-29)</td>
<td>SJ</td>
<td>55.6±7.1</td>
<td>54±6.3</td>
<td>54.3±7</td>
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</tr>
<tr>
<td>Casajus (2001)</td>
<td>Elite/Spain</td>
<td>15(25.8±3.2)</td>
<td>SJWAS</td>
<td>47.8±2.9</td>
<td>46.7±2.8</td>
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</tr>
<tr>
<td>Aziz et al. (2005)</td>
<td>Elite/Singapore</td>
<td>41(25.7±3.9)</td>
<td>SJWAS</td>
<td>55±5</td>
<td>59±5†</td>
<td>62±6†</td>
<td>62±6†</td>
<td></td>
</tr>
<tr>
<td>Casajus (2001)</td>
<td>Elite/Spain</td>
<td>15(25.8±3.2)</td>
<td>CMJ</td>
<td>26.1±3.7</td>
<td>27.8±2.9</td>
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<td></td>
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</tr>
<tr>
<td>Clark et al. (2008)</td>
<td>Elite/England</td>
<td>42(25±3.5)</td>
<td>CMJ20 s (mean)</td>
<td>≈ 42</td>
<td>≈ 43</td>
<td>≈ 48.5</td>
<td>≈ 49</td>
<td>≈ 46</td>
</tr>
<tr>
<td>Thomas and Reilly (1979)</td>
<td>Professional/England</td>
<td>31 (r: 18-29)</td>
<td>SBJ</td>
<td>211±17.4</td>
<td>225±11.6†</td>
<td>225±15.2†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD; ECP1 = end of competition period; PPS = Prior pre-season; EPS/BCP = end pre-season period/beginning competition period; MCP = Middle competition period; ECP2 = end of competition period 2; r = ranging; ≈ = approximately; CMJ = countermovement jump; CMJWAS = countermovement jump with arm swing; SJ = squat jump; SJWAS = Squat jump with arm swing; CMJ15-20s = countermovement jump performed during 15-20 seconds; SBJ = standing broad jump; * sig. dif. from ECP1; † sig. dif. from PPS; ‡ sig. dif. from EPS/BCP;
Table 7. Summary of studies examining changes in sprint ability exercises through different periods of a soccer season

<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country</th>
<th>N (years)</th>
<th>Test</th>
<th>Training Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aziz et al. (2005)</td>
<td>Elite/Singapore</td>
<td>41(25.7±3.9)</td>
<td>T5(s)</td>
<td>1.04±0.06</td>
</tr>
<tr>
<td>Silva et al. (2011)</td>
<td>Elite/Portugal</td>
<td>18 (25.7±4.6)</td>
<td>T5(s)</td>
<td>1.03±0.06</td>
</tr>
<tr>
<td>Silvestre et al. (2006)</td>
<td>NCAAI/USA</td>
<td>25(19.9±1.3)</td>
<td>T9.1(s)</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>Magal et al. (2011)</td>
<td>NCAAI/USA</td>
<td>12(20±0.9)</td>
<td>T10(s)</td>
<td>2.03±0.15</td>
</tr>
<tr>
<td>Caldwell and Peters (2009)</td>
<td>Semi-professional/England</td>
<td>13(24±4.4)</td>
<td>T15(s)</td>
<td>2.43±0.9</td>
</tr>
<tr>
<td>Aziz et al. (2005)</td>
<td>Elite/Singapore</td>
<td>41(25.7±3.9)</td>
<td>T20(s)</td>
<td>3.04±0.16</td>
</tr>
<tr>
<td>Magal et al. (2009)</td>
<td>NCAAI/USA</td>
<td>12(20±0.9)</td>
<td>T30(s)</td>
<td>4.72±0.26</td>
</tr>
<tr>
<td>Silva, et al. (2011)</td>
<td>Elite/Portugal</td>
<td>18 (25.7±4.6)</td>
<td>T30(s)</td>
<td>4.16±0.18</td>
</tr>
<tr>
<td>Zoppi et al. (2006)</td>
<td>Professional/Brazil</td>
<td>5(18.3±0.5)</td>
<td>30 m (m.s)</td>
<td>7.1±1.0</td>
</tr>
<tr>
<td>Silvestre et al. (2006)</td>
<td>NCAAI/USA</td>
<td>25(19.9±1.3)</td>
<td>T36.5(s)</td>
<td>5±0.2</td>
</tr>
<tr>
<td>Magal et al. (2009)</td>
<td>NCAAI/USA</td>
<td>12(20±0.9)</td>
<td>T40(s)</td>
<td>5.86±0.52</td>
</tr>
<tr>
<td>Ostojic (2003)</td>
<td>Elite/Serbia</td>
<td>30(23.5±3.1)</td>
<td>T50(s)</td>
<td>7.5±0.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD; ECP1 – end of competition period; PPS – Prior pre-season; EPS/BCP – end pre-season period/beginning competition period; MCP – Middle competition period; ECP2 – end of competition period 2; T5-50 – 5 to 50 meters sprint time; *sig. dif from ECP1; † sig. dif from PPS1; ‡ sig. dif from EPS/BCP1; § sig. dif from MCP1; ‡ sig. dif from ECP2;
### Table 8. Summary of studies examining change of direction speed through different periods of a soccer season

<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country</th>
<th>n (years)</th>
<th>COD Test</th>
<th>Training Period</th>
<th>ECP1</th>
<th>PPS</th>
<th>EPS/BCP</th>
<th>MID</th>
<th>ECP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercer et al. (1997)</td>
<td>Amateurs</td>
<td>15(24.7±3.8)</td>
<td>Illinois Agility run</td>
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<td>16.5±0.4</td>
<td>16±0.4</td>
<td>✷</td>
<td></td>
<td></td>
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<tr>
<td>Caldwell and Peters</td>
<td>Semiprofessional/England</td>
<td>13(24±4.4)</td>
<td>Illinois Agility run</td>
<td></td>
<td>14.73±0.37</td>
<td>14.97±0.38*</td>
<td>14.76±0.38Ω</td>
<td>14.68±0.34Ω</td>
<td>14.63±0.37Ω</td>
</tr>
<tr>
<td>Silva et al. (2011)</td>
<td>Elite/Portugal</td>
<td>18 (25.7±4.6)</td>
<td>T-test Agility run</td>
<td></td>
<td>8.71±0.33</td>
<td>8.74±0.36</td>
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<td>8.41±0.27Ω</td>
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<tr>
<td>Magal et al. (2009)</td>
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<td>12(20±0.9)</td>
<td>pro-agility test (5-10-5)</td>
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<td>4.96±0.19</td>
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<td></td>
<td></td>
<td>4.80±0.33</td>
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</tbody>
</table>

Values are mean ± SD; **ECP1** – end of competition period; **PPS** – Prior pre-season; **EPS/BCP** - end pre-season period/beginning competition period; **MCP** – Middle competition period; **ECP2** – end of competition period 2; ✷ sig. dif from ECP1; Ω sig. dif from PPS; † sig. dif from EPS/BCP1
<table>
<thead>
<tr>
<th>Study</th>
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<th>n(years)</th>
<th>S Test</th>
<th>Training Period</th>
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</thead>
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<tr>
<td>Mercer et al.</td>
<td>Professional/</td>
<td>15(24.7±3.8)</td>
<td>T IS (1.05rad/s:Nm)</td>
<td>KER 226±28 223±23</td>
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<td>(1997)</td>
<td>ND</td>
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<td>KEL 222±21 218±32</td>
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<td>KFL 145±35 139±32</td>
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<td></td>
<td>H/Qright 61±6 62±10</td>
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<td></td>
<td>H/Qleft 65±13 63±09</td>
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<td></td>
<td></td>
<td>BDext 2±09 3±10</td>
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<td></td>
<td></td>
<td></td>
<td>BDflex 4±28 0±18</td>
</tr>
<tr>
<td></td>
<td>Elite/Portugal</td>
<td>18 (25.7±4.6)</td>
<td>IS (90°/s; Nm)</td>
<td>KED 238.9±39.8 240.7±51.4 239.1±45 243.9±52.8</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>KEND 241.7±39 241.3±47 241.8±40 243.8±53.3</td>
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<td>KFD 131.22±2 131.58±21.9 135.36±25.1 138.03±24.2</td>
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<td>KFND 128.9±19 128.561±23.4 133.01±20.1 133.86±23.6</td>
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<td>H/QD 54.91±6 54.65±5 56.6±8.5 57.46±7.3</td>
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<td>H/QND 53.4±4.9 53.2±5.2 55.02±5.4 54.9±6.1</td>
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<td>BDext 5.76±5.36 5.57±5.42 5.70±4.95 8.54±4.91</td>
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<td>BDflex 6.64±6.3 5.59±4.7 7.66±5.4 7.6±5.8</td>
</tr>
<tr>
<td>Malliou et al.</td>
<td>Professional/</td>
<td>19(27.2±3.2)</td>
<td>T IS(60°/s;Nm)</td>
<td>KER 233.8±26.9 234±30 229±22</td>
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<td>(2003)</td>
<td>Greek</td>
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<td></td>
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<td>KEL 230.9±25 227±28 222±33</td>
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<td></td>
<td>IS(180°/s;Nm)</td>
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<td></td>
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<td>KEL 162±16 155.9±18 157.8±15</td>
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<td>n(years)</td>
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<td>Test</td>
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<tr>
<td>Kraemer et al. (2004)</td>
<td>NCAAI/USA</td>
<td>11(19.91±30.9)</td>
<td>S</td>
<td>IS(1.05rad/s;Nm)</td>
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<tr>
<td></td>
<td></td>
<td>11</td>
<td>S</td>
<td>KF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14(18.71±0.9)</td>
<td>NS</td>
<td>KE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14(18.71±0.9)</td>
<td>NS</td>
<td>KF</td>
</tr>
</tbody>
</table>

Values are mean ± SD; S - sample; ECP1 – end of competition period; PPS – Prior pre-season; EPS/BCP - end pre-season period/beginning competition period; MCP –Middle competition period; ECP2 – end of competition period 2; T- team; S- starter players; NS- non-starter players; IS – isokinetic dynamometer; KERight- Peak Torque Knee extensor right leg; KELeft - Peak Torque Knee extensor left leg; KED- Peak Torque Knee extensor dominant leg; KEND- Peak Torque Knee extensor non-dominant leg; KFRight- Peak Torque Knee flexor right leg; KFLeft - Peak Torque Knee flexor left leg; KFD - Peak Torque Knee flexor dominant leg; KFND - Peak Torque Knee flexor non-dominant leg; H/Qright- Hamstrings quadriceps strength ratio right leg; H/Qleft- Hamstrings quadriceps strength ratio left leg; BDext – bilateral strength different in extension; BDflex – bilateral strength different in flexion; RHG – right hand grip strength; LHG – Left hand grip strength; † sig. dif from PPS; † sig. different from EPS/BCP; ** sig. dif. Between groups
TITLE: Strength and muscle power training in soccer

Authors:

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Contents

Abstract

1. Strength and muscle power

2. Strength and muscle power training in soccer

2.1 Physiological adaptations

2.2 Adaptations in sport specific efforts
2.2.1 Sprint ability
2.2.2 Jump ability
2.2.3 Change of direction ability
2.2.4 Sport specific skills

2.3 Comparing different modes of intervention

3. Strength and endurance training

3.1 Strength and endurance training in soccer

4. References
Abstract

Data regarding the activity pattern of soccer players and soccer’s physiological demands have shown that high-level physical performance requires a well-developed neuromuscular system in order to cope with several essential power-based efforts during games and trainings, i.e., jumping, accelerations, decelerations and changes of direction while sprinting. Neuromuscular improvements may be of relevant importance to increase players’ performance during the game and particularly during the most intense periods. Research with different athletic populations have shown that improvements at the neuromuscular level not only result in an enhanced neuromuscular performance as well as in an optimized short and long term endurance capacity. Although research is scarce, coaches, medical staffs and players have contributed to an increase in the number of investigations in the field of strength and muscle power interventions in soccer. This progress in scientific knowledge has generally shown that distinct strength and muscle power training programs can induce significant performance improvements in a wide range of non-specific and soccer specific-neuromuscular parameters. Moreover, it has been shown that the training of neuromuscular function and soccer specific endurance can be combined and advised since it can promote, among other factors, improvements in non-specific and soccer specific-endurance and neuromuscular parameters.

1. Strength and muscle power training

Strength training has become an integral component of the physical preparation for enhancement of sports performance (Young, 2006). While strength is defined as the integrated result of several force-producing muscles performing maximally, either isometrically or dynamically during a single voluntary effort of a defined task (Hoff & Helgerud, 2004), power is a product of force and the inverse of time, i.e., the ability to produce as much force as possible in the
shortest possible time (Hoff & Helgerud, 2004). According with Schmidtleicher (1992), maximal muscle strength and power are not distinct entities, as power performance is influenced by training methods that maximize both strength and stretch-shorten cycle activity (SSC).

Scientific literature examining the mechanisms underlying strength adaptations effects is marked largely by references to the role of muscle mass (Gabriel et al., 2006). The strength and performance improvements as result of hypertrophy programs are usually related to an increase in cross-sectional area of the muscle. However, increases in muscular strength without noticeable hypertrophy have served as the first line of evidence for the neural involvement in acquisition of muscular strength (Gabriel et al., 2006). As such, training programs targeting neural adaptations focus the increase in the capacity of the neuromuscular system to produce force and improve performance by the promotion of physiological adaptations related to increases in motor unit recruitment and in rate coding (Gabriel et al., 2006). Although both muscle hypertrophy and neural adaptations are the basis for development of muscle strength (Toigo & Boutellier, 2006), their respective mechanisms of adaptation in the neuromuscular system are distinct (Hakkinen et al., 2003). In fact “more strength,” i.e., the adaptational effect, did not necessarily result of increased muscle mass, since several distinct adaptations can lead to the same effect (Toigo & Boutellier, 2006). Moreover, the trainable effects of explosive-type strength training causing enhanced explosive force production, result primarily by the increased neural activation of the trained muscles, i.e., probably related to increases of number and/or firing frequencies of active motor units as well as of changes in the recruitment pattern of the motor units (Hakkinen et al., 1985). Also, gains in explosive muscle strength after heavy-resistance strength training programs have been attributed to enhanced contractile rate of force development, impulse, and efferent neuromuscular drive of human skeletal muscle (Aagaard et al., 2002).

One of the central goals of strength training in a high competition sport, in this specific case in soccer, is related to the capacity of improving players’ specific-relevant athletic activities that are inherent to their sport. With this concern,
methodologies that consist of a combination of weight training, plyometric training, and sport-specific force-based actions, have being used. This type of methodological interventions have been applied with the belief that these modes of strength training allow optimal power development and transfer of training adaptations to athletic activity (Ebben & Watts, 1998). In fact, soccer entails a varied range of actions, involving breaking and propulsive forces and distinct contraction modes and velocities that require all force-velocity potential of neuromuscular system. One possible advantage of the adoption of training modes that comprise stretch-shorten cycle activity in soccer, among other factors, is related to adaptation to the eccentric component of the SSC actions present in a wide range of soccer-explosive specific efforts. This type of contraction is usually associated to muscle damage which may delay recovery of the condition player’s capacity to successfully perform during periods of heavy match commitments (Ascensao et al., 2008; Ebbeling & Clarkson, 1989; Ispirlidis et al., 2008; Magalhaes et al., 2010; Reilly et al., 2008; Silva et al., 2011b; Twist & Eston, 2005). This would imply that muscles adapt to training regimes that engage them in stretch shortening cycles and will be less impaired with repeated exposure (Reilly et al., 2008).

2. Strength and muscle power training in soccer

The effects of different modes of strength training in soccer players has been matter of increased research in the last decade. The effects of combined high-intensity strength training (S) and plyometric training (S+P; 4 sets x 5RM squats + 10-min rest + 4 stets x 8 rep CMJ) (Ronnestad et al., 2008), combined S and sprint training (S+SP; e.g. 4 sets x 5RM squats + 10-min rest + 4 x 40m sprints)(Kotzamanidis et al., 2005) and complex strength training (CT) programs (Maio Alves et al., 2010; Manolopoulos et al., 2006; Mujika et al., 2009) have been investigated. Although some similarity between modes of strength and muscle power training exist (combined weight and plyometric or sprint training vs. complex training), there are also important differences. In this review, as early defined by Ebben and Watts (1998), complex training refers to training
protocols comprising the alternation of biomechanically comparable exercises in the same workout and sport specific drills (e.g., 6 rep. of calf extension exercise at 90% 1RM + 5 sec. rest + 8 vertical jumps or /+ 5sec. rest + 3 high ball header’s). Moreover, the effect of CT involving explosive exercises (plyometric) and soccer specific skills (soccer kicking), with or without resistive loads (e.g., kicking against resistance imposed by rubber bands) in biomechanical, neuromuscular and in performance parameters, has been analysed (Manolopoulos et al., 2006). These methods have been used because of a belief that favours specific power development, due to continued neural and morphological adaptations typically associated with advanced training (Ebben & Watts, 1998). Also, the effect of other strength training methodologies, such as electrostimulation training (EMS), in soccer players’ physical fitness has been analyzed (Billot et al., 2010).

The short-term impact of different strength and/or muscle power training modes, H vs. S (Bogdanis et al., 2009), S vs. S+P (Ronnestad et al., 2008), S vs. S+SP (Kotzamanidis et al., 2005), CT vs. SP (Mujika et al., 2009) has also been matter of research. Moreover, the effects of plyometric training performed on different ground surfaces (grass vs. sand), and of different plyometric training techniques (Drop jumps vs. CMJ) has been also investigated (Impellizzeri et al., 2008; Sedano et al., 2011; Thomas et al., 2009).

These training programs primarily have analysed the effects of different short-term protocols, implemented in distinct season time-lines and with different weekly volumes. In this matter, research has been developed in preseason (Bogdanis et al., 2009; Impellizzeri et al., 2008; Maio Alves et al., 2010; Ronnestad et al., 2008) and in-season periods (Billot et al., 2010; Chelly et al., 2009; Gorostiaga et al., 2004; Mujika et al., 2009; Sedano et al., 2011; Thomas et al., 2009). Also, training programs examining soccer players’ functional and muscle structure (e.g., myosin heavy chain composition) adaptations to high intensity strength training performed in the isokinetic contraction mode have also been implemented in the off-season period (Andersen et al., 1994). The length of the muscle strength and/or power training program ranged from 4-wks (Impellizzeri et al., 2008), 5wks (Billot et al., 2010), 6-wks (Bogdanis et al.,
2009; Maio Alves et al., 2010; Thomas et al., 2009), 7-wks (Mujika et al., 2009; Ronnestad et al., 2008), 8-wks (Chelly et al., 2009), 9-wks (Kotzamanidis et al., 2005), 10 wks (Manolopoulos et al., 2006; Sedano et al., 2011), 11-wks (Gorostiaga et al., 2004) to 12-wks of intervention (Aagaard et al., 1993; Trolle et al., 1993). These studies frequently examined the training-induced performance effects of 2 (Chelly et al., 2009; Gorostiaga et al., 2004; Kotzamanidis et al., 2005; Maio Alves et al., 2010; Ronnestad et al., 2008; Ronnestad et al., 2011; Thomas et al., 2009) to 3 sessions (Billot et al., 2010; Bogdanis et al., 2009; Impellizzeri et al., 2008; Manolopoulos et al., 2006; Sedano et al., 2011) of strength and muscle power training per week. Nevertheless, the short-term effect of a lower weekly volume programs (1 session per week) (Maio Alves et al., 2010; Mujika et al., 2009; Ronnestad et al., 2011) and the training-induced adaptations of different weekly training volumes (1 vs. 2 sessions per week) has been analyzed (Maio Alves et al., 2010). In addition, the effects of the training frequency (one session per week vs. one session every second week) on the maintenance of the strength and performance pre-season gains throughout the in-season period has also been examined (Ronnestad et al., 2011).

2.1 Physiological adaptations

Different strength-related methodological interventions are able to induce adaptations in distinct qualities of players’ neuromuscular systems (table1). Enhanced dynamic (Aagaard et al., 1993; Andersen et al., 1994; Billot et al., 2010; Bogdanis et al., 2009; Chelly et al., 2009; Kotzamanidis et al., 2005; Ronnestad et al., 2008; Ronnestad et al., 2011; Trolle et al., 1993) and static maximum force production (Aagaard et al., 1993; Billot et al., 2010; Trolle et al., 1993) and as well as increased muscle power outputs, during different physical attributes, have been reported (Aagaard et al., 1993; Andersen et al., 1994; Bogdanis et al., 2009; Chelly et al., 2009; Gorostiaga et al., 2004; Ronnestad et al., 2008; Ronnestad et al., 2011; Trolle et al., 1993). Increments in one repetition maximum (1RM) in isoinertial modes as the half-squat exercise
(Bogdanis et al., 2009; Bogdanis et al., 2011; Chelly et al., 2009; Kotzamanidis et al., 2005; Ronnestad et al., 2008; Ronnestad et al., 2011), in hamstrings leg curls, and in the one leg step up bench exercises (Kotzamanidis et al., 2005) were analysed. Moreover, increments in maximal isometric voluntary contraction (MIVC) in the leg press task after CT training (Manolopoulos et al., 2006) and in knee extension task after electrostimulation (Billot et al., 2010) and isokinetic training (Aagaard et al., 1993; Trolle et al., 1993) were also examined. Improvements in 1RM per lean leg volume (LLV) 1RM/LLV after high-intensity (S) and hypertrophy (H) strength training (Bogdanis et al., 2009) and in the relative force (maximum force divided per body weight) after CT have been reported (Manolopoulos et al., 2006).

According to Harris et al., (2007), training studies should use a specific isoenertial loading scheme, and test protocols should assess performance over the force-velocity continuum to gain a greater understanding of the effect of load on muscular function. Moreover, certain neuromuscular-related qualities such as impulse, rate of force development, and explosive strength can predict athletic performance better and hence its development should be targeted (Harris et al., 2007). One such muscle strength and power training-induced adaptation is an observed increase (moving up) in the force-velocity relationships and mechanical parabolic curves between power and velocity after high-intensity training programs, both in the type of isoinertial (Chelly et al., 2009) and isokinetic exercises (Aagaard et al., 1993). However, contradictory results were observed when (Bogdanis et al., 2009) applying the same methodology for analysis alterations in force-velocity relationships (cycling force velocity tests in a cycle ergometer against resistive loads ranging from 2% to 10% of body mass), after H or S training. In fact, only maximal force increased, while maximal cycling speed corresponding to zero load ($V_0$) and the rate at which the highest value of power is achieved ($V_{opt}$) remained unchanged (Bogdanis et al., 2009). Nevertheless, Ronnestad et al., (2008) and Gorostiaga et al., (2004) observe increases in force-velocity curve after high-intensity strength training and/or explosive-type strength training, respectively. In Ronnestad et al., (2008), the results regarding the squat jump tests with loads
of 20, 35 and 50 Kg were used for analysis of the effect of S and S+P programs on force-velocity relationships. When the researchers pooled the groups to increase the statistical power, increases in all measures of peak power were observed. It seems that high-intensity strength training significantly increases performance in professional soccer players at both the high force end (observed increases in 1RM and sprint acceleration) and the high velocity end (improvements analyzed in peak sprint velocity and the 4 bounce tests (4BT)), but only as long as the subjects perform concurrent plyometric and explosive exercises during their soccer sessions (Ronnestad et al., 2008). However, Gorostiaga et al., (2004) using the CMJ performance with loads of 20, 30, 40, 50, 60 and 70 kg, observed that increases were primarily observed in the low-force portions of the load vertical jumping weight curve (CMJ, CMJ$_{20kg}$, CMJ$_{30kg}$) after low-frequency, low-intensity explosive-type strength training. The analysis of load-vertical jumping height relationships have been shown to characterize the force-velocity relationship in CMJ jumps (Viitasalo, 1985).

Improvements in explosive strength and in rate of force development evaluated during isometric leg press after CT were observed (Manolopoulos et al., 2006). Also, after soccer-specific strength and skill training (soccer kick) interventions, an increase in electromyography (EMG) activity of certain muscles (vastus medialis) involved in the task were analyzed (Manolopoulos et al., 2006).

### 2.2 Adaptations in sport specific efforts

In soccer, as in other sports, the effectiveness of a strength/power training program aims to translate into improvement in the sport-specific actions that require good levels of muscle strength and power. In fact, even though low to medium intensity running are the predominant activities during the match, power-based efforts such as sprints, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power of the neuromuscular system, are essential skills to successfully perform in soccer (Cometti et al., 2001). In fact, a high degree of stress is set on soccer players neuromuscular systems in order to cope with this essential power-based efforts
required during training and competition. In this matter, different strength-related training interventions have shown to work as a powerful training stimulus in the promotion of adaptations through a large range of explosive sport specific skills (e.g., jump, COD; table 1) (Bogdanis et al., 2009; Chelly et al., 2009; Gorostiaga et al., 2004; Impellizzeri et al., 2008; Kotzamanidis et al., 2005; Maio Alves et al., 2010; Mujika et al., 2009; Ronnestad et al., 2008; Ronnestad et al., 2011; Sedano et al., 2011) and explosive soccer-specific skills (soccer kick) (Billot et al., 2010; Sedano et al., 2011). These improvements were confirmed in observations of a positive transfer into the acceleration, maximum speed, COD, diverse forms of jump ability exercises, and in the eccentric component of the stretch shortening cycle of players. As an example, in Ronnestad et al., (2008) although the performance of high-intensity strength (S) and S plus Plyometric (P) training did not reveal improvements in certain functional parameters, when the groups were pooled the results revealed that from a large diversity of functional parameters, only CMJ performance did not improve after training.

### 2.3.1 Sprint Ability

Regarding adaptations in sprint qualities (e.g., acceleration and maximal speed, table 1), improvements in 5-m (Chelly et al., 2009; Maio Alves et al., 2010), 10-m (Bogdanis et al., 2009; Impellizzeri et al., 2008; Manolopoulos et al., 2006; Sedano et al., 2011), 15-m (Maio Alves et al., 2010; Mujika et al., 2009), 20-m (Impellizzeri et al., 2008), 30-m (Kotzamanidis et al., 2005), 40-m (Bogdanis et al., 2009; Ronnestad et al., 2008; Ronnestad et al., 2011) and splits time from 30-m to 40-m (Ronnestad et al., 2008) were reported after different training interventions. Others did not observe improvements in 5-m (Gorostiaga et al., 2004; Thomas et al., 2009), 10-m (Billot et al., 2010; Thomas et al., 2009), 15-m (Gorostiaga et al., 2004; Thomas et al., 2009), 20-m (Thomas et al., 2009) and 30-m sprint performance (Kotzamanidis et al., 2005). The observed change differences in sprint performance between studies may be related with a wide range of circumstances as player training status, players’ background and/or training modes. In the training program applied in the study
of Thomas et al., (2009) players performed solely one type of plyometric
exercise, while in the study of Billot et al., (2010) players were just submitted to
EMS training, which has an evident lower level of specificity. As such, these
previous limitations existent in the interventional protocols may have
contributed, at least in part, to a lack of transfer of training adaptations to
dynamic and complex activities, that require the coordination and force
production of different body muscles as is the case of sprint performance.

2.3.2. Jump ability

Research focusing on the training-effects of different strength-related
interventions, reported an enhancement in jump performance (table 1).
Improvements in squat jump (SJ) (Chelly et al., 2009; Impellizzeri et al., 2008;
Kotzamanidis et al., 2005; Maio Alves et al., 2010; Ronnestad et al., 2011), 4-
bounce test (4BT)(Ronnestad et al., 2008), 5- jump test (5-JT)(Chelly et al.,
2009), countermovement jump test (CMJ)(Bogdanis et al., 2009; Gorostiaga et
al., 2004; Impellizzeri et al., 2008; Kotzamanidis et al., 2005; Sedano et al.,
2011; Thomas et al., 2009), in CMJ with free arms (Sedano et al., 2011) and in
eccentric utilization ratio (CMJ/SJ)(Impellizzeri et al., 2008) have been
observed. Nevertheless, contradictory reports regarding improvements in SJ
after plyometric training (Sedano et al., 2011) and in CMJ performance after
high-intensity strength protocols (Chelly et al., 2009; Ronnestad et al., 2011)
can be found in literature. Also, no significant increments in CMJ were observed
after CT involving workouts with high (Maio Alves et al., 2010) or low loads
(Mujika et al., 2009) and in drop jumps from a 40 cm height (DJ_{40})(Kotzamanidis
et al., 2005) after S and S+Sp.

2.3.3 Change of direction speed (COD)

According to the literature, it is difficult to discern which force/power qualities
(e.g., horizontal and lateral) and technique factors influence event- or sport-
specific COD ability (Brughelli et al., 2008). Regarding training-induced
adaptations in the COD speed, Bogdanis et al., (2009) observed that both hypertrophy (H) and high-intensity (S) modes of strength training were effective in increasing COD evaluated through different COD tests (Table 1). Nevertheless, the improvements in COD that were evaluated by the 505 agility test performance after different plyometric training techniques (Thomas et al., 2009) were not found after CT (Maio Alves et al., 2010). Also, in a study of Mujika et al., (2009) where players perform CT, no improvements in COD evaluated by agility 15-m test were observed. The differences in results can be related with diverse factors, such as the period of the season when the intervention was carried out, the structure of the training intervention, soccer players’ previous training status and as already mentioned, the players’ training background. In fact, as an example, the study of Maio Alves et al., (2010) was implemented in preseason and the research of Thomas et al., (2009) was carried out during in season. Consequently, the accumulated effect of COD actions performed during trainings and games may influence the results. In this matter, recent results observed that COD has a tendency to improve towards the soccer competition period in professional players (Silva et al., 2011b). Also, although from the same age groups, the differences in players’ competitive level (elite vs. semi-professional) between former studies should not be ignored. Moreover, the fact that Mujika et al., (2009) did not examine COD improvements after in-season CT may be related to only six sessions of CT being performed in a seven week period. This fact may suggest that higher training volumes may be necessary to induce adaptations in COD.

### 2.3.4 Sport specific skills

One of the most important indicators of a successful soccer kick is the speed of the ball. Studies involving amateur soccer players observed that CT (Manolopoulos et al., 2006) and EMS training (Billot et al., 2010) resulted in increases in ball speed with (Billot et al., 2010; Manolopoulos et al., 2006) and without run up (table 1) (Billot et al., 2010). In addition, elite under 19 players performing plyometric training improve ball speed with the dominant and non-
dominant leg (Sedano et al., 2011). Nevertheless, other studies involving elite soccer players, performing different modes of strength training (high intensity or low intensity isokinetic strength training or functional training-loaded kicking movements without the ball) did not result in significant improvements in ball speed (Aagaard et al., 1993; Trolle et al., 1993). In fact, although some groups increased certain strength parameters, these did not result in a positive transference to consecutive gains in ball speed.

2.4 Comparing different modes of muscle strength and power interventions

Recent research has examined physiological and performance alterations resulting from distinct modes of strength training in players of the same standard (table 1). Bogdanis et al., (2009) analyzed the training effects of H and S programs on speed, muscle mass, force and power production. Data from this research (Bogdanis et al., 2009) have shown that significant increases in muscle mass of the lower limbs only occurred in H group. One the other hand, S type of strength training was more effective in increase maximum strength by lean leg volume and the functional capacity of soccer players in sprint and COD tests (Bogdanis et al., 2009). These improvements were not associated with increases in muscle volume and were attributed to neural adaptations, i.e., changes in the pattern of motor unit recruitment and increase rate coding (Bogdanis et al., 2009; Gabriel et al., 2006). In another study, Bogdanis et al., (2011) examined alterations in the total work over a repeated cycle ergometer sprint test (10 x 6-sec sprint with 24-sec passive recovery) after H and S training. Data reveal that even though both groups improved test performance, the S group had a significantly greater improvement in total work during the second half (sprint 6-10; 8.9 ± 2.6%) compared with the first half of the sprint test (sprint1-5; 3.2 ± 1.7%). The distinct results observed after H and S training may highlight that S training results in an high capability to sustain fatigue during a repeated sprint test (Bogdanis et al., 2011). In addition, mean power output (MPO) expressed per lean leg volume (LLV) was better maintained
during the last 6 sprints post-training only in the S group and there was no change in MPO per LLV in the H-group over the 10 sprints (Bogdanis et al., 2011). According to these results, the researchers concluded that training protocols targeting neural adaptations may be superior to hypertrophy protocols, not only due to increased strength without changes in muscle mass, but above all because it appears to promote higher adaptations in soccer-specific field tests (Bogdanis et al., 2009; Bogdanis et al., 2011) and increase fatigue resistance during repeated sprint exercise (Bogdanis et al., 2011).

The analysis of the impact of high vs. low intensity isokinetic strength vs. functional strength showed that the players who performed a high load, low angular velocity program had a higher improvement in maximal isometric and isokinetic strength and also in peak power outputs measured at different knee angles and angular velocities (Aagaard et al., 1993; Trolle et al., 1993). Even though the increases in dynamic muscle strength were generally observed at the specific velocities used in the training programs, the high load/low velocity group also improved muscle force and power at high knee extension velocities (Aagaard et al., 1993; Trolle et al., 1993). According the researchers, the greater training adaptations in a wide range of velocities, examined in the high load/low velocity strength training program, may be hypothetically related to changes in neural and morphological factors (e.g., increases in rate of force development, increases in muscle mass and/or increases in angle of fiber pennation).

The training-induced adaptations of high-intensity strength training (S) vs. S plus plyometric (P) training programs (S+P)(Ronnestad et al., 2008) and S vs. S plus sprint training (SP) programs (S+SP)(Kotzamanidis et al., 2005) were also examined. In the study of Ronnestad et al., (2008) although no significant differences between groups were observed, the group of players who performed S+P improved in a higher range of functional parameters. Kotzamanidis et al.,(2005) observed that only soccer players who performed sprint training in addition to the strength training (S+SP) improved jump and sprint performance. As such, it seems that combining heavy and light training schemes may be an effective mode for improving muscular function and may be
particularly useful where force application is required in a range of functional tasks (Harris et al., 2007). Moreover, data suggest that combining resistance- and speed-training programs and resistance and plyometric training programs in the same session seems to be more effective than the resistance training program per se. Thus, strength training designs should focus both the intramuscular and intermuscular aspects of athletic performance. In fact, hypertrophy and general power exercises can enhance sports performance, but optimal transfer from training also requires a specific exercise programs (Young, 2006). Also, as observed by Mujika et al., (2009) there is evidence that a low volume of combined forms of strength/power training is more effective in improving sprint performance (15-m sprint time) than the sole performance of lower volumes of sprint training.

As already mentioned, soccer players are usually involved in weekly matches of the national leagues and very often in international commitments, limiting the time for fitness training. Maio Alves et al.,(2010) examined the effect of the performance of higher (2 sessions peer week) and lower (1 session peer week) weekly volume of CT in sprint, jump and COD ability of high-level junior players. The authors observed that there were not significant differences between groups, even though higher percentages of improvements were analyzed in the group subject to a lower weekly volume. Recently, Ronnestad et al., (2011) examined that the performance of one high-intensity strength training session per week during the first 12-wks of in-season period was enough to maintain the initial pre-season (two wks sessions throughout 10 wks ) gain in strength, jump and sprint performance. On the other hand, a lower in-season volume (one session each second week) only maintained the initial gains in jump performance, i.e., strength and sprint performance decrease (Ronnestad et al., 2011).

There is also evidence that adaptations resulting from explosive strength training in the mode of plyometric training can differ as result of the ground surface used during training (sand vs. grass) (Impellizzeri et al., 2008). In this matter, Impellizzeri et al.,(2008) observed that plyometric training performed on grass produced greater effects in CMJ and in the eccentric utilization ratio
CMJ/SJ than when performed on sand. On the other hand, a trend towards higher adaptations was observed in SJ in the group that performed the training program on sand. Additionally, plyometric training performed on sand surfaces was shown to induce lower levels of muscle soreness than when performed on grass. Soccer players are affected by fatigue during and after the game (Bangsbo et al., 2007; Krstrup et al., 2011; Mohr et al., 2005; Rampinini et al., 2011; Reilly et al., 2008). Moreover, the observed reduction in soccer players' ability to produce force towards the end of the match and in the periods subsequent to the match (Andersson et al., 2008; Ascensao et al., 2008; Greig, 2008; Ispirlidis et al., 2008; Krstrup et al., 2011; Magalhaes et al., 2010; Rampinini et al., 2011; Rebelo et al., 1998) follows the increase in some indirect markers of muscle damage (Andersson et al., 2008; Ascensao et al., 2011; Ascensao et al., 2008; Ispirlidis et al., 2008; Magalhaes et al., 2010) and are also characterized by longer periods of muscle soreness (Ascensao et al., 2011; Ascensao et al., 2008; Ispirlidis et al., 2008; Magalhaes et al., 2010; Thompson et al., 1999). In view of that, it may be expected that during periods in which players are submitted to high volumes and intensity of training (e.g., preseason) as well as in periods in which athletes who recover from injury and are trying to regain physical capacity, this kind of surface (sand) might be a good alternative for the execution of plyometric programs. In fact, in addition to improving neuromuscular capabilities, it has shown to produce lower levels of muscle soreness (Impellizzeri et al., 2008).

3. Strength and endurance training

There is consensus in the literature that soccer players' performance is intimately associated to the efficiency of different energy-related systems. In fact, the preparation for elite soccer competition requires that athletes develop high levels of physical fitness. With this aim, soccer players perform intense training programs with multiple goals of increasing strength, power, speed, speed endurance, agility, aerobic fitness and game skills. Nevertheless, contradictory results have been examined in different athletic populations concerning the effectiveness of the simultaneous stress of different
physiological systems during training cycles in order to increase neuromuscular and endurance-related parameters (Docherty & Sporer, 2000; Gorostiaga et al., 2004; Hakkinen et al., 2003; Kraemer et al., 1995; Lopez-Segovia et al., 2010; Nunez et al., 2008; Wong et al., 2010). According to Hakkinen et al., (2003) the physiological stimuli directed at skeletal muscles as a result of strength training and endurance training is divergent in nature. The compatibility between endurance training and strength training may depend upon the methodology applied for the improvement of strength and endurance (Docherty & Sporer, 2000; Hakkinen et al., 2003). Thus, the magnitude of the observed antagonism as result of combined strength and endurance training may differ based on the nature of the resistance training program and the target goal (Docherty & Sporer, 2000; Hakkinen et al., 2003). In fact, when the modes of strength and endurance training focus on the same location of adaptation (e.g., peripheral adaptations), the muscle is required to adapt in distinctly different physiological and anatomical ways which may reduce the adaptation of one of the systems (Docherty & Sporer, 2000). In addition, the volume and frequency of training may also influence the amount of incompatibility observed (Hakkinen et al., 2003). Some authors have proposed a hypothesis to explain the interference phenomenon or incapability of achieve/maintain higher levels of strength when strength and endurance are simultaneously trained. This hypothesis suggests that combined training leads to excessive fatigue, increment in catabolic environment, differences in motor unit recruitment patterns, possible shift in fiber type and conflict in the direction of adaptation pathways required to the muscle (Docherty & Sporer, 2000; Hakkinen et al., 2003; Kraemer et al., 1995).

3.1 Strength and endurance training in soccer

Data from different studies highlight that enhancements in neuromuscular function can be a determinant in improving short and long-term endurance capacity in different athletic populations (Aagaard & Andersen, 2010; Nummela et al., 2006; Paavolainen et al., 1999a; Paavolainen et al., 1999b; Saunders et al., 2006). Moreover, soccer players with greater neuromuscular capabilities
were shown to perform at high levels in certain game-related physical parameters (Silva et al., 2012).

The summary of changes in physiological and functional parameters resulting of performance of concurrent strength and endurance training are present in table 2. In a study of Wong et al., (2010) professional male soccer players performed 2 sessions per week of high-intensity resistance training and speed endurance throughout the 8-wks of preseason training. The training program resulted in significant improvements in endurance fitness, soccer-specific endurance (SSE) and soccer-specific-neuromuscular parameters (SSN). Helgerud et al.,(2002) applied concurrent high-intensity aerobic long-interval training and maximal strength training for neural adaptation during 8-wks of preseason. This type of training protocol resulted in improvements in VO$_{2}$max (8.9%), running economy (4.7) and 1RM during half squat strength exercise (52%). Also, this training intervention resulted in certain SSN adaptations assessed by 10-m sprint performance (0.06-s improvement) and vertical jumping height (3-cm increments). Another group of research (Bogdanis et al., 2011) examined the strength training-effects of hypertrophy (H) and high-intensity strength training (S) programs in professional soccer players during preseason where the weekly cycle also involved a considerable amount of interval training and small-sided games. These seem to be effective methodologies targeting endurance fitness and SSE development [for a review see (Hill-Haas et al., 2011; Iaia et al., 2009)]. The authors observed that both aerobic fitness parameters (e.g., VO$_{2}$max and MAS) and SSE evaluated by the YYIET and Hoff`s dribbling track test significant improved in both groups. Nevertheless, the question regarding the compatibility of endurance and strength is highlighted in the distinct results. In fact, only S improved RE significantly, and a trend to a better performance in the YYIET in the S than in H group was reported.

In the research of Nunez et al., (2008), semi-professional male soccer players performed both endurance and strength sessions in order to develop players’ physical fitness throughout the season (4 cycles of 12 weeks). In the endurance and strength block the ratio between the different physical fitness sessions were 2:1 (endurance block composed of 2 endurance training sessions and 1
strength training session and vice versa). This type of periodization was effective in improving both the endurance performance assessed by the Probst test as well as SSN evaluated through different jump exercises throughout the different season time-points. The results suggested that no conflict of adaptations occur when high volumes and low volumes of the different physical capacities were simultaneously combined during a soccer training cycle. Also, Lopez-Segovia et al., (2010) examined the training-induced adaptations in elite U-19 players during 4 months of the season. The training program consisted of 4 training sessions per week, in which 2 were focused on development of physical condition. In these 2 sessions the training content was focused on the improvement of players’ aerobic performance. Training was complemented with one or two specific-strength training session per week performed at the start of the training session. This type of periodization improved loaded CMJ and the speed of movement in full squats with loads ranging from 20kg to 40kg. Nevertheless, significant decrements in different sprint abilities were examined. According to the researchers, the high volume of aerobic work performed by the team might explain the lack of improvement in the former sprint variables. Regarding soccer players’ endurance fitness, it was observed that MAS increased by 3.2% after the intervention period (Lopez-Segovia et al., 2010). Gorostiaga et al., (2004) observed a similar pattern response in sub-maximal blood lactate values in both the explosive strength training plus soccer training group and in the group performing only soccer training. These findings suggest that concurrent explosive-type strength training and soccer training did not interfere with the development of endurance (Gorostiaga et al., 2004).

It seems logical to conclude that in soccer, the training of neuromuscular function and soccer specific endurance can be combined and advised since it can promote, among other factors, improvements in non-specific and soccer specific endurance and explosive performance. Nevertheless, compatibility seems to be greater when high-intensity or explosive strength is combined with high-intensity endurance training.
4. References


Patla (Eds.), *Biomechanics IX-A* (pp. 96-101): Human Kinetics, Champaign.


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<tr>
<th>Study</th>
<th>Country/ n (age)</th>
<th>Type of training</th>
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<th>P</th>
<th>Physiological adaptations</th>
<th>Performance changes</th>
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<tr>
<td>Bogdanis et al.,</td>
<td>Professional/</td>
<td>HIST: 8-12 upper and lower body exercises + 4 sets of half-squats at</td>
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<td>PS</td>
<td>↑17.3% 1RM†</td>
<td>↑=1.6% 10m sprint†</td>
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<td>Greek/ 9 (22.9 ± 1.1)</td>
<td>90%1RM / 5rep / 3min rest between sets / emphasis on maximal mobilization during concentric action</td>
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<td></td>
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<td>6wks</td>
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<td>↑≈1.6 % 10m sprint †</td>
<td>↑=10% CMJ†</td>
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<td>↑=1.2% T-TEST</td>
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<td>↔V_w(VER.min⁻¹); V_o(VER.min⁻¹); F_i(kg)</td>
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<td>↑≈1% 10m sprint †</td>
<td>↑=5.3%CMJ</td>
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<td>PS</td>
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<td>Ronnestad et al.,</td>
<td>Senior/ Norway/ 14</td>
<td><strong>HIST- PS:</strong> wk_{1,3} (1st session - 3 x 10RM + 2nd session - 3 x 6RM); wk_{6} (1st session - 3 x 8RM + 2nd session - 3 x 5RM); wk_{2,10} (1st session - 3 x 6RM + 2nd session 3 x 4RM); <strong>IN:</strong> wk_{1,2} (1 session wk - 3 x 4RM) half squats emphasizing maximal mobilization in concentric phase and slower eccentric phase</td>
<td>2x/wk/10wks</td>
<td>PS</td>
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<td>1x/wk/12wks</td>
<td>IN</td>
<td>↔ 1RM</td>
<td>↑ 3.3% SJ; ↔ CMJ;</td>
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<td>↑ 10 % SJ</td>
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<td><strong>HIST</strong> (half-squats): wk_{3,5} (3 sets x 6RM); wk_{3.5} (4 sets x 5RM); wk_{5.5} (5 sets x 4RM) emphasizing maximal mobilization in concentric phase and slower eccentric phase (i.e. ≈ 2sec);</td>
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<td>↑ 9.1% SJ</td>
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<td>↑ 9.5% PPO_{50}</td>
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<td>↔ CMJ; 10m sprint</td>
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**Table 1. Contd**
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<th>Study</th>
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<td>ND/Greece/12(17.0 + 1.1)</td>
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<td>2x/ wk/ 9wks</td>
<td>ND ↑ 8.6% 1RM of BHS ↑17.5% 1RM of SU ↑18% 1RM of LCH ↑7.8 % SJ↑ 16.6 % CMJ↑ 13.5% 30m sprint↑ ↔ DJ40cm</td>
<td>Only perform the previous defined HIST program</td>
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<td>HIST in isokinetic mode</td>
<td>32 ses/ 12wks</td>
<td>OS ↑ 10-26% CON IKE(0. 4.18 and 5.24 rad/s) ↑ 9-14% CON IKE(0 and 0.52 rad/s) ↑ 5-29% PPO(3.14 rad/s) ↑ 15-29% PPO(3.14 rad/s) ↑ 24-42% CON IKE Vpeak(5.24 rad/s) ↑ 18-32% PPO Vpeak (15.24 rad/s) ↑ MIVC90° knee extension ↑ 9% CON IKE(2.09 rad/s) ↔ PPO; PPO; MIVC90° knee extension; CON IKE Vpeak; PPO Vpeak (15.24 rad/s) ↔BS without run up</td>
<td>Low resistance strength training in isokinetic mode (low intensity high speed contraction group)</td>
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<tr>
<td></td>
<td></td>
<td>Functional strength training in the form of loaded kicking movements without ball</td>
<td>4 sets x 24 RM</td>
<td>4 sets x 16 RM</td>
<td>↑ 7-13% CON IKE(0.52-2.09-3.14 rad/s) ↑ 9-14% CON IKE(0 and 0.52 rad/s) ↑ 7%PPO (4.18 rad/s) ↑ 9-12% PPO(0.52-2.09-3.14 rad/s) ↔ BS without run up</td>
<td></td>
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</tbody>
</table>

**Table 1. Contd**
<table>
<thead>
<tr>
<th>Study</th>
<th>Level/Country/ n (age)</th>
<th>Type of training</th>
<th>F/D</th>
<th>P</th>
<th>Physiological adaptations</th>
<th>Performance changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maio Alves et al., (2010)</td>
<td>Elite/ Portugal/ 9 (17.4 ± 0.6)</td>
<td>Complex and contrast strength training: 1&lt;sup&gt;st&lt;/sup&gt; Station: 6 rep. of 90º Squats at 85% 1RM then 1 set of 5m high skipping, in a Straight line and then 5m sprint. 2&lt;sup&gt;nd&lt;/sup&gt; Station: 6 rep. of calf extension at 90% 1RM then 8 vertical jumps and then 3 high ball header’s. 3&lt;sup&gt;rd&lt;/sup&gt; Station: 6 rep. of leg extension exercise at 80% 1RM then 6 jump from the seated position than 3 drops jumps (60 cm), executing a soccer heading.</td>
<td>1x/ wk/ 6wks</td>
<td>PS</td>
<td>↑ 9.2% 5m sprint</td>
<td>↑ 6.2% 15m sprint</td>
</tr>
<tr>
<td>Mujika et al., (2009)</td>
<td>Elite / Spain / 10 (18 ± 0.5)</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; session - introduction session of hill sprinting (8% slope); 2&lt;sup&gt;nd&lt;/sup&gt; session - dedicated to sled pulling sprint training, towing ≈18% BM; 3&lt;sup&gt;rd&lt;/sup&gt; 4&lt;sup&gt;th&lt;/sup&gt; and 5&lt;sup&gt;th&lt;/sup&gt; session (weeks 3 to 5) 3 series of 4 reps of calf rises (≈ 35% BM) and parallel squats (≈50% BM) and 2 repetitions per leg of hip flexions (≈ 15% BM); 6&lt;sup&gt;th&lt;/sup&gt; session – Stair climbing: 18 x (18 steps x 22.5 cm)/120-sec rec. (alternating single leg, double leg, single, double, frontal and lateral step). Weight training emphasizing maximal concentric mobilization. Strength and power exercises in sessions 3-5 immediately followed soccer-specific activities such as jumps, accelerations, ball kicks, and offensive and defensive actions</td>
<td>1x/ wk/ 7wks</td>
<td>IS</td>
<td>↑≈2.8% 15m sprint†</td>
<td>↔ CMJ; CMJWAS; CMJ&lt;sub&gt;15.5&lt;/sub&gt;; Agility 15m</td>
</tr>
<tr>
<td>10 (18 ± 0.7)</td>
<td>Sprint training: 1&lt;sup&gt;st&lt;/sup&gt; and 2&lt;sup&gt;nd&lt;/sup&gt; session- 2 x(4x 30-m); 3&lt;sup&gt;rd&lt;/sup&gt; and 4&lt;sup&gt;th&lt;/sup&gt; session- 3 x(4x 30-m); 5&lt;sup&gt;th&lt;/sup&gt; and 6&lt;sup&gt;th&lt;/sup&gt; session- 4 x(4x 30-m); 90-s recovery between rep/180-sec recovery between sets;</td>
<td>1x/ wk/ 7wks</td>
<td>↔ CMJ; CMJWAS; CMJ&lt;sub&gt;15.5&lt;/sub&gt;; Agility 15m; 15m sprint</td>
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<td>Study</td>
<td>Level/Country/ n (age)</td>
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<tr>
<td>Manopoulos et al., (2006)</td>
<td>Amateurs/ NS/ 10 (19.9 ± 0.4)</td>
<td>wk1:2 : general strength (10 exercises/3 sets/15-20 rep); wk3:4: 3sets/6 rep (5 different exercises as skipping, jumping on one leg and on both legs, jumping running forwards, backwards and to the side, jumping obstacles and kicking); wk5:6: (a) 3 sets x 6 instep kicks within a time of 5 s (b) 6 kicking’s with a 5m run-up approach against resistance provided by a rubber band (RRB) attached on the ankle of the swinging leg (c) 3x10-min/5- or 8-a SSG, with or without loads (d) series of modified exercise sequences: 1st) 6 kicking’s (RRB), 3 jumps, isometry trunk with a player on the back (PB) in a semi-seated position for 6 s, 4 sideward jumps; 2nd) 6 leg extensions RRB, 3 headers, isometry ankle musculature, carrying PB for 6 s, 1 kicking; 3rd) 6 knee flexion repetitions RRB, 4 sideward jumps, 3 x 5-m sprints and a soccer kick.</td>
<td>3x/ wk/ 10wks</td>
<td>NS</td>
<td>↑ 13.9% MIVC_leg press</td>
<td>↑=4% 10-m sprint</td>
</tr>
<tr>
<td>Impellizzeri et al., (2008)</td>
<td>Amateurs/ Italian/ 37 (25± 4)</td>
<td>PI on grass; Vertical jumping: 15sets in wk1; 20 sets wk2; 25sets in wk3; always 10rep per wk; Bounding: 3sets wk1; 4sets wk2; 5sets per wk in wk3; always 10rep per wk; Broad jumping: 5sets x 8rep wk1; 5sets wk2; 7sets wk3; always 10 rep per wk wk3; Drop jump : 3sets x 5rep wk1; 5sets x 9rep wk2; 6sets x 15rep per wk in wk3; recovery 15-30s between repetitions 1-2-min between sets</td>
<td>3x/ wk/ 4wks</td>
<td>PS</td>
<td>↑ 3.7% 10m sprint</td>
<td>↑= 2.8% 20m sprint</td>
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<td></td>
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<td>Same PI protocol but performed on a different ground surface (sand).</td>
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<td>↑ 4.7% SJ</td>
<td>↑= 14.5% CMJ†</td>
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<td></td>
<td>↑ 19% CMJ/SJ†</td>
<td>†= 4.3% 10m sprint</td>
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<td></td>
<td>↑ 2.5% 20m sprint</td>
<td>†= 10% SJ†</td>
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<td>16.4% CMJ;</td>
<td>13.7% CMJ/SJ</td>
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<tr>
<td>Study</td>
<td>Level/ Country/ n (age)</td>
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<tr>
<td>Sedano et al., (2011)</td>
<td>Elite U-19/ Spain/ 11(18.4±1.1)</td>
<td>PI: Jump over hurdles: 16-26 sets/5rep; Horizontal jumps: 16-26 sets/5rep; Lateral jumps over hurdles: 16-26 sets/5rep; wk1,2,4 and 5 – 300 jumps; wk3 and 6 – 240 jumps; wk5,7 – 330 jumps; wk6,8 – 180 jumps; wk7,9 – 390 jumps; 30 sec recovery between sets of 5rep and 5min after 4sets of 5reps</td>
<td>3x/ wk/ 10wks</td>
<td>IS</td>
<td>↑ 8% CMJ</td>
<td>↑ 15% CMJ, ↓ 5% BSdl</td>
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<td>↑ 6.4% BSndl</td>
<td>↑ 0.32% 10m sprint</td>
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<tr>
<td>Thomas et al., (2009)</td>
<td>Semi-professional/ 12(17±0.4)</td>
<td>PI: DJ&lt;sub&gt;group&lt;/sub&gt; session began at 80 foot contacts and progressed to 120 by end of training program</td>
<td>2x/ wk/ 6wks</td>
<td>IS</td>
<td>↑ ≈ 5% CMJ</td>
<td>↑ = 5% CMJ, ↑ ≈ 5% 505 agility test</td>
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<td>↓ 5m,10m,15m and 20m sprint time</td>
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<tr>
<td>Gorostiaga et al., (2004)</td>
<td>Amateurs/ Spain/ 10 (17.3 ± 0.5)</td>
<td>Explosive-strength training (Low loads weight training and plyometric and sprint exercises): full squat-lift (2-3 sets/2-6 rep/ 20-52 Kg) and power clean (3-4 sets/ 3-4 rep / 16-28 kg) 2x/wk; vertical CMJ to box (3-5 sets / 5-8 rep/ only in wk&lt;sub&gt;4&lt;/sub&gt;); hurdle vertical jumps (3sets/ 4rep/ only in the wk&lt;sub&gt;9&lt;/sub&gt;); sprints (1set/ 3-5 rep/ 15-40m) performed 1x/wk; 2min recovery between sets and exercises</td>
<td>2x/ wk/ 11wks</td>
<td>IS</td>
<td>↓ ≈ Hr&lt;sub&gt;15-14 km.h⁻¹ bpm&lt;/sub&gt;</td>
<td>↑ 5.1% CMJ</td>
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<td>↑ 9.9% CON IKE&lt;sub&gt;60º.s⁻¹&lt;/sub&gt;</td>
<td>↑ 7.5% CMJ&lt;sub&gt;30kg&lt;/sub&gt;</td>
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<td></td>
<td>↑ 13.9% CMJ&lt;sub&gt;30kg&lt;/sub&gt;</td>
<td>↓ 5 and 15m sprint; CMJ&lt;sub&gt;40,60,60-70kg&lt;/sub&gt;;</td>
</tr>
<tr>
<td>Billot et al., (2010)</td>
<td>Amateurs/ French/ 10 (20 ± 2)</td>
<td>Electroestimulation training: 2-min session on both quadriceps femoris muscle (36 contractions per session); Knee fixed at 60º(0º corresponding to full extension of the leg); EMS 3-sec long followed by a rest period of 17-sec (duty cycle 15%); Intensity range 60-120 mA (higher than 60% of Muscle voluntary contraction)</td>
<td>3x/ wk/ 5wks</td>
<td>IS</td>
<td>↑ 22.1% ECC IKE&lt;sub&gt;150º.s⁻¹&lt;/sub&gt;</td>
<td>↑ 9.6% BS without run-up</td>
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<td></td>
<td></td>
<td></td>
<td>↑ 9.9% CON IKE&lt;sub&gt;60º.s⁻¹&lt;/sub&gt;</td>
<td>↑ 5.6% BS with run-up</td>
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<td></td>
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<td></td>
<td></td>
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<td>↓ ≈ SJ; CMJ; CMJWAS;</td>
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<td></td>
<td></td>
<td>↓ ≈ 27.1% MIVC&lt;sub&gt;60º&lt;/sub&gt;</td>
<td>10m sprint; V&lt;sub&gt;10m&lt;/sub&gt;</td>
</tr>
</tbody>
</table>
↑ - significant improvement; ↔ - no significant alterations; ≈ - approximately; NS - not specified; F/D - frequency and duration of training protocols; P - period of the soccer season; rec - recovery; HIST - high-intensity strength training; PI - plyometric training; wk - week; PS - performed during preseason; IS - performed during in-season; ND - not defined; ALB - alternate leg bound; DLHJ - double leg hurdle jump; SLFH - single leg forward hop; BHS - back half squat at 90°; SU - Step up on a bench with one leg; LCH - Leg curls for hamstrings; Rep. - repetitions; LLV - lean leg volume; 1RM - repetition maximum in half-squat exercise; 1RM/LLV - maximal strength in half-squat strength per lean leg volume; PPO (W) - peak power output; COD - change of direction; Vopt (ver.min^-1) - speed were the highest value of power is achieve; V (ver.min^-1) - maximal cycling speed corresponding to zero load; F_0 (kg) - individual theoretical maximal force generated at zero pedal speed; SJ - squat jump; DJ - drop jump from 40-cm height; 5-J - 5-jump test; 4BT = 4-bounce test; CMJ/SJ - eccentric utilization ratio; CMJ = countermovement jump; CMJ10-20-30-40-50-60kg - countermovement jump height with extra loads ranging from 10 to 60 kg; CMJA - counter movement jump with free arms; PP020-35-50kg (watts) - peak power in squat jumps with loads of 20 to 50 kg; PRV = peak running velocity; Hr_{13-14km.h^-1} (bpm) - heart rate at 13 and 14 km.h^-1; La_{13-14km.h^-1}(mM) - Blood lactate concentration at 13 and 14 km.h^-1; LMV - leg muscle volume; TMV - thigh muscle volume; MTCSA - mean thigh cross sectional area; Wpeak - leg cycling peak power; V_{first step} - velocity during the first step after the start of sprint test; V_{first5m} - average running velocity during the first 5-m of the sprint test; V_{max} - maximal running velocity; MPV (rpm) - maximal pedaling velocity; BM - body mass; RBB - resistance provided by a rubber band; MIVC - maximal isometric voluntary contraction in the leg press machine (knee and hip angles of 110° and 90°, respectively; 180°= full extension); MIVC/BW - maximal force divided by body weight; EMG VL - electromyography activity of vastus medialis of the swinging leg (phase 3) normalized relatively to the maximal EMG value during kick; F_{100} - maximal force value during the first 60 ms of the contraction; F_{100} - maximal force value during the first 100 ms of the contraction; BS with run up - ball speed after kicking with previous run up; BS without run up - ball speed after kicking without previous run up; BSnd - ball speed after kicking with dominant leg; BSndl - ball speed after kicking with non-dominant leg; MCS - maximal cycling speed (revs.min^-1); EMS - electrostimulation training; ECC IKE - eccentric isokinetic knee extensor peak torque (angular velocity); CON IKE - concentric isokinetic knee extensor peak torque (angular velocity); CON IKE50 - concentric isokinetic knee extensor peak torque at 50° knee extension (angular velocity); PPO_50 - peak power at 50° knee extension (angular velocity); CON IKE50 Vpeak(5.24 rad/s) - concentric isokinetic knee extensor peak torque exerted at the instance of peak velocity (angular velocities higher than 5.24 rad/s); PPO Vpeak(5.24 rad/s) - peak power output exerted at the instance of peak velocity (angular velocities higher than 5.24 rad/s); MIVC_{KE} - maximal isometric voluntary contraction of knee extensors (angle); V_{10m} - velocity at 10 meters sprint; VO_2max - maximal oxygen consumption; MAS - maximal aerobic speed; RE - running economy; RSA - repeated sprint ability test; DTT - Holf’s dribbling track test.
Table 2. Physiological and functional adaptations to concurrent strength and endurance training

<table>
<thead>
<tr>
<th>Study</th>
<th>Level /Country/ n (age)</th>
<th>Type of training</th>
<th>D</th>
<th>P</th>
<th>Physiological adaptations</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Nunez et al., (2008)</td>
<td>Semi-professional/ Spain/ 16 (28 ± 3.7)</td>
<td><strong>ST and ET</strong> - a sequence of general, special, and specific exercises incorporated in different training blocks. ET followed the time-line sequence of variable trajectory, medium extensive, intensive and short intensive intervals. ST followed the sequence of maximal holds, fast holds, horizontal and vertical jumps. ET block (2 sessions ET + 1 session ST) ST block (1 session ET + 2 session ST)</td>
<td>4x12wks</td>
<td>S</td>
<td>↑73% -80% Probst test</td>
<td>↑11.1-16.2% SJ, ↑8.7% CMJ, ↑8.4% CMJWAS</td>
</tr>
<tr>
<td>Wong et al., (2010)</td>
<td>Professional/ Hong-Kong/ 9 (24.6 ± 1.5)</td>
<td><strong>ST</strong>: 5 exercises; high-pull, jump squat, bench press, back half squat and chin-up; 4 sets at 6RM with 3 min rest between sets</td>
<td>2x/wk/8wks</td>
<td>PS</td>
<td></td>
<td>↑14% VJ, ↑5.9% T10, ↑2.8% T30, ↑19.7% YYIR1, ↑3.1% MAS, ↑19.2% MAS distance</td>
</tr>
<tr>
<td>Lopez-Segovia et al., (2010)</td>
<td>Elite/ Spain/ U-19</td>
<td><strong>ET</strong>: high-intensity runs, physical-technical circuits and SSG, with maximal intensity during 4-6-min periods. <strong>ST</strong>: jumps with and without external training loads, half-squats and full-squats. The speed of movement ranged from 0.8 to 1.2 m.s⁻¹. ST complemented with sprint exercises with loads (5kg) including change of direction movements, and 15 to 20-m take-offs with resisted sled towing (10kg)</td>
<td>2x/wk/16-wks</td>
<td>PS-1S</td>
<td></td>
<td>↑16.8%CMJ20kg, ↑15.8%Fsquats20kg, ↑17.1%Fsquats30kg, ↑15.2%Fsquats40kg, ↓2.3%T20, ↓2.4%T30, ↓3.2%T10-20-m, ↓1.6%T10-30-m, ↓2.6%T20-30-m</td>
</tr>
<tr>
<td>Bogdanis et al., (2009)</td>
<td>Professional/ Greek/ 9(22.9 ± 1.1)</td>
<td><strong>ST and ET</strong>: (8-12 upper and lower body exercises + 4 sets of half-squats at 90%1RM / 5rep / 3min rest between sets) + SSG/IR (Wk1: 20-min SSG; Wk2: 72-min IR + 60min SSG; Wk3: 72min IR + 40min SSG; Wk4: 40min IR + 54min SSG; Wk5: 20min IR + 48min SSG; Wk6: 20min IR + 48min SSG)</td>
<td>6-wks</td>
<td>PS</td>
<td>↑5.4%Total work in RSA</td>
<td>↑29.4%YYIET, ↑10%DTT, ↑7%VO₂max</td>
</tr>
</tbody>
</table>

**ST and ET**: (8 - 12 upper and lower body exercises + 4 sets of half-squat at 70%1RM / 12rep / 1.5min rest / + the same SSG/IR protocol | 4.5% Total work in RSA, 16.2%VO₂max, 15.8%VO₂max ↔ RE | 21.5%YYIET, 19.6% DTT |
↓ - significant decrement; ↑ - significant improvement; ↔ - no significant improvements; F/D - frequency and duration of training protocols; P - period of the soccer season; ST - strength training; ET - endurance training; HIAT - high-intensity aerobic training; HIST - high intensity strength training; SE - speed endurance; S - performed throughout the soccer season; PS - performed during preseason; IS - performed during in-season; SJ - squat jump; CMJ - countermovement jump; CMJWAS - countermovement jump with arm swing; VJ - vertical jump height; T10 - 10 meters time; T20 - 20 meters sprint time; T30 - 30 meters sprint time; T10-20 - split time between 10 and 20 meters sprint performance; T10-30 - split time between 10 and 30 meters sprint performance; T20-30 - split time between 20 and 30 meters sprint performance; YYIR1 - yo-yo intermittent recovery level one; MAS - maximal aerobic speed; MASdistance - maximal aerobic distance; VO2max - maximal oxygen consumption; 1RMHF - one repetition maxim in half squat strength exercise; wk1-6 - week 1, week 2…; IR - interval running; SSG - small-side-games; Fsquats20-40kg - speed of movement during full squats exercise with loads ranging from 20 to 40 kg;
Experimental Work
TITLE: Individual match playing time during the season affects fitness-related parameters of male professional soccer players

RUNNING HEAD: Seasonal variations in physical fitness

Authors:

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Affiliations:

¹ Faculty of Sport, University of Porto, Portugal
² Center of Research, Education, Innovation and Intervention in Sport (CIFI2D)
³ Research Center in Physical Activity, Health and Leisure (CIAFEL)

Abstract

The purpose of this study was to analyse the effects of an entire season on physical fitness parameters (PFP) in male professional soccer players (N=18). Performance in 5m and 30m sprint (T5 and T30), countermovement jump (CMJ), agility (T-test), knee extensors (KE) and flexors (KF) isokinetic strength, hamstrings/quadriceps strength ratio (H/Q) and bilateral differences (BD), and Yo-Yo intermittent endurance test 2 (YYIE2) were evaluated in four moments (E1-E4) throughout the season. Individual match playing time (IMTC) was quantified. Significant improvements in CMJ and YYIE2 from E1 to E2 were observed (p<0.05-0.01). T30 improved from E2 to E3 (p<0.01). CMJ decreased from E2 to E3 and E4, and YYIE2 from E2 to E4 (p<0.05). There were increments in H/Q ratio and Agility from E1 and E2 to E3 and E4 (p<0.05-0.01). Significant correlations were found in all evaluation points between different PFP and between changes in strength parameters and agility, T5 and T30, CMJ and YYIE2 (p<0.05-0.001). IMPT was correlated to changes in T5 (E1 to E3; r = -0.705), knee extensors non-dominant leg (KEND; E2 to E3; r = 0.786) and KF (E3 to E4; r = 0.575-0.590). The interrelationship between muscle strength (e.g. KE), sprint (e.g. T5) and jump abilities (CMJ) suggests the importance of muscle strength and power training for soccer. The present study suggests that the systematic participation of the players in soccer matches favors the increase and maintenance of soccer players KE and KF muscle strength and sprint ability (T5). Thus, given the unique demands of actual match play, coaches should try to incorporate a competitive friendly match in the weekly training cycle of non starter players.

Keywords: Soccer Season; Training Schedule; Training status; Strength; Muscle Power; Intermittent endurance.
INTRODUCTION

The regular evaluation of soccer players' physical fitness using different tests is of special importance as players should be able to successfully compete during 10-11 months. Data from different studies suggest that elite soccer players cover 8 to 13 km during matches (7) at a mean intensity close to the anaerobic threshold (AT) (43). Moreover, energy expenditure during match averages 70-75% of maximal oxygen consumption (VO_{2}max) (7,38), which suggests that performance at elite level may, in part, be determined by aerobic fitness (38). Furthermore, despite low to medium intensity running are the predominant activity patterns of soccer players, muscle power-based efforts such as sprints, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power of the neuromuscular system (15), are essential factors to successfully perform in soccer. Therefore, it is consensual among the literature that soccer players' performance is intimately related to the efficiency of different energy-related systems (43).

During season players are usually under prolonged physiological stress, as has been described in different studies. Analysis of soccer player during the season have revealed signs of oxidative (9) and functional stress (7,25), and immune system (36) and hormonal stress-related imbalances (25). In such conditions, the maintenance or improvement of player performance is not solely determined by appropriate conditioning, but also by the ability of the body systems to recover and regenerate following multiple stress stimuli (25). Therefore, to better understand the recovery pattern of the player under such demanding competitive stress conditions, a longitudinal coverage of physical performance in several time points throughout the season may be useful. However, there is a lack of studies concerning the effects of an entire season on physical fitness of professional players engaged in sport activities, including soccer. Most of the studies rely on particular functional or physiological data such as anthropometrics (11,13,25,31), VO_{2}max and anaerobic threshold measurements (11,13,31,32), specific intermittent endurance test (6,36),
exercise performance in incremental treadmill tests until exhaustion (11), muscle power through jump and sprint tests (4,11,30) and lower limb strength evaluated by isokinetic devices (30,31). Inconsistency has been revealed in the literature regarding the impact of training and competition in the variation of certain fitness parameters (4,10,11,13,25,30-32). Moreover, these observations resulting from longitudinal scanning of players fitness, as well as from the analysis of the short term impact of diverse forms of strength (29,49) and endurance training (42,49), did not considered the influence of the cumulative amount of competition playing time. A study performed by Impellizzeri et al. (23), where rating of perceived exertion (RPE) was used to quantify the internal training load (RPE-Tld), showed that in weeks comprising two official matches, the RPE-Tld can represent about 50% of total weekly training load decreasing to 25% in weeks with only one match performed. As so, a possible determinant factor of physical fitness over the season could be the cumulative amount of match playing time, a subject scarcely explored. Therefore, the purposes of this study were to examine the impact of the season in a group of fitness-related parameters and the association between their changes, considering the influence of individual match playing time (IMPT).

METHODS

Experimental approach to the problem
As presented in figure 1, the different physical tests were performed in the same order and in the same period of the day on four time points of a soccer season (10-11 months) as follows: 1\textsuperscript{st} (E1) – before the soccer season; early July); 2\textsuperscript{nd} (E2) - end of the preseason period (early August); 3\textsuperscript{rd} (E3) - middle of the season (January); 4\textsuperscript{th} (E4) - end of the competitive season (May).

In each moment, players were evaluated for basic anthropometry, countermovement vertical jump (CMJ), sprint and agility abilities, leg extensor and flexor maximal strength, and Yo-Yo intermittent endurance test 2 (YYIE2). However, no YYIE2 data were collected at the mid-season (E3) due to Club heavy match commitments at that time. Additionally, the players’ individual time
spent in the matches was recorded. The physical fitness measurements throughout different time points of the season allowed the analysis of seasonal fitness alterations, the relationship between the different fitness parameters, and the influence of individual match playing time (IMPT) in physical fitness. The selected variables provide valid and reliable data for the evaluation of physical fitness parameters, known to be directly (agility, sprint, jump, intermittent endurance) and indirectly (isokinetic strength parameters) related to soccer performance.

*figure 1 about here

**Subjects**

A group of 23 male professional players competing in the Portuguese elite championship (rank 6 of the Union of European Football Associations - UEFA) were initially involved in the protocol. As a result of injuries (n=2) and transfers to other clubs (n=3), 5 players were not considered in the analysis (n=18; table1). Only players free of injury involved in the full training schedules were tested. In accordance with the Club policy, all soccer players underwent usual physical examination in the beginning of the season (e.g. blood sample analyzes, rest ECG, lung X-Ray).

The experimental protocol followed the Declaration of Helsinki of the World Medical Association for research with humans and was approved by the local Ethics Committee. All participants were fully informed about the aims, experimental protocol and procedures, and provided written informed consent.

*table 1 about here

**Evaluation Procedures**

The CMJ, sprint ability and agility tests were conducted in indoor facilities to exclude possible ground surface variations in the soccer pitch throughout the season. Before the tests, all the players performed a 10 to 15 minutes (min) warm-up consisting of light jogging, specific mobility exercises and stretching routine, and 10 meters (m) sprints. Players completed two rounds of each test.
in the same sequence and the best result was considered for analysis. Each subject was allowed to a minimum of 5-min rest between tests to ensure adequate recovery. All the evaluations took place at the same time of the day, after a regular overnight sleep. Players were instructed to maintain normal routines for daily food and water intake, and followed the same dietary recommendations defined by the medical staff. In addition, during the days of physical tests, players were instructed to refrain from drinking beverages containing caffeine and/or alcohol and from consuming food during the 3 hours before testing. In the two days preceding evaluations, the players had a day-off (1st day) and a training session (2nd day) including low intensity exercises aiming to improve post-match recovery.

**Physical Performance Tests**

**Counter movement Jump**
The CMJ was performed using a platform, Ergojump (Digitime 1000, Digest Finland) according to Bosco et al., (8), whereby the highest vertical jump (cm) and the longest flying time (s) were registered. The best trial of the two jumps was considered.
The apparatus consisted of a digital timer connected by a cable to a jump platform, Ergojump (Digitime 1000, Digest Finland). The timer is triggered at take-off and then by participants’ feet at the moment of touchdown. The subject started from an upright standing position on the platform, and immediately after an eccentric phase (corresponding to a semi squatting position), the participants jumped vertically without using arms (arms remained at both sides, hands on the hip throughout the tests).

**Sprint Time**
Sprint measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA) mounted on tripods positioned approximately 0.75-m above the floor and situated 3-m apart facing each other on either side of the starting line (0 m), at 5 and at 30-m. The players stood 0.3-
Agility
Agility was evaluated through the T-test following protocol of Semenick (41) with modifications (figure 2). Subjects began with both feet 0.3-m behind the starting point A. At their own discretion, each subject sprinted forward 9.14-m (10 yd) to point B and touched the base of the cone with the right hand. They then sprinted to the left 4.57-m (5 yd) and touched the base of a cone (C) with the left and. Subjects then sprinted to the right 9.14-m (10 yd) and touched the base of a cone (D) with the right hand. They then sprinted to the left 4.57-m back to point B and touched the base of a cone with the left hand. They turn 270° and then ran to point A, passing the finishing line. Two test trials were performed, and times were recorded to the nearest one-hundredth of a second. As described for sprint ability, measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA). The players stood 0.3-m behind the starting line, being time activated when they passed the electronic sensors, and the clock stopped the instant players again crossed the Point A. The fastest trial was considered.

*Figure 2 about here

Isokinetic Strength
In order to evaluate players’ lower limb muscle function, maximal gravity corrected concentric peak torque of quadriceps and hamstring, bilateral leg strength differences (BD) and ratio between concentric hamstring (H) and quadriceps (Q) peak torque values (H/Q) were measured during isokinetic knee joint movement (Biodex System 2, NY, USA) of the dominant and non-dominant leg at the angular velocity of 90°.s⁻¹ (1.57 rad.s⁻¹), according to Magalhães et al., (27). After individual self-report, the dominant leg was determined by a routine visual inspection in a simple target kicking test requiring accuracy. Prior
to muscle function measurements, subjects perform a standardized warm-up consisting of 5-min period on a cycle ergometer (Monark E-824) with a fixed load corresponding to 2% of body weight. Players were then seated on the dynamometer chair at 85º inclination (external angle from the horizontal) with stabilization straps at the trunk, abdomen and thigh to prevent inaccurate joint movements. The contralateral leg was not secured to avoid influencing the strength developed by the knee being tested. The knee to be tested was positioned at 90º of flexion (0º= fully extended knee) and the axis of the dynamometer lever arm was aligned with the distal point of the lateral femoral condyle. Before the anatomical alignments and procedures, all the subjects were instructed to kick and also to bend the tested leg as hard and as fast as they could through a complete range of motion (from 90º to 0º). Subjects were also instructed to hold their arms comfortably across their chest to further isolate knee joint flexion and extension movements. All subjects also performed a specific sub-maximal warm-up protocol on the Biodex device in order to familiarize with the isokinetic device and test procedure. Three maximal repetitions at angular velocity 90º.s⁻¹ (1.57 rad.s⁻¹) were therefore carried out. The highest peak torque found during all the repetitions was chosen for the calculation of the bilateral leg strength differences (BD). This parameter was calculated as follows: [(dominant concentric leg strength - non-dominant concentric leg strength)] / dominant concentric leg strength *100 and is expressed from absolute values as percentage i.e., independently of the difference direction [from dominant (D) to non-dominant (ND) or ND to D]. The ratio between concentric hamstring (H) and quadriceps (Q) peak torque values (H/Q) was also determined and expressed as percentage.

**Yo-Yo Intermittent Endurance Test Level 2**

The Yo-Yo tests were designed to measure the ability to perform bouts of repeated intense intermittent exercise. After a 10-min warm-up, the players performed the test, which consists of repeated 2 x 20-m runs back and forth between the start and finish line at a progressively increased speed controlled by audio beeps from a CD-rom (5). The initial speed is 11.5 km/h (12.5 s for 2 x
20-m) and between running bouts the participants have a 5s rest period. The test was considered ended when the subjects failed twice to reach the starting line (objective evaluation) or the participant felt unable to complete another shuttle at dictated speed (subjective evaluation) (12). The total distance covered during the YYIE2 (including the last incomplete shuttle) was considered as the testing score. Heart rate was measured during the YYIE2 and recorded every 5s using a HR monitor (POLAR TEAM SYSTEM™, Polar Electro, Kempele, Finland). Field testing sessions were performed on the football pitch where players undertake their daily training sessions on several marked 2-m wide and 20-m long running lanes.

Intraclass correlation coefficients of all physical fitness tests were estimated using a test-retest procedure, with a random sub-sample of 9 subjects in each evaluation moment. The intraclass correlation coefficients (R) of all variables were high as follows. Height, weight and fat mass: 0.93 ≤ R ≤ 0.99; counter movement jump: 0.80 ≤ R ≤ 0.88; sprint time: 0.71 ≤ R ≤ 0.87; agility: 0.70 ≤ R ≤ 0.85; Yo-Yo intermittent: 0.80 ≤ R ≤ 0.97; and isokinetic strength: 0.78 ≤ R ≤ 0.98.

Playing schedule and training program

The generic training and competition plan completed by the soccer players involved in this study was supplied by the technical staff of the team. As can be seen by the season time-line (figure 1), 32 training sessions (6.4/wk) each lasting for 90 to 120-min per session, and 8 “friendly” matches for a total duration of 3400-min were comprised between E1 and E2 (5-wks). Training contents during this period consisted of 2 sessions per week of aerobic and strength training from week 1 to week 3 and 2 “friendly” matches. From week 4 to 5, one session per week of aerobic and strength training and 6 “friendly” matches were performed, 3 matches in each week. Aerobic training sessions consisted of general (interval running) and specific exercises (small-sided games and soccer specific circuits). Strength training sessions followed the specificity of strength training based on complex and contrast training (14).
Before training sessions players performed a warm-up lasting approximately 20 to 30-min (5 to 10-min jogging and low-intensity running, flexibility and mobility exercises, technical drills, short and brief explosive actions). After training sessions a warm-down that lasted approximately 10 to 15-min (jogging and stretching) was fulfilled.

Between E2 and E3 (25-wks), first half of in season, the team performed 150 training sessions (6/wk) with a total duration of 13300-min and played 26 official matches (1.04 matches/wk). In this period, players performed in each week one session of aerobic high intensity training (high intensity interval training and small-sided games), one session of functional strength training (plyometric training, resistive sprints, agility drills) and one session of others sprint ability exercises (with and without changes of directions).

Between E3 and E4 (17-wks), second half of in season, players were engaged in 90 training sessions (5.3/wk) with a total duration of 7200-min and played 26 official matches (1.5/wk). In this period, the number of matches increased, and the number of fitness training sessions decreased.

**Statistical Analysis**

All data are reported as means and standard deviations (SD). Normality was tested with the Shapiro-Wilks test. Intraclass correlation coefficient was calculated to estimate the reliability of the physical fitness tests. Analysis of variance for repeated measures was used to compare differences between evaluations. Pearson correlation coefficients ($r$) were used to determine association between tests, tests changes and their relationship with individual match playing time. The SPSS statistical package (version 14.0; Inc., Chicago, Ill) was used. Statistical significance was set at $p \leq 0.05$.

**RESULTS**

**Physical performance tests**

The results of the physical tests performed in the four time points throughout the season are shown in tables 2, 3 and 4.
The best 5-m (T5) and 30-m (T30) results were obtained at E3, but significant differences were only observed between E2 and E3 in the T30 (table 2). The results of CMJ were significantly higher in the E2 compared with the other three time periods (Table 2). No differences were found between any other moments.

T-test performance showed a significant improvement in E3 and E4 when compared with E1 and E2 (table 2). No differences were found between E3 and E4 neither between E1 and E2.

No significant changes were found in knee extension (KE) and flexion (KF) strength in both dominant (D) and non-dominant (ND) legs (KED, KEND, KFD, KFND), as well as in bilateral strength differences (BD) in leg extension (BDE) and flexion (BDF) in the four time points (Table 3). However, a significant increase in the H/Q ratio was found from E1 and E2 to E3 and E4.

*Table 2 about here*
*Table 3 about here*

As mentioned YYIE2 was only evaluated in E1, E2 and E4. The total distance covered during the YYIE2 significantly increased in E2 and E4 compared with E1. However, a significant decrease was also observed from E2 to E4 (Table 4).

*table4 about here*

**Association between tests**

In E1, T5 was correlated with T30 ($r=0.662; p<0.001$) and T-test time ($r=0.577; p<0.01$). The T30 was also correlated with CMJ ($r=-0.456; p<0.05$) and T-test ($r=0.441; p<0.05$). Moreover, significant correlations were observed between CMJ and KE values [(r= 0.524 and 0.534 for KED ($p<0.05$) and KEND ($p<0.01$), respectively]. In E2, significant correlations were found between CMJ and T30 ($r=-0.723; p<0.01$), T5 ($r=-0.526; p<0.01$) and T-test time ($r=-0.556; p<0.01$). The T30 was also correlated with T5 ($r= 0.667; p<0.01$) and T-test time.
(r=0.725; p<0.01). In E3, correlations were found between CMJ and T5 (r=-0.508; p<0.05) and T30 (r=-0.686; p<0.01), and also between T5 and T30 (r=0.801; p<0.001). Additionally, significant correlations between H/QND and the T-test were found (r= 0.607; p<0.01). In E4, correlations between T5 and CMJ (r=-0.730; p<0.01; Fig. 3) were observed. The KED was correlated with the T5 (r=-0.547; p<0.05) and H/QND was correlated with the CMJ (r= 0.623; p<0.01). In all the evaluations, body mass was correlated with the peak torques from both KE and KF (r ranging from 0.6 to 0.8 and p<0.01 to 0.001).

*Figure 3 about here

**Association between changes in the tests**

From E1 to E2, significant correlations were observed between individual changes in T30 and changes in CMJ (r=-0.546; p<0.05) and KED (r=-0.623; p<0.01). Changes in T-test time were correlated with changes in KFD (r=0.522; p<0.05) and H/QD (r=0.768; p<0.001). Also, a significant correlation between individual changes in KED and YYIE2 was observed (r=-0.568; p<0.05). From E2 to E3, significant correlations were observed between individual changes in T-test time and H/QD (r= 0.726; p<0.05). Also, changes in KEND were correlated with T5 (r=-0.638; p<0.05; Fig. 4), and changes in T-test time were correlated with H/QND (r=0.622; p<0.05) from E3 and E4.

*Figure 4 about here

**Association between match playing time and changes in the tests**

The individual match playing time (IMPT) from E1 to E3 was correlated with the individual changes in T5 (r=-0.705; p<0.01; Fig. 5). From E2 to E3, the IMPT was significantly correlated with KEND (r=0.786; p<0.05) and H/QND (r=-0.738; p<0.05). From E3 to E4, the IMPT was correlated with KFD (r=0.590; p<0.05), KFND (r=0.575; p<0.05) and H/QND (r =0.794; p<0.05, Fig. 6).

*Figure 5 about here
DISCUSSION
The purposes of this study were to examine the impact of the season in a group of fitness-related parameters and the association between their changes, considering the influence of individual match playing time. 

Our data showed that very short sprint performance measured by the T5 is a stable physical ability throughout the soccer season. However, significant differences were observed in the T30, where the time to perform the 30-m distance decreased from E2 to E3 (p<0.01) evidencing better sprint performance in the middle of the competitive period. In contrast with our data, Aziz et al. (4) reported a gradual improvement in sprint performance over the season in both 5 and 20-m sprint tests.

The soccer players showed a significantly higher CMJ performance in E2 than in E1, E3 and E4 (Table 2). Accordingly, Clark, et al. (13) also found variations in CMJ throughout the soccer season. However, discrepancies regarding seasonal variation in CMJ performance have been reported in literature. In fact, in contrast with our results, others did not report variations in CMJ during the season (11,30,31). The increase in CMJ performance in E2 could possibly be explained by the higher number of sessions dedicated to improve strength and muscle power during preseason. During this period, high training volumes are usual aiming to improve the general fitness levels (45) including muscle power. Moreover, around December before E3, and from E3 to E4, it was observed an increase in the number of games (see figure 1) requiring more time given to recovery interventions, which implies a reduction in the volume of strength and power training and potentially explain CMJ decrement from E2 to E3 and E4. The predominant use of functional strength or solely functional type of muscle training during in season may result in a decrement of the jump ability gains attained during preseason training (10,39). Nevertheless, in a study by Caldwell & Peters (10), semi-professional players only performing functional strength training during in season were able to further increase and maintain the improvements obtained during preseason.
Some researchers have suggested the importance of the agility as an independent physical variable in the assessment of male football players’ performance (44,48). Our data showed improvements in agility performance from E1 and E2 to E3 and E4, with the highest scores being observed in E3. A better coordination between agonist and antagonist muscles allows an improved ability to stop, start and turn rapidly while maintaining balance without loss of speed (41), which may explain, at least in part, our results. These actions that are systematically performed during training and games could likely contribute to the agility increase observed in E3 and E4.

All together, the results of the agility and sprint tests suggest that activities related with the stretch-shortening cycle (26) were improved in the mid-season (E3). However, CMJ was not improved in this period. One possible reason is that CMJ might not fulfil the criteria of SSC as would running-based exercises, which lie in a very fast transition from stretch to shortening phases as well as in a greater stretch velocity, both critical for stretch reflex activation (47).

Generally, data from our study showed that players maintain the same levels of strength both on knee extension and flexor muscles as well as bilateral strength differences throughout the soccer season. In fact, only significant increases in the H/Q ratio from E1 and E2 to E3 and E4 were observed. A stability of isokinetic strength parameters (e.g. KE) of professional soccer players over the season was also observed by other investigators (30). In contrast, amateur players showed a significant decrease of KE during an 11 week competitive soccer season (25). This fact may highlight the possible influence of training time in exercises specifically targeting muscle strength. Curiously, the KE peak torques observed in the present study during the season were higher than those reported in professional soccer players even at lower angular velocities (60º/s⁻¹) (30,31). Possible differences in time devoted to strength training may contribute to explain, at least partially, the referred differences between studies. Nevertheless, it is important to refer that despite the popularity of isokinetic tests in research, they are less predictive of performance and longitudinally changes than isoinertial measures, as a high sensibility is expected when the mode of training matched the mode of testing (22).
Despite the multi-factorial aetiology of muscle injury, some evidences suggest that imbalances in H/Q, as well as BD (2,16), previous injury (2,17) and lower resistance to fatigue (e.g. hamstrings) (40), are major risk factors for soft tissues injuries of soccer players. Throughout the time course of our study, the H/Q ratios observed in the different time points were identical to those reported previously by our group (27) and lower than the results reported by others (31). Furthermore, Mercer et al., (31) did not report any significant changes in H/Q from the beginning to the end of pre-season (E1 to E2). According with the improvements in CMJ during preseason and in agility during in season, it seems reasonable to suggest that a detraining effect may occur during offseason. In fact, there is scientific evidence that detraining effects are more marked in muscle power than in strength, being the former more dependent on muscle coordination (24,34).

The Yo-Yo Test is widely used to evaluate the ability of soccer players to intermittently perform exercise and has been considered a sensitive tool to detect seasonal changes in the fitness of soccer players (6). Our data showed an increase in the YYIE2 performance after preseason period followed by a decrease at the end of season (E4). These results are in agreement with previous data reporting soccer players’ lower intermittent endurance performance at the beginning of the season in field and laboratory tests. In fact, data obtained through different tests, such as the incremental treadmill test to exhaustion (32), exercise tolerance at VO$_2$max, Intermittent Field Test (36) and the Yo-Yo Intermittent Recovery Test (6) expectedly revealed lower performance at the beginning of the season than during the competitive period. Unfortunately, due to practical constrains related to the competitive calendar of the team engaged in the present study, no data have been collected during the mid-season (E3), which unable to assess the impact of the first half of the season in the ability to perform intermittent endurance exercise.

Despite some controversies regarding the eventual contribution of the players’ aerobic power, the increments in YYIE2 performance in E2 and E4 compared to E1 may also be related to improvements in other endurance-related physiological features. Rampinini et al. (35) recently observed that time
constant of VO\textsubscript{2} Kinetics and the ability to maintain acid-base balance are also important physiological factors to the performance in Yo-Yo tests. As so, improvements in the former factors throughout the season may have increase the capacity to repeatedly perform intermittent endurance exercise. On the other hand, the lower levels of intermittent endurance observed in E1 may also explain the significant increment observed during preseason (E2), since in such conditions higher adaptations should be expected.

The decline in YYIE2 performance observed in our study at E4 compared to E2 might be related with the specificity of the competition schedule. In fact, Mohr et al., (33) reported decreases in the ability to perform high-intensity running during games in elite soccer players involved in National and European competitions (two matches per week) towards the middle of the season, during which there is limited time to fitness training and more time to recovery is needed. Accordingly, Kraemer et al., (25) observed that starter players who usually accumulate more match playing time experience greater performance decrements in some fitness parameters. Curiously, no relationship was observed in the present study between changes in YYIE2 and IMPT.

An intriguing finding was that players with lower increments or higher decrements in KFD (E1 to E2), H/QD (E1 to E2 and E2 to E3) or H/QND ratio (E3 to E4) showed higher improvements in agility performance. These results suggest that improvements in flexors strength and H/Q ratios seem to be on the opposite direction of agility development. A possible explanation could be that the decrease in H/Q strength ratio, allowed the increment of acceleration capacity resulting from the increase of KE activation and from the reduction of the antagonist KF coactivation during the knee extension task (50).

The present results showed that improvements in KED from E1 to E2, KEND from E3 to E4 (fig. 4), and CMJ from E1 to E2 were significantly correlated with the performance improvement in short sprints. KE also showed significant correlations with the CMJ (E1) and T5 (E4; KED). Moreover, high correlations between CMJ and T30 (E1, E2 and E3) and T5 (E3 and E4; fig.4) were observed. Previous studies (19,48) showed that players with improvements in CMJ and in strength experienced improvements in short-sprint performance. As
proposed by other researchers (19), this may suggest a possible transfer from leg power strength gains into enhanced sprint performance. In fact, there were significant associations between the different functional tests that rely in power activities (CMJ, T5, T30 and Agility; Fig. 3) in the different evaluation time points. Moreover, from E1 to E2 we observed that the players with higher improvements in KED showed lower improvements in YYIE2 ($r = -0.568$; $p<0.05$). However, during the same period a higher improvement in YYIE2 was parallel to an improvement in a muscle power test (CMJ). It has been reported that concurrent muscle training and high intensity interval training performed during preseason could lead both to improvements in muscle power (10 and 30-m sprint, vertical jump) and in intermittent endurance performance (YYIR1)(49). However, after the observation of an increment in both CMJ and YYIE2 performance during preseason in the present study, no relationships between the two physical fitness parameters and/or their changes were found.

Top level soccer players are usually involved in the weekly matches of the national leagues and very often in international commitments. These competitive demands may impose strains to various physiological systems including musculoskeletal, nervous, immune and metabolic (7,9,25,36), to a point where the managing of player’s physical fitness through training and recovery strategies became influential to maximize match performance. Thus, the knowledge on the relationship between IMPT and PFP throughout the season would be helpful in the management of training programs including the time scheduling of official and “friendly” matches and specific training sessions. During the 90 min of the match, soccer players can perform 10-20 sprints, a high intensity running every 70s, about 15 tackles, 10 headings, 50 involvements with the ball, and 30 passes, as well as changing pace and sustaining forceful contractions to maintain balance and control of the ball against defensive pressure (43). Therefore, high stress levels are imposed on the neuromuscular system in order to cope with this essential muscle power based efforts. As already observed in other intermittent team sports such as handball (18,20), individual match playing time (IMPT) seems to influence players’ fitness during season. In fact, according with our results, players with
more accumulated IMPT from E1 to E3 showed higher improvements in T5 (r = -0.705; p < 0.01; Fig. 5). This suggests that the short bursts of acceleration required during games could have a positive effect in developing players` ability to accelerate (15,48). It was observed that players with higher IMPT showed both higher increments and lower decrements in KEND (r = 0.786; p < 0.05; E2 to E3), KFD and KFND (r = 0.575 and 0.590, respectively; p < 0.05; E3 to E4). Accordingly with our data, Kraemer et al., (25) observed that during and after an 11-week competitive soccer season, collegiate soccer players` KF strength did not significantly decreased as much has KE. Moreover, the authors observed that starter players showed higher values of KF in the different evaluation points. Additionally, we observed that the correlation coefficients between IMPT and individual changes in H/Q ratio were r = -0.738 (p < 0.05) from E2 to E3 and changed to a positive value of r = 0.794 (p < 0.05) from E3 to E4 (Fig. 6), reflecting the different impact of IMPT in KE and KF in the different evaluations throughout the competitive period. The observed relationship between IMPT and these PFP suggests that male professional soccer players with higher IMPT have higher capabilities to increase or maintain muscle strength (KE and KF) and sprint (T5) throughout the season, i.e., competition time may possibly contribute to influence certain physical characteristics of professional soccer players. In fact, it was evidenced that after the game KF is affected by fatigue and muscle damage and the KF reduction follows the increase in some indirect markers of muscle damage (1,3,28). Moreover, KF were also referred as a muscle group less resistant to fatigue than KE (40) that shows after a match higher force decrements (1,21) and longer period of muscle soreness (46) than extensors muscles. This may explain at least partially the higher adaptations found in this muscle group in players with more IMPT between E3 and E4. In fact, muscle adapts to training regimens that engage them in stretch-shortening cycles and are impaired less with repeated exposure (37).
This study showed that changes in different parameters of soccer players’ fitness are observed throughout a soccer season. In this way, a proper control of training and competition workloads and their impact should be monitored. The preseason improvements in players’ physical fitness from start (E1) to the end of the season (E4), makes logical to conclude that during offseason players experiment pronounced effects of detraining in jump ability and agility, and intermittent endurance capacity. Thus, male professional soccer players should perform a specific training program or participate in active leisure activities in order to attenuate reductions in training status that results from offseason period as well as to better cope preseason training loads. Coaches should be aware that the interrelationship between KE strength, 5-m and 30-m sprint time and CMJ performance suggest that muscle strength and power training should be an important component of soccer training. Moreover, given that during the in-season, the isolated completion of functional strength exercises may be insufficient to maintain the jump ability of professional players, the incorporation of some form of weight training (e.g. combined weight and plyometric training) might be beneficial. The present study suggests that the systematic participation in soccer matches favors the increase and maintenance of male professional soccer players’ muscle strength and sprint ability. Thus, given the unique demands of actual match play, coaches should try to incorporate a competitive friendly match in the weekly training cycle of non-starter players.

ACKNOWLEDGEMENTS
We thank to the technical staff and to the soccer players of the team participating in the study. The results of the present study do not constitute endorsement by NSCA.
References

31. Mercer, TH, Gleeson, NP, and Mitchell, J. Fitness profiles of professional soccer players before and after pre-season conditioning. In: *Science and*


Table 1. Physical characteristics of the professional soccer players at the four test occasions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>25.7 ± 4.6</td>
<td>25.4 ± 4.7</td>
<td>26.9 ± 4.3</td>
<td>27.1 ± 4.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.5 ± 9.2</td>
<td>76.2 ± 9.3</td>
<td>76.3 ± 8.4</td>
<td>77.7 ± 9.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.1 ± 5.7</td>
<td>177.8 ± 5.7</td>
<td>178.7 ± 6.6</td>
<td>179.1 ± 6.6</td>
</tr>
<tr>
<td>Fat Mass (%)</td>
<td>9.8±3.7</td>
<td>9.5±2.7</td>
<td>9.3±3.0</td>
<td>9.1±3.1</td>
</tr>
</tbody>
</table>

Values are mean ± SD. **E1** – evaluation 1 (Prior to pre-season), **E2** – evaluation 2 (End of pre-season) **E3** - evaluation 3 (Mid-season), **E4** – evaluation 4 (End-of-season)
Table 2. Variation of different performance measurements in the four evaluation moments throughout the soccer season

<table>
<thead>
<tr>
<th>Variables</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>G. S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 5 m (s)</td>
<td>1.032 ± 0.11</td>
<td>1.056 ± 0.05</td>
<td>1.017 ± 0.06</td>
<td>1.033 ± 0.07</td>
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</tr>
<tr>
<td>T 30 m (s)</td>
<td>4.164 ± 0.18</td>
<td>4.217 ± 0.15</td>
<td>4.137 ± 0.14</td>
<td>4.16 ± 0.17</td>
<td>4.169</td>
</tr>
<tr>
<td>CMJ (cm)</td>
<td>42.44 ± 4.04</td>
<td>44.84 ± 4.5</td>
<td>42.84 ± 4.4</td>
<td>42.23 ± 4.38</td>
<td>43.08</td>
</tr>
<tr>
<td>T-test (s)</td>
<td>8.714 ± 0.33</td>
<td>8.745 ± 0.36</td>
<td>8.408 ± 0.27†</td>
<td>8.498 ± 0.27†</td>
<td>8.591</td>
</tr>
</tbody>
</table>

Values are mean ± SD. *p< 0.01 E3 vs E2; **p< 0.05 E2 vs. E1, E3 and E4; †p< 0.01 E3 and E4 vs E1 and E2; E1 – evaluation moment 1 (Prior to pre-season), E2 – evaluation moment 2 (End of pre-season) E3 - evaluation moment 3 (Mid-season), E4 – evaluation moment 4 (End-of-season); G.S.- Global Sample; T5m – 5 meters sprint time; T30m – 30 meters sprint time; CMJ – countermovement jump.
Table 3. Variation of isokinetic strength measurements in the four evaluation moments throughout the soccer season.

<table>
<thead>
<tr>
<th>Variables</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>G. S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KED 90°.s(^{-1}) (Nm)</td>
<td>238.9 ± 39.8</td>
<td>240.7 ± 51.4</td>
<td>239.13 ± 45.2</td>
<td>243.9 ± 52.8</td>
<td>240.64</td>
</tr>
<tr>
<td>KFD 90°.s(^{-1}) (Nm)</td>
<td>131.2 ± 22.1</td>
<td>131.6 ± 21.9</td>
<td>135.4 ± 25.1</td>
<td>138.03 ± 24.2</td>
<td>134.05</td>
</tr>
<tr>
<td>H/Q D (%)</td>
<td>54.91 ± 6</td>
<td>54.65 ± 5</td>
<td>56.6 ± 8.5 *</td>
<td>56.4 ± 7.3 *</td>
<td>55.64</td>
</tr>
<tr>
<td>KEND90°.s(^{-1})(Nm)</td>
<td>241.746 ± 39</td>
<td>241.338 ± 47</td>
<td>241.85 ± 40</td>
<td>243.8 ± 53.3</td>
<td>241.417</td>
</tr>
<tr>
<td>KFNd 90°.s(^{-1})(Nm)</td>
<td>128.99 ± 19.6</td>
<td>128.6 ± 23.4</td>
<td>133 ± 20.1</td>
<td>133.8 ± 23.6</td>
<td>130.74</td>
</tr>
<tr>
<td>H/Q ND (%)</td>
<td>53.35 ± 4.9</td>
<td>53.2 ± 5.2</td>
<td>55.02 ± 5.4 *</td>
<td>54.9 ± 6.1 *</td>
<td>54.1</td>
</tr>
<tr>
<td>BDKE (%)</td>
<td>5.76 ± 5.36</td>
<td>5.57 ± 5.42</td>
<td>5.70 ± 4.95</td>
<td>8.54 ± 4.91</td>
<td>6.39</td>
</tr>
<tr>
<td>BDKF (%)</td>
<td>6.64 ± 6.3</td>
<td>5.59 ± 4.7</td>
<td>7.66 ± 5.4</td>
<td>7.6 ± 5.8</td>
<td>6.87</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * p< 0.05 E3 and E4 vs E1 and E2; E1 – evaluation moment 1 (Prior to pre-season), E2 – evaluation moment 2 (End of pre-season) E3 - evaluation moment 3 (Mid-season), E4 – evaluation moment 4 (End-of-season); G.S. - Global Sample; KED- peak torque in knee extension dominant legs; KFD- peak torque in knee flexion dominant legs; H/Q D- concentric hamstrings/quadriceps strength ratio dominant leg; KEND- peak torque in knee extension non-dominant legs; KFNd- peak torque in knee flexion non-dominant legs; H/Q ND- concentric hamstrings quadriceps strength ratio non-dominant leg; BDKE- bilateral strength differences in extensors muscles; BDKF- bilateral strength differences in flexors muscles;
Table 4. Variation of YYIET2 measurements in the four evaluation moments throughout the soccer season.

<table>
<thead>
<tr>
<th>Variables</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>G. S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>YYIET2 (m)</td>
<td>1120 ± 187.6</td>
<td>2250 ± 296</td>
<td>-</td>
<td>1640 ± 196**</td>
<td>1670</td>
</tr>
<tr>
<td>HRmax (bpm)</td>
<td>197.9 ± 8.3</td>
<td>196 ± 7.1</td>
<td>-</td>
<td>196.1 ± 9.0</td>
<td>196.9</td>
</tr>
<tr>
<td>HRmean (bpm)</td>
<td>181.6 ± 9.6</td>
<td>180.9 ± 7</td>
<td>-</td>
<td>176 ± 9.8</td>
<td>179.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * p< 0.01 E2 vs E1; ** p< 0.05 E4 vs E1 and E2; E1 – evaluation moment 1 (Prior to pre-season), E2 – evaluation moment 2 (End of pre-season) E3 - evaluation moment 3 (Mid-season), E4 – evaluation moment 4 (End-of-season); G.S.- Global sample; YYIET2 – Yo-Yo intermittent endurance test level 2; HRmax – maximal heart rate; HRmean – mean heart rate, m – meters, bpm – beats per minute.
Figure 1 – Season time-line. \textbf{E1} – evaluation moment 1 (Prior to pre-season); \textbf{E2} – evaluation moment 2 (End of pre-season); \textbf{E3} – evaluation moment 3 (Mid-season); \textbf{E4} – evaluation moment 4 (End-of-season); \textbf{YYIE2} – Yo-Yo intermittent endurance test level 2; IS – isokinetic strength; CMJ – countermovement jump; T5 – 5 meters sprint time; T30 – 30 meters sprint time; W – week; 8FM – eight friendly matches; 1 OM – one official match per week; 2 OM – two official matches per week; 1FM – one friendly match per week; 2FM – two friendly matches per week; 3FM – three friendly matches per week.
**Figure 2** - Layout of the T-test. Modified from Semenick (1990). Brief explanation

**Figure 3** – Relationship between counter movement jump (CMJ) and 5 meters sprint time (T5) in E4;
Figure 4 – Relationship between changes in peak torque knee extensors non-dominant leg (KEND) and 5 meters sprint time (T5) from E3 to E4;

Figure 5 – Relationship between individual match playing time (IMPT) and changes in 5 meters sprint time (T5) from E1 to E3;
Figure 6 – Relationship between individual match playing time (IMPT) and changes in hamstrings/quadriceps strength ratio non-dominant leg (H/Q ND) from E3 to E4;
TITLE: Neuromuscular function, hormonal, redox status and muscle damage markers in high-level players throughout a competitive soccer season.

RUNNING HEAD: Seasonal variations in stress and performance indicators

Authors:

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Under Review
Abstract
This study aims to analyze the changes in performance and stress-related markers of professional players throughout the season (N=14). 5 and 30-m sprint, jump and change of direction performance, and maximal isokinetic knee extension (KE) and flexion (KF) were evaluated in four time-points (E1-E4). Plasma testosterone/cortisol ratio (T/C), creatine kinase (CK), superoxide dismutase (SOD), glutathione peroxidase (GPX) and reductase (GR) activities, myoglobin (Mb), C-reactive protein (CRP), uric acid (UA), protein sulfhydryls (-SH), malondialdehyde (MDA) contents and total antioxidant status (TAS) were also measured. Cortisol (C) decreased and T/C increased in E2 and E3 (P<0.01-0.05). Mb, CK and PCR increased at E2 and PCR (P<0.01-0.05) in E3. SOD, SOD/GPX, -SH and MDA increased at E2 and E3 (P<0.01-0.05). From E2-E3, T/C and SOD/GPX increased and C, Mb and GR (P<0.01-0.05) decreased. Off-season resulted in decreased T/C, CK, SOD, SOD/GPX, -SH and MDA an increased C and GR (P<0.05). Changes in T/C were correlated with changes in KF dominant (KFD_{E2-E3}; r=0.858) and non-dominant leg (KFND_{E2-E3}; r=0.848) from E2-E3. In E3, T/C ratio was associated with KFND and KE dominant leg (KED; r=0.563-0.636). A negative association between C and KE and KF (r=-0.636-0.677) was found in E3. In E2, significant correlations were observed between individual match playing time (IMPT_{E2}), performance and biochemical variables (r=0.456-0.615). Our data suggest that professional players although submitted to high level of stress possess an efficient capability to cope with the season stressful demands. Additionally, the systematic participation of the players in matches influences performance and biochemical responses.

**Keywords:** training status; training period; performance testing; neuromuscular function; blood monitoring; anabolic:catabolic status; oxidative stress; muscle damage; recovery;
Introduction

Soccer players are submitted to a high level of physical and physiological stress during training and matches. These high demands are confirmed by players’ activity pattern analysis during the matches (Rampinini et al. 2007; Osgnach et al. 2010), as well as by the magnitude of the post-match performance impairments, biochemical responses and temporal kinetics of recovery (Magalhaes et al. 2010; Andersson et al. 2008). Despite the high demands imposed to players during nine months of regular match play (competitive season), the integrative analysis of the longitudinal changes in players’ physical performance and in stress bio-markers are limited. In fact, high volumes of training and/or competition interspersed by insufficient recovery may induce fatigue (Filaire et al. 2003) and ultimately an overreaching state (Kraemer and Ratamess 2005). Nevertheless, different investigations using distinct methodological designs to analyse longitudinal changes in soccer players physical fitness challenged the common concept amongst the soccer population (e.g. technical and medical staff) that players may accumulate “fatigue” during periods of heavy match commitments (Meister et al. 2011) and/or as the season progresses (Silva et al. 2012; Silva et al. 2011; Faude et al. 2011; Rampinini et al. 2007). In fact, although contradictory reports regarding performance impairments have been published (Kraemer et al. 2004; Silva et al. 2011; Jensen et al. 2009; Rampinini et al. 2007; Faude et al. 2011) there are increasing evidence of psychometric deteriorations (e.g. increased tension, anger) towards the end of season (Rebelo 1999; Filaire et al. 2003; Faude et al. 2011) that may suggest a state of non-functional overreaching/overtraining. Additionally, the occurrence of inadequate immune function during periods of high physical stress has been reported (Reinke et al. 2009; Rebelo et al. 1998). Moreover, alterations in soccer players’ anabolic-catabolic hormonal environment (Kraemer et al. 2004; Handziski et al. 2006) and increased levels of oxidative stress were reported after high-intensity periods of soccer training (pre-season) (Arent et al. 2010). Therefore, in research protocols aimed to study physical performance, fatigue and recovery in sport, a multi-dimensional
test panel should be included to achieve a higher efficiency of the monitoring process (Bishop et al. 2008; Faude et al. 2011). In fact, in addition to sport-specific performance parameters, hormonal, oxidative and muscle damage markers have been suggested as important bio-markers during training monitoring in different athletic populations (Kraemer and Ratamess 2005; Bishop et al. 2008; Rebelo 1999).

Evidence-based scientific data regarding the putative relationships between soccer players’ physical fitness, hormonal environment and oxidative status under demanding soccer competitive stress conditions are limited. Moreover, despite the growing number of match commitments in the national and international context, the influence of the individual match playing time during season on the referred parameters is lacking. On the other hand, one of the less understood and more scarcely studied periods of the soccer season is the off-season. Therefore, studies investigating if off-season allow players to recover their organic homeostasis are needed. Knowledge clarifying this gap in literature may allow improving the management of training periodization, competition scheduling, and, eventually, of specific ergogenic interventions (e.g., antioxidants supplementation).

The present study intends to examine, for the first time, alterations in performance, hormonal, oxidative and muscle damage parameters throughout the season and during the recovery period in the same cohort of professional soccer players. We also aim to determine the relationships between players’ individual match playing time and physiological and physical performance changes.

Methods

Subjects
A group of 20 male professional outfield soccer players (mean ± standard deviation: 25.7 ± 4.6 years, body mass 76.5 ± 9.2 kg, height 178.1 ± 5.7 cm, fat percentage 9.8 ± 3.7%) from a team competing in the Portuguese Professional Soccer League were involved in this study. As a result of injuries (n=2) and
transferences to other clubs (n=4), 6 players were not considered in the analysis (n=14). In each evaluation, only free of injury players involved in the full training schedules were tested. In accordance with the club policy, all soccer players underwent usual physical examination in the beginning and throughout the season (e.g. blood sample analyzes, rest ECG, lung x-Ray). The subjects were not taking exogenous anabolic–androgenic steroids or other drugs or substances expected to affect physical performance or hormonal balance throughout the different assessment moments. The players perform several in or out of competition doping control tests undertaken by the Portuguese Anti-doping Authority. The subjects were not taking any medications that would have an impact on the results of the study.

The experimental protocol was approved by the local board, and followed the Declaration of Helsinki of the World Medical Associations for research with humans. All participants were fully informed about the aims, experimental protocol and procedures, and provided written informed consent.

**Experimental design**

As presented in figure 1, the different physical tests and blood sample collections were performed in the same order and in the same period of the day on four time points of a soccer season as follows: 1st evaluation (E1) – before the beginning of the preparation period of the 1st soccer season (July); 2nd evaluation (E2) - middle of the competitive season (January); 3rd evaluation (E3) end of the competitive season (May); 4th evaluation (E4) – after the end of the recovery period and before the beginning of the preparation period of the 2nd soccer season (July). All the evaluations were performed after 72-h of the last official match. In the two days preceding evaluations, the players had a day-off (1st day) and a training session (2nd day) including low intensity exercises aiming to improve post-match recovery. In E1 E2 and E3, blood samples were collected and players were evaluated for basic anthropometry, countermovement vertical jump (CMJ), sprint and change of direction abilities, knee extensor and flexor maximal strength. In E4, blood samples collections were again obtained. Players’ individual match playing time was registered
throughout the season. The final individual match playing time of each player (IMPT; sum of all minutes played by a player throughout all the competition period) was used to analyse the association between performance and biochemical variables.

*figure 1 about here

**Procedures**
In order to match the circadian rhythms of the different variables, the different time points of blood sample and functional data measurements were coincident. As so, blood samples and functional data were obtained after an overnight fast between 09.00 and 12.00. Procedures order were as follows: 1st - Blood samples collection; 2nd - CMJ, 3rd - T5 and T30, 4th - COD and 5th - Isokinetic strength. In the laboratory, a resting blood sample was taken after subjects have been standing for at least 15 min, after which subjects consumed a light standardized meal and drink and rested for 2 h. The meal consisted of 1.7 g white bread and 0.3 g of low-fat spread; both values are per kilogram of body mass (Thompson et al. 2003) The CMJ, sprint ability and COD tests were conducted in indoor facilities. Before the tests, all the players performed a 10 to 15 minutes (min) warm-up consisting of light jogging, specific mobility exercises and stretching routine, and 10 meters (m) sprints. Players completed two rounds of each test in the same sequence and the best result was considered for analysis. Each subject was allowed to a minimum of 5-min rest between tests to ensure adequate recovery. All the evaluations took place at the same time of the day, after a regular overnight sleep (0800-11.00 a.m.). Players were instructed to maintain normal routines for daily food and water intake, and followed the same dietary recommendations defined by the medical staff. In addition, during the days of physical tests, players were instructed to refrain from drinking beverages containing caffeine and/or alcohol and from consuming food during the 2 hours before testing.

**Physical Performance Tests**

**Counter-movement Jump (CMJ)**
The CMJ was performed using a platform, Ergojump (Digitime 1000, Digest Finland) according to (Bosco et al. 1983) whereby the highest vertical jump (cm) and the longest flying time (s) were registered. The best trial of the two jumps was used for data analyses. The apparatus consisted of a digital timer connected by a cable to a jump platform, Ergojump (Digitime 1000, Digest Finland).

Sprint ability

Sprint measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA) mounted on tripods positioned approximately 0.75-m above the floor and situated 3-m apart facing each other on either side of the starting line (0 m), at 5 and at 30-m. The players stood 0.3-m behind the starting line, started at their own discretion, being time activated when players cross the first pair of photocells, and they ran as fast as they could to complete 30-m distance. The fastest trial was used for data analyses.

Change of direction ability (COD)

Change of direction ability (COD) was evaluated using an agility test (T-test). T-test does not include a perceptual or decision making component and can be used to measure change of direction ability. An adapted version of the T-test by Semenick (Semenick 1990) was performed. Subjects began with both feet 0.3-m behind the starting point A. At their own discretion, each subject sprinted forward 9.14-m (10 yd) to point B and touched the base of the cone with the right hand. They then sprinted to the left 4.57-m (5 yd) and touched the base of a cone (C) with the left and. Subjects then sprinted to the right 9.14-m (10 yd) and touched the base of a cone (D) with the right hand. They then sprinted to the left 4.57-m back to point B and touched the base of a cone with the left hand. They turn 270º and then ran to point A, passing the finishing line. Two test trials were performed, and times were recorded to the nearest one-hundredth of a second. As described for sprint ability, measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA). The players stood 0.3-m behind the starting line, being time
activated when they passed the electronic sensors, and the clock stopped the instant players again crossed the Point A. The fastest trial was used for data analyses.

**Strength assessment**

In order to evaluate muscle function, subjects were familiarized with the muscle function test on at least two occasions during preliminary visits to the laboratory. Maximal gravity corrected concentric peak torque of quadriceps and hamstrings was measured during isokinetic knee joint movement of dominant leg at an angular velocity of 90°.s\(^{-1}\) (1.57 rad.s\(^{-1}\)) using an isokinetic dynamometer (*Biodex* System 2, USA). After individual self-report, the dominant leg was determined by a routine visual inspection in a simple target kicking test requiring accuracy. Prior to muscle function measurements, subjects perform a standardized warm-up consisting of 5-min period on a cycle ergometer (Monark E-824) with a fixed load corresponding to 2% of body weight. Players were then seated on the dynamometer chair at 85° inclination (external angle from the horizontal) with stabilization straps at the trunk, abdomen and thigh to prevent inaccurate joint movements. The contralateral leg was not secured to avoid influencing the strength developed by the knee being tested. The knee to be tested was positioned at 90° of flexion (0°= fully extended knee) and the axis of the dynamometer lever arm was aligned with the distal point of the lateral femoral condyle. Before the anatomical alignments and procedures, all the subjects were instructed to kick and also to bend the tested leg as hard and as fast as they could through a complete range of motion (from 90° to 0°). Subjects were also instructed to hold their arms comfortably across their chest to further isolate knee joint flexion and extension movements. All subjects also performed a specific sub-maximal warm-up protocol on the Biodex device in order to familiarize with the isokinetic device and test procedure. Three maximal repetitions at angular velocity 90°.s\(^{-1}\) (1.57 rad.s\(^{-1}\)) were therefore carried out. The higher peak torque value was used for data analyses.
Blood sampling and preparations

All the venous blood samples were taken by conventional clinical procedures using EDTA as anticoagulant. Nevertheless, no tourniquet was used in order to minimize potentially oxidative stress induced by an ischemia-reperfusion maneuver.

The freshly withdrawn blood (~10ml) was immediately centrifuged at 3000 rpm during 10 minutes for careful removal of the plasma. Plasma was separated into several aliquots and rapidly frozen at – 80°C for later biochemical analysis of plasma total testosterone (T), cortisol (C), myoglobin (Mb), creatine kinase (CK), uric acid (UA), C-reactive protein (CRP), total antioxidant status (TAS), superoxide dismutase (SOD) glutathione peroxidase (GPX) and glutathione reductase (GR), sulfhydryl groups (-SH) and malondialdehyde (MDA).

Biochemical Assays

Plasma testosterone (T) and cortisol (C) were measured immune-enzymatically using commercial test kits VIDAS® testosterone (REF. 30418) and VIDAS® cortisol S (REF. 30451).

Plasma creatine kinase (CK) activity was determined spectrophotometrically using a commercial test kit (ABX A11A01632, Montpellier, FR).

Plasma myoglobin concentration was assessed using a commercial test kit (myoglobin bioMerieux 30446, Carnaxide, PT).

C-reactive protein (CRP) was measured using an enzyme-linked immune sorbent assay system (PENTRA 400, Horiba ABX, Montpellier, FR).

Total antioxidant status (TAS) was measured spectrophotometrically using a commercial kit (Randox NX2332 Crumlin, UK). Uric acid (UA) was determined by an enzymatic method using a commercial kit (Horiba ABX A11A01670, Montpellier, France).

Regarding enzyme activities in plasma, superoxide dismutase (SOD) activity was measured spectrophotometrically at 550 nm using a commercial Ransod kit from Randox (catalogue no. SD 125, Crumlin, UK). The activity of glutathione peroxidase (GPX) was assayed by a spectrophotometric technique at 340 nm using a commercial Ransel kit from Randox (catalogue no. RS 505). The
activity of glutathione reductase (GR) was measured with a spectrophotometric procedure at 340 nm using a commercial GR kit from Randox (catalogue no. GR 2368).

Plasma MDA was assayed according to Rohn et al., (1993) with some modifications and measured by the formation of thiobarbituric acid reactive substances at 535 nm. Plasma SH was spectrophotometrically evaluated at 414 nm according to Hu (1990). Protein content was spectrophotometrically assayed using bovine serum albumin as standard according to Lowry et al., (1951) Samples were analyzed in duplicate and the mean of the two values was used for statistical analysis.

**Statistical Analysis**

Mean, standard deviation (SD) and/or standard error mean (SEM) were calculated. Normality was tested with the Shapiro-Wilks test. After this assumption, One-way analysis of variance (ANOVA) with repeated measures was used to establish whether there were differences between evaluations. Intraclass correlation coefficient was calculated to estimate the reliability of the physical fitness tests. ICC of all physical fitness tests were estimated using a test-retest procedure, with a random sub-sample of 9 subjects in each evaluation moment. The intraclass correlation coefficients (R) of all variables were high as follows. Counter movement jump: 0.80 ≤ R ≤ 0.88; sprint time: 0.71 ≤ R ≤ 0.87; agility: 0.70 ≤ R ≤ 0.85; Yo-Yo intermittent: 0.80 ≤ R ≤0.97; and isokinetic strength: 0.78 ≤ R ≤ 0.98. All data analysis was performed using SPSS 18.0 package. A significance level of 0.05 was chosen.

**Results**

**Performance parameters**

Anthropometric data and results of performance in the different physical fitness tests are present in Table 1.
Biochemical parameters

Figure 2 shows the hormonal changes during the four season time points. No significant alterations were observed in plasma total testosterone (T; Fig. 2) concentration in the different time points. Plasma cortisol (C) concentration was significantly lower in end-of-season than in the remaining time-points (E3; $P < 0.01$-$0.05$; Fig. 2). The T/C ratio progressively increased towards competitive season (E1 and E2 vs E3; $P < 0.05$) and decreased throughout the recovery period ($P < 0.05$; Fig. 2).

Plasma Myoglobin (Mb) and C-reactive protein (CRP) contents and creatine kinase (CK) activity can be examined in Figure 3. Mb content was higher in E2 ($P < 0.05$) than in the other time-points (E1 E3 and E4). Plasma CK activity were higher in E2 than in E1 and E4 ($P < 0.01$) and in E3 than in E4 ($P < 0.05$). Plasma CRP content was higher in E2 and E3 than in E1 ($P < 0.05$).

Data concerning the variation on the different redox state parameters throughout the examination period are observed in Figure 4. No significant alterations in plasma total antioxidant status (TAS) were examined between E1 (1.12 ± 0.07 nmol/L), E2 (1.23 ± 0.07 nmol/L), E3 (1.16 ± 0.05 nmol/L) and E4 (1.11 ± 0.066 nmol/L). Plasma uric acid (UA) contents were stable throughout the different time-points, E1 (5.41 ± 0.37 mg/dl), E2 (5.44 ± 0.25 mg/dl), E3 (5 ± 0.15 mg/dl) and E4 (5.5 ± 0.3 mg/dl).

Plasma superoxide dismutase (SOD) activity was elevated in the middle (E2; $P < 0.05$; Figure 4) and at the end of competition period (E3; $P < 0.01$; Figure 4) than in the beginning of season one (E1). Also a decrement in SOD activity was observed from E3 to the end of the recovery period (E4; $P < 0.05$; Figure 4). No significant alterations in plasma glutathione peroxidase (GPX; Figure 4) activity during season were examined. Nevertheless, the SOD and GPX kinetics resulted in a lower SOD/GPX ratio throughout competition (E2 vs E1; $P < 0.05$; Figure 4 and E3 vs. E1, E2 and E4; $P < 0.05$; Figure 4). Moreover, a significant increment in SOD/GPX ratio was analysed within the competition phase (E2 vs E3; $P < 0.05$; Figure 4). Plasma activity of glutathione reductase were lower in E3 than in E2 ($P < 0.01$; Figure 4) and E4 ($P < 0.05$; Figure 4). Plasma protein sulfhydryl content was higher in E2 and E3 than in E1 and E4 ($P < 0.05$). Also,
an increase in plasma malondialdehyde (MDA) content was analysed in E2 and E3 than in E1 and E4 ($P < 0.01$-$0.05$; Figure 4)

Relationships between biochemical parameters and individual match playing time (IMPT) and performance in the different time points

We examined that players with the greater individual changes in T/C from E2 to E3 had the higher individual changes in KFD ($KFD_{E2-E3}$; $r = 0.858$; $p = 0.003$), KFND ($KFND_{E2-E3}$; $r = 0.848$; $p = 0.004$) from E2-E3. Also, in E3 an association between T/C ration and KED ($r = 0.563$; $p = 0.015$) and KFND ($r = 0.636$; $p = 0.005$) were examined. We also observed a negative association between plasma concentrations of C and KED ($r = -0.646$; $p = 0.009$), KEND ($r = -0.662$; $p = 0.004$); KFD ($r = -0.677$; $p = 0.004$), and KFND ($r = -0.636$; $p = 0.006$) in E3.

In the middle of the season (E2) we examined that players with higher match playing time ($IMPT_{E2}$) were also those with better T30 ($r = -0.531$; $p = 0.019$) and CMJ ($r = 0.615$; $p = 0.009$) performance. $IMPT_{E2}$ were also related to higher cortisol concentration ($r = 0.560$; $p = 0.024$) and lower T/C ratio ($r = -0.553$; $p = 0.033$). Also correlations between TAS ($r = 0.647$; $p = 0.003$), GPX ($r = 0.544$; $p = 0.016$) and SOD/GPX ($r = -0.456$; $p = 0.050$) and $IMPT_{E2}$ were analysed.

Discussion

Our data confirm that professional soccer players are submitted to high level of stress throughout the season expressed by increased muscle damage and oxidative stress markers; however, seem to have an efficient capability to deal with and sustain these stressful demands. In addition, the recovery period is related to a decrease in markers of muscle damage and oxidative stress and players' individual match playing time influences the response of physical, hormonal and oxidative stress-related parameters.

In the present study, the professional players improved change of direction speed (COD) throughout the season without significant alterations in isokinetic force, sprint or CMJ performance. Improvements in in-season COD ability, evaluated by other tests, have also been reported in semi-professional players (Caldwell and Peters 2009). Enhancement in COD speed is an important in-
season performance adaptation due to the large number of non-linear high-intensity running-based activities performed during matches (Bloomfield et al. 2007) and to the high levels of mechanical (e.g. eccentric actions) and metabolic stress that such actions involve (e.g. acceleration-deceleration dynamics) (Dellal et al. 2010; Osgnach et al. 2010). Additionally, the better scores of the different activities based on stretch-shortening cycle actions in E2 may suggest that in the middle of the in-season period players’ neuromuscular system is more “tuned” to the soccer-specific activities than in end of the season (E3). Curiously, this better performance scores in E2 occurred in parallel with peak values of some stress markers (Mb, CK, CRP).

Testosterone (T) and cortisol (C) are two major endogenous indicators of tissue adaptation, and their ratio represents a biological marker of homeostasis reflecting the relationship between the anabolic and catabolic hormonal environment within the body (Hayes et al. 2010; Kraemer et al. 2004; Kraemer and Ratamess 2005). Throughout the analysed period of the present study, total plasma T concentration was stable. Similar findings were reported by Filaire et al., (2003) regarding salivary testosterone alterations throughout the same season time-points. Moreover, Kraemer et al., (2004) also observed that T did not change during the soccer competitive period. However, an higher T was found just one week after the end of the competitive period than in the initial phase of competition period (Kraemer et al. 2004). Nevertheless, others did not corroborate this data. For example, Handziski et al., (2006) showed that in the week of taper that preceded the beginning of competitive season, T was higher than in the start of pre-season training, but was lower at the end-of-season than in the former time-points. Regarding C concentration, our results showed a progressive decline throughout the competitive period with significantly lower values at the end-of-season (E3) than in the start of both seasons (E1 and E4) and middle-of-season (E2). Kraemer et al., (2004) did not examine changes in C concentrations during an 11-week competitive period of academic soccer players. In contrast with ours and previous data (Kraemer et al. 2004), low values after the pre-season training (Handziski et al. 2006) and increases in the end-of season periods have been reported (Filaire et al. 2003;
Handziski et al. 2006). As a result of the T and C kinetics, we observed a progressive increase in T/C until the end-of-season and a decrease during off-season. Although stable T/C have been reported throughout the soccer season (Filaire et al. 2003), others (Handziski et al. 2006) observed an increment in T/C after the preparatory period (week of taper preceding competitive phase) followed by a decrement in the end-of-season (end-of-season vs pre-and-post preseason). The different results between studies can be explained, at least partially, by the differences in the study designs. Handziski et al., (2006) and Kraemer et al., (2004) analysed the soccer season with different periodization’s structures (e.g., half-season and 11-wk competitive season, respectively). Perhaps the length of the preparation period and/or the different densities of match commitments during the weekly in-season cycles that characterize distinct periodization structures, may among other factors, explain the different hormonal kinetics. In line with our observations, studies in other football codes (Cormack et al. 2008) and team sports (Martinez et al. 2010) reported a decrease in C and an increase in T/C during the competitive period. The progressive decrease in C and increase in T/C ratio observed in our study may reflect an enhanced anabolic environment throughout the competition phase (Kraemer et al. 1998). Nevertheless, resting hormonal concentration reflects the current state of muscle tissue and elevations or reductions may occur at various stages depending on substantial changes in the volume and intensity of the training phases (Hakkinen et al. 1988), e.g. number of weekly match commitments. In this regard, data collected by Martinez et al., (2010) in several in-season time-points showed that high-level basketball players may alternately increase or decrease catabolic and anabolic-related hormones throughout in-season. This data seem to highlight that it is possible to correct or avoid situations of accumulated stress in high competitive contexts. Moreover, it is appealing to speculate that players’ physical adaptations and/or specificities of their phenotype resulting from training background may led to a higher ability to cope with the stress induced by the intermittent nature of the activity in soccer. Another factor that cannot be excluded is a decreased influence of psychophysiological factors. In fact, the success of the studied team in this
season may have contributed, at least in part, for the decrease in C concentration, found in the end of season. This same factors may also influence the E1 and E4 scores, since the beginning of season can be associated with a high anticipatory level of psychophysiological stress that may influence C concentrations (Hoffman et al. 2005). In fact, it is recognized that preseason is a period devoted to players’ selection and squad definitions. On other hand, Reinke et al., (2009) observed that the reduction of stress levels of professional soccer players during off-season was related to an increase in catabolic metabolism (e.g., increased in leukocytes counts and IL-8) and a decrease in soccer players lean body mass (Reinke et al. 2009). Therefore, since resting cortisol concentrations generally reflect long-term training stress (Kraemer and Ratamess 2005), the higher C values found during off-season can be related, among other factors, to a process related to the metabolization of the damage muscle cells during in-season (Reinke et al. 2009).

On the other hand, reports from academic starter players showed higher C in the middle of the competition phase than in other time points within the competition period (Kraemer et al. 2004). Additionally, Kraemer et al., (2004) observed that T/C increased in non-starters players after one week of recovery during off-season. However, no changes were examined in starter players (Kraemer et al. 2004). In this regard, we observed a positive association between individual match playing time in E2 (IMPT_{E2}) and C values, and a negative association with T/C ratio at E2. Nevertheless, in this time-point, we observed that players with higher IMPT were those with greater performance in power-based efforts (T30 and CMJ) and no association between the hormonal data and performance was found. It is likely that these increased levels of catabolic hormonal-related evidences (increased cortisol and decreased T/C ratio) in players with higher IMPT might have been influenced by muscle damage induced by training and competitions. Since IMPT_{E2} was associated with lower T/C and with a higher performance (T30 and CMJ) its plausible to question to which extent anabolic/catabolic ratio can be related with performance, as well as to the magnitude of variation than can influence certain performance measurements. Although a decrease in anabolic/catabolic ratio
have been suggested to decrease performance (Adlercreutz et al. 1986), different investigations observed that soccer players’ performance increments can be related with both decrements (Gorostiaga et al. 2004) and higher values of T/C (Kraemer et al. 2004). In our study, players with greater individual changes in $T/C_{E2-E3}$ had the higher individual changes in $KFD_{E2-E3}$ and $KFNDE2-E3$ between the two periods. Furthermore, a positive association between $T/C$ ratio and KED and KFNND values and a negative association between plasma C and isokinetic knee extensor and flexor force production were examined in E3. However, since we did not examine the hypothalamo-pituitary-testicular and adrenal axis-related hormones that mediate the mechanism of T and C production, as well as other important factors that influence T and C role in the muscle tissue (e.g., hormone bioavailability, binding proteins, membrane receptors), the present results should be interpreted with caution.

Soccer competitions and trainings impose an activity pattern that request high levels of mechanical (e.g. acceleration and decelerations) and/or metabolic demands (e.g. elevated mitochondrial oxygen consumption). These characteristics are considered a prone condition to tissue damage (Teague and Schwane 1995; Vina et al. 2000). As an example, plasma concentration of certain intracellular proteins and serum enzyme activities were shown to be elevated during soccer match recovery periods (Magalhaes et al. 2010) and after high-intensity training periods (pre-season) (Arent et al. 2010). In our study, we examined a higher in-season increment in Mb content and CK activity in E2 than in E1 and E4. We also analyzed higher CK in E3 than after the off-season recovery period (E4). Interestingly, we observed a decrease in Mb content from E2 to E3 and higher peak values in both bio-markers in the middle of the season, which might be associated with “residual” levels of muscle damage due to the regular training sessions that players undergone (McLellan et al. 2010). Nevertheless, in line with others analyzing a distinct team sport (Hoffman et al. 2005), the plasma kinetic of certain intracellular proteins and enzymes (i.e., plasma myoglobin (Mb) was lower and creatine kinase tend to be lower towards the end-of-season), seems to suggest that a “contact adaptation” of skeletal muscles to the repeated mechanical and/or metabolic stress during
season (Hoffman et al. 2005). Accordingly, we examined that although C-reactive protein (CRP) increased from E1 to E2 and E3, peak values of this biomarker as well as from CK and Mb were observed in E2. Nevertheless, values observed in E2 and E3 were not statistically higher than the analyzed at the beginning of the 2nd season. Other study (Reinke et al. 2009) did not find differences in professional soccer players’ plasma CRP between the end-of-season, beginning and end of pre-season period. Curiously, longitudinal analysis of players’ game-physical performance showed improved short (high-intensity running in the peak 5 minutes period of the match) (Silva et al. 2012) and extended match performance (overall match high-intensity running) towards the end-of-season (Rampinini et al. 2007; Silva et al. 2012).

The high absolute levels of mitochondrial oxygen consumption, the increased oxidation of circulating catecholamines, the intermittent and repeated sprint actions-causing temporary ischemia-reperfusion events in skeletal muscle are plausible factors that may induce reactive oxygen and nitrogen species (RONS) (Ascensao et al. 2008) during the soccer season. Although with scarce and some contradictory results, studies involving other team sports showed that training may induce improvements in both athletes total antioxidant enzymatic and non-enzymatic capacity [for refs see (Finaud et al. 2006)]. Nevertheless, data gathered in soccer players (Arent et al. 2010) revealed that the increasing free radical scavenging capacity by means of ergogenic supplementation or by endogenous up-regulation of antioxidant defences due to the training-related pro-oxidant insults, is not a guarantee of sufficient protection or a condition for a decrement in exercise-induced oxidative damage during periods of high-intensity training and competition. In our study, we did not observe significant alterations in TAS and UA levels throughout the soccer season. To our best knowledge, no reports have been published regarding TAS kinetics during the soccer season. Filaire et al., (2003) observed higher plasma UA levels in professional soccer players in the beginning and at the end-of competitive period than before the beginning of the pre-season. Data examining post-match UA (Krustrup et al. 2006; Magalhaes et al. 2010) and IMP responses (Krustrup et al. 2006) seem to suggest that the completion of a soccer match is
associated with a significant degradation of purine nucleotides. Despite no changes were observed in UA contents during season, data from Reinke et al. (2009) suggests a compensated hypo-perfusion (and hypoxia) during the playing season i.e. observed decreased muscle resting and post-ischemic blood flow. Consequently, this hypoxia-related condition may increase XDH and XO activity in endothelium cells and predispose the endothelium to oxidative and inflammatory damage (Terada et al. 1992), which is consistent with the in-season increases in Mb, CK, CRP and MDA.

Regarding chronic alterations in antioxidant enzymatic activity, we observed a progressive increase in SOD activity in E2 and E3. This up-regulation of SOD may result from a continuous pro-oxidant insult. In fact, SOD is a major defence upon superoxide radicals and is the first defence line against oxidative stress (Finaud et al. 2006). Moreover, in conjunction with the protective role against free radical induced muscle damage during intense exercise, antioxidants may have a positive effect on performance and on the prevention of fatigue (Vina et al. 2000). Arent et al., (2010) examined the effects of pre-season supplementation with a dietary containing an orally available form of SOD as a part of an antioxidant and anti-catabolic nutriceutical mixture. Although no differences between supplemented and control players were found in performance capacity, players submitted to the ergogenic intervention presented lower CK and 8-isoprostranes responses during the post-exercise recovery period (Arent et al. 2010). Curiously, although we did not observe any relationship, the lowest CK activity in-season was coincident with the higher SOD activity (E3). Concerning the chronic alterations in resting antioxidant glutathione cycle-related enzyme activities, no significant changes in GPX activity were observed. However, GR activity decreased from E2 to E3. Taking into account the critical physiological role of the balance between the first (SOD) and second step (GPX and/or catalase) antioxidant enzymes, the progressive increase in plasma SOD/GPX ratio during in-season, seems to favor H$_2$O$_2$ accumulation. This fact could be indicative of a low plasma scavenging efficiency contributing to enhanced oxidative stress (Gaeta et al. 2002) that is consistent with the increased levels of MDA found in present study. As
previously mentioned, the increase in oxidative stress by-products (e.g. MDA) may suggest that an imbalance exists between oxidants and antioxidants due to the continuous pro-oxidant state associated with the soccer demands (e.g., muscle high metabolic rates).

Concerning IMPT, it seems that it not only influences some physical (Silva et al. 2011; Sporis et al. 2011) and hormonal parameters but might also have some impact on the endogenous enzymatic and non-enzymatic antioxidant system. Our study showed that in E2, players with higher IMPT had increased levels of TAS, GPX activity and a lower ratio between the 1st and 2nd line of antioxidant enzymatic defense. The increased levels of TAS and GPX activity in players with higher IMPT_E2 may act as a mechanism to strengthen the endogenous antioxidant defence and restore the redox balance (Anderssson et al. 2010) due to the increase in the intensity and volume of exercise exposure (Powers et al. 1999).

Regarding plasma sulfhydryl residues (-SH), we observed an increase during the competitive phase. These results suggest a decreased disulphide linkages (-S-S-) concentration from both proteins and reduced glutathione, and ultimately prevention of protein oxidation. The higher training background (Martinovic et al. 2009) (e.g. years of practice) and standard (Nakagami et al. 2009) of professional players cannot be ruled out as factors that may influence the results, since they have been associated with higher concentrations of sulfhydryl groups. Probably, the impact of vigorous training and competition on oxidative stress may lead to a up regulation in antioxidant defences and associated shift in redox balance in favour of a more reducing environment, thereby protecting athlete from excessive oxidative damage during subsequent competitions and training sessions (Fisher-Wellman and Bloomer 2009).

According to our results, it seems that professional soccer players, although submitted to a high level of stress signalled by increase muscle damage and oxidative stress-related markers, have an efficient capability to sustain the soccer season stressful demands. In fact, despite these stress-related alterations throughout the competitive season no significant performance decreases were observed, i.e. at least in the examined neuromuscular
parameters and competition results (1.7 and 1.6 match average points from E1 to E2 and from E2 to E3 respectively). In addition, the recovery period is related to a decrease in markers of muscle damage and oxidative stress. As expected, the reduction in the functional and metabolic demands during off-season produce a decrease in lipid peroxidation by-products (MDA) and in –SH contents.

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**Ethical standards**

The authors declare that all the procedures of the experiments comply with the current laws of the country in which they were performed.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**


mechanism of production, and protection by antioxidants. IUBMB Life 50 (4-5):271-277. doi:10.1080/713803729
Table 1. Functional data of the professional soccer players at the three test occasions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>T5 (s)</td>
<td>1.03 ± 0.06</td>
<td>1.01 ± 0.05</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>T30 (s)</td>
<td>4.16 ± 0.17</td>
<td>4.14 ± 0.14</td>
<td>4.16 ± 0.17</td>
</tr>
<tr>
<td>COD (s)</td>
<td>8.71 ± 0.27</td>
<td>8.41 ± 0.23*</td>
<td>8.49 ± 0.27*</td>
</tr>
<tr>
<td>CMJ (cm)</td>
<td>42.4 ± 4.0</td>
<td>42.8 ± 4.4</td>
<td>42.2 ± 4.4</td>
</tr>
<tr>
<td>KED (Nm)</td>
<td>239 ± 24.7</td>
<td>239 ± 13.8</td>
<td>244 ± 194</td>
</tr>
<tr>
<td>KEND (Nm)</td>
<td>242 ± 22.6</td>
<td>242 ± 31.4</td>
<td>244 ± 34</td>
</tr>
<tr>
<td>KFD (Nm)</td>
<td>131 ± 12</td>
<td>135 ± 9.7</td>
<td>138 ± 9.6</td>
</tr>
<tr>
<td>KFND (Nm)</td>
<td>129 ± 11</td>
<td>133 ± 8.1</td>
<td>134 ± 6.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD. * p < 0.01 E1 vs E2 and E3; ** E1 – evaluation 1 (Prior to pre-season 1), E2 – evaluation 2 (Middle of competitive season) E3 - evaluation 3 (End-of-season), T5 – 5 meters sprint time; T30 – 30 meters sprint time; COD – change of direction ability; KED – knee extensor dominant leg; KEND – knee extensor non-dominant leg; KFD – knee flexor dominant leg; KFND – knee flexor non-dominant leg; ND – not determined;
Fig. 1 Schematic representation of the study design
Fig. 2 Hormonal responses to a soccer season. Values represent mean ± SEM.
Fig. 3 Plasma Myoglobin content, Creatine Kinase activity and CRP content during the soccer season. Values represent mean ± SEM.
Fig. 4 Superoxide dismutase, glutathione peroxidase and glutathione reductase activities and SOD/GPX ratio during the soccer season. Values represent mean ± SEM.
Fig. 5 Malondialdehyde and sulfhydryl contents during the soccer season. Values represent mean $\pm$ SEM
**TITLE:** Training status and match activity of professional soccer players throughout a season

**RUNNING HEAD:** Training status and match related physical performance

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ABSTRACT

The purpose of this study was to examine match activity (MA) and fatigue development (FD) during official soccer games in different moments of a season and the influence of training status (TS) on MA and FD. MA of 13 professional players was examined by time-motion analysis at four time points of a competitive season. In addition, per time point within the two-week period between the two games video-filmed, players performed the following physical tests: countermovement jump, 5-m and 30-m sprints, change of direction, knee extensor and flexor isokinetic strength, and Yo-Yo intermittent endurance test-level two. Players covered a greater high-intensity distance running (HI; $p < 0.05$) in the last quarter of the season than in the second (E2) and third (E3). Within each assessment period, a greater distance was covered in HI during the peak five-minute period of the match (P5-min) than in the five-minute period after P5-min (Next5-min) and the remaining five-minute periods (Av5-min; $p < 0.05$) of the match. Also, P5-min was higher in E4 than in the beginning of the season (E1, E2 and E3; $p < 0.05$). The physical fitness variables, composites scores of power-related and isokinetic strength tests were correlated ($r$ ranging: 0.59-0.73, $p < 0.05$) with game physical parameters (GPP) analyzed by time-motion. Soccer players showed to cover more HI during the game and in the P5-min towards end-of-season. Players with greater muscle strength and power expressed lower performance decrements in GPP. In conclusion, results highlight the relevance of players’ neuromuscular function on game physical performance.

Keywords: performance analysis, competitive period, strength, muscle power, fatigue
INTRODUCTION

Match analysis is a widely used instrument in professional soccer to study tactical and physical performance of players and referees (2). Research with some of the most up-to-date technologies such as the multi-camera method (2,42,44), global position systems (44) and video based time-motion analysis (30,44) revealed detailed information about players’ movement patterns (8,26,30). Moreover, these methods seem to be able to detect performance decrements during soccer games and thereby enable the study of game-induced fatigue (44). Data revealed that several signs of fatigue can be manifested temporally during a game (6,30,31), toward the end of the game (6,30,31), and persist afterwards (4,28,32,45) with a time-dependency for the fitness parameter evaluated. Also, it has been observed through these methods that performance during the match is dependent on multiple factors (7,25,26,30,42).

Recent investigations observed that physical performance during the game changes throughout the season (30,42) and are related to players’ training status (25,26,41). In fact, some studies showed that soccer players’ performance in the Yo-Yo intermittent recovery test both in males (25) and females (26), in incremental field tests (shorter version of the University Montreal Track Test) (41) and in the repeated shuttle sprint ability test (41) are indicators of game-related physical performance.

However, data on non-locomotive activity as well as unorthodox movements (e.g. shuffling, diving), soccer specific movements (e.g. heading, blocking), and accelerations and decelerations are generally omitted or not taken into account (8). In fact, a massive metabolic load is imposed on players, not only during the more intense parts of the game, but every time acceleration occurs, even when speed is low (36). The regular evaluation of soccer physical fitness using different tests is of special importance, as soccer players should be able to successfully perform over competitive seasons of around 10-11 months. Although seasonal variations in game-physical performance have been analyzed (30,42), these investigations usually involve three time points of the
season (beginning, middle and end of season). Therefore, research assessing a higher range of time points throughout the season will be more fruitful when analyzing seasonal variations in game-related physical parameters. Moreover, scrutinized research of seasonal alterations in fatigue patterns (e.g. temporary fatigue) of soccer players during games has never been reported. Thus, we aimed to analyze game-related physical performance and fatigue development during official soccer games during four time points of the season.

Rapid force production is considered essential for a wide range of athletes (1,20,34). In fact, soccer players’ muscle strength (3,16) and performance in sport-specific muscle power efforts (16,33) were reported to be related to the player’s and team’s competitive level. Moreover, improvements in coordination specificities (12) and a greater agility (19) have been positively associated with performance and the ability to delay fatigue. In accordance with recent reports, enhancement of neuromuscular function can be a determinant in improving short and long term endurance capacity (1). In spite of these indications and although high stresses are imposed on the neuromuscular system during soccer games, until now, no study addressed whether muscle strength and power can be indicators of game-related physical performance. Therefore, we also aim to investigate whether physical conditioning evaluated in field and laboratory tests is related to game-physical performance and fatigue development. We hypothesized that during the season soccer players may experience performance alterations in certain game physical parameters analyzed by time-motion. Moreover, given the high stress levels imposed on the neuromuscular system, soccer players` training status might be associated with some game-related physical parameters.

METHODS

Experimental approach to the problem

Match activity and fatigue during games has been a topic of increased research in recent years (4,6-8,28,30,40). Moreover, players’ physical and physiological characteristics have already been extensively described (6,16,20). However,
the seasonal alterations in match activity and the influence of players` training status in game physical performance had been scarcely investigated. Therefore, in order to analyze seasonal alterations in game physical parameters and in fatigue patterns (e.g temporary fatigue), a sequence of time-motion analysis of 13 players were performed over eight videotaped matches during four time points of a competitive season (Figure 1). Temperature and relative humidity during matches at different time points were as follows: E1 – 1st and 3rd official games, temperature ranged from 24-26°C and relative humidity from 40-44%; E2 - 8th and 10th official games, temperature ranging from 19-21°C and relative humidity from 55-60%; E3 - 15th and 16th official games, temperature ranging from 13-15°C and relative humidity from 60-65%; E4 - 24th and 26th official game, temperature ranging from 14-16°C and relative humidity from 60-75%. In addition, to analyze the influence of player training status in game physical performance, players performed a group of laboratory and field physical tests within two-weeks of each of the two time points of competitive games video-filmed (E1 and E3; Figure 1). The players performed the countermovement jump (CMJ), 5-m (T5) and 30-m (T30) sprints, change of direction ability (t-test), knee extensor (KE) and flexor (KF) maximal isokinetic strength, and the Yo-Yo intermittent endurance test level two (YYIE2). These tests provide valid and reliable data allowing an evaluation of physical fitness parameters directly (agility, sprint, jump, intermittent endurance) and indirectly (isokinetic strength)-related to soccer physical performance. In addition, composite scores were determined to provide a more complex operational indicator of physical fitness. With this option, we aimed to investigate the association between game physical parameters (GPP) and certain soccer players` specific physical fitness parameters to provide a more global indicator of soccer players` training status (e.g the sum of the scores in the soccer specific muscle power-related tests).

*Figure 1 about here
Subjects
A group of 13 male professional soccer players (four defenders, five midfielders and four attackers (mean ± SD: 25.7 ± 4.6 years, body mass 76.5 ± 9.2 kg, 178.1 ± 5.7 cm and 9.8± 3.7% fat percentage) from a professional team competing in the Portuguese championship (Professional Soccer League) was involved. All the subjects had a minimum of three and a maximum of 10 years of senior soccer professional activity. Only injury free players participating in full training schedules were tested. In accordance with the professional Club policy and medical requirements, all soccer players underwent physical examinations both at the beginning and throughout the season (e.g. blood sample analysis, resting ECG, lung x-Ray). All subjects were told of the purpose of the study and written informed consent was obtained according to the Declaration of Helsinki. The study was approved by the Scientific Committee of the Sports Faculty of the University of Porto.

Evaluation procedures
Match analyses
In order to avoid variations in pitch dimensions, all videotaped records were restricted to home-played matches (as Host team), and were filmed by the same group of researchers. Game score and rank of opposing team data are presented in Table 1.

Time-motion was performed according to the procedures defined by Krstrup et al., (26). Each player was filmed close up during the entire match by a VHS movie camera (DCR-HC53E, SONY, JAPAN) positioned at the side of the field, at a height of about 15-m and at a distance of 30 to 40-m from the touchline. The videotapes were later replayed on a monitor for computerized coding of activity patterns. The following locomotor categories were used: standing (0 km·h⁻¹), walking (6 km·h⁻¹), jogging (8 km·h⁻¹), low-speed running (12 km·h⁻¹), moderate-speed running (15 km·h⁻¹), high-speed running (18 km·h⁻¹), sprinting (30 km·h⁻¹) and backward running (10 km·h⁻¹). The locomotor categories were chosen in accordance with Bangsbo et al. (7), whereas the mean speed for each category was determined after detailed studies of video tapes. Thus, the
time for the players to pass landmarks in the grass, center circle and other know
distances was used to calculate the speed for each locomotor activity. The
above activities were later divided into four locomotor categories: (1) standing;
(2) walking; (3) low-intensity running, encompassing jogging, low-speed running
and backward running; and (4) high-intensity running, consisting of moderate-
speed running, high-speed running, and sprinting. The frequency and duration
of each activity were recorded in 5-, 15-, 45- and 90-minutes periods throughout
the game. The distance covered by each locomotor activity was determined in
five-minute intervals as the product of the total time and mean speed for that
activity. The total distance covered during a match was calculated as the sum of
the distances covered during each type of activity. The peak distance covered
in high-intensity running in a five-minute period is also presented. This period
represents that five-minute which contains the most high-intensity running in a
game and is specific to each of the monitored players. All the match recordings
were analyzed by an experienced observer. Krustrup and Bangsbo (24)
observed that the coefficients of variation for test-retest analysis were 1, 2, 5, 3,
and 3%, respectively, for total distance covered, walking, low-intensity running,
high-intensity running, and backward running. In the present study, the
coefficient of variation (CV) for test-retest analysis in different locomotors
parameters was lower than 5%. Each players` locomotive style was previously
extensively analyzed and several validation-tests were performed according to
the predetermined locomotor categories (26). Both halves were analyzed in a
random order.

Physical fitness testing
All evaluations took place at the same time of day, after the players had a
regular overnight sleep. Players were instructed to maintain normal routines for
daily food and water intake, and followed the same dietary recommendations
defined by the medical staff. In addition, during the days of physical tests,
players were instructed to refrain from drinking beverages containing caffeine
and/or alcohol and from consuming food during the three hours before testing.
In the two days preceding evaluations, the players had a day-off (first day) and
a training session (second day) involving low intensity exercises aimed to improve post-match recovery.

Countermovement vertical jump (CMJ), sprint and change of direction (COD) tests were conducted at indoor facilities to exclude the influence of ground surface variations of the soccer pitch on results. The YYIE2 was performed on a field of natural grass where the team normally conducted their training sessions. The order of the tests was as follows: (1) CMJ, (2) sprint, (3) change of direction ability (COD), (4) knee extensor and flexor isokinetic strength and (5) YYIE2. Before the tests, all players performed a ten to fifteen minutes warm-up consisting of light jogging, specific mobility exercises and stretching routines, and three 10-m sprints. Players completed two rounds of each test in the same order. Each subject was allowed a minimum of five-minute rest between tests to ensure adequate recovery.

The CMJ was performed using an Ergojump platform (Digitime 1000, Digest Finland) according to Bosco et al.,(9) whereby the highest vertical jump (centimeters) and the longest flight time (seconds) were registered. Sprint ability measurements were carried out with telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA) placed at the starting line (0 m), at 5 and at 30-m. Change of direction ability (COD), defined as the ability to decelerate, reverse or change movement direction and accelerate again (23) was evaluated using an agility test (T-test; Figure 2). T-test does not include a perceptual or decision making component and can be used to measure change of direction ability. An adapted version of the T-test by Semenick (46) was performed. Subjects began with both feet 0.3-m behind the starting point A. At their own discretion, each subject sprinted forward 9.14-m (10 yd) to point B and touched the base of the cone with their right hand. They then sprinted to the left 4.57-m (5 yd) and touched the base of a cone (C) with their left hand. Subjects then sprinted to the right 9.14-m (10 yd) and touched the base of a cone (D) with the right hand. Next they sprinted to the left 4.57-m back to point B and touched the base of a cone with the left hand. They turned 270° and then ran to point A, passing the finishing line. Two test trials were performed, and times were recorded to the nearest one-hundredth of a second. As described for sprint
ability, measurements were carried out with telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA). Test time was activated when players passed the electronic sensors in Point A, and the clock stopped the instant players again crossed this point. In sprint and COD tests players were instructed to run as quickly as possible from a standing start 0.3-m behind the starting line.

*Figure 2 about here*

To evaluate players’ lower limb muscle function, maximal gravity corrected concentric peak torque of quadriceps and hamstrings was measured during isokinetic knee joint movement (Biodex System 2, NY, USA) of the dominant and nondominant leg at the angular velocity of $90^\circ \cdot \text{sec}^{-1}$ (1.57 rad/sec$^{-1}$), according to Magalhães et al., (27). Prior to muscle function measurements, subjects perform a standardized warm-up consisting of five-minute period on a cycle ergometer (Monark E-824) with a fixed load corresponding to 2% of body weight. Players were then seated on the dynamometer chair at 85$^\circ$ inclination (external angle from the horizontal) with stabilization straps at the trunk, abdomen and thigh to prevent inaccurate joint movements. The contralateral leg was not secured to avoid influencing the strength developed by the knee muscles being tested. The tested knee was positioned at 90$^\circ$ of flexion (0$^\circ$= fully extended knee) and the axis of the dynamometer lever arm was aligned with the distal point of the lateral femoral condyle. Before the anatomical alignments and procedures, all the subjects were instructed to kick and bend the tested leg as hard and as fast as they could through a complete range of motion (from 90$^\circ$ to 0$^\circ$). Subjects were also instructed to hold their arms comfortably across their chest to further isolate knee joint flexion and extension movements. All subjects also performed a specific sub-maximal warm-up protocol on the Biodex device in order to familiarize with the isokinetic device and test procedure that entailed three maximal repetitions at angular velocity $90^\circ \cdot \text{sec}^{-1}$ (1.57 rad/sec$^{-1}$). The YYIE2 was performed after a ten-minutes warm-up consisting of repeated 2x20-m runs back and forth between the start and finish line at a progressively
increased speed controlled by audio bleeps from a CD-ROM according to Bangsbo (5). The initial speed is 11.5 km/h (12.5 s for 2 x 20-m) and between running bouts the participants have a five second rest period. The total distance covered during the YYIE2 was considered as the testing score. Heart rate was measured during the YYIE2 and recorded every five seconds using a HR monitor (POLAR TEAM SYSTEM™, Polar Electro, Kempele, Finland).

Intraclass correlation coefficients of all physical fitness tests were estimated using a test-retest procedure, with a random sub-sample of eight subjects in each evaluation moment. The intraclass correlation coefficients (R) for all variables were as follows. Height, weight and fat mass: 0.93 ≤ R ≤ 0.99; counter movement jump: 0.83 ≤ R ≤ 0.88; sprint time: 0.76 ≤ R ≤ 0.87; agility: 0.75 ≤ R ≤ 0.85; Yo-Yo intermittent: 0.80 ≤ R ≤ 0.97; and isokinetic strength: 0.81 ≤ R ≤ 0.98.

**Statistical Analysis**

All data were reported as means and standard deviations (SD) for each variable. The assumptions of normality were assumed using the Shapiro-Wilks test and Mauchly test, respectively. After these assumptions, analysis of variance for repeated measures was used to determine if there were differences between the different 15-minutes and five-minute periods within the game, as well between the match activity parameters in the different season time points. The Bonferroni test for multiple comparisons was used to identify specific differences between the means in locomotor activities in the different time-points. Intraclass correlation coefficient and coefficient of variation was calculated to estimate the reliability of the physical fitness tests and locomotor parameters, respectively. Z scores were calculated for each test; scores were reversed for the three timed items (T5, T30 and Agility) since lower times reflect better performance. The composite scores were determined to provide a more complex operational indicator of physical fitness. Composite scores of performance in muscle power-related (CSPRT; T5, T30, Agility and CMJ) and isokinetic strength tests were calculated and their association with match analysis data was determined. Correlation coefficients (r) were used to
determine association between physical tests, physical tests composite scores and time-motion data. The SPSS statistical package (version 14.0; Inc., Chicago, Ill) was used. Statistical significance was set at $p \leq 0.05$. 

RESULTS

Seasonal variations

Results regarding seasonal variations in the frequency of activity changes, number of runs in low (FLI) and high intensity (FHI), total distance (TD) covered during game, as well as in each type of movement during the two halves are presented in Tables 1 and 2. The TD in the first 15-minutes period was 10%, 7% and 17% lower ($p < 0.05$) in E2 than in E1, E3 and E4 respectively, and 11% higher ($p < 0.05$) in E4 than in E3. In the last 15-minutes of the game the TD was 14% and 8% higher in E4 than in E2 and E3, respectively ($p < 0.01$). The distance covered in high-intensity running (HI) in the last 15-minutes of the first half of the match in E4 was 49%, 37% and 33% higher ($p < 0.05$) than in E1, E2 and E3, respectively. Also, the HI in the last 15-minutes period of the game in E4 was 43% higher than in E3 ($p < 0.05$). The sum of the distance covered at HI in the two last 15-minutes periods of each half was 36% and 38% higher in E4 than E2 and E3, respectively ($p < 0.05$). The peak 15-minutes period of HI running was 30%, 47% and 43% higher in E4 than in E1, E2, and E3, respectively ($p < 0.05$), while the lower 15-minutes period of HI running during the game was 58%, 46% and 41% higher in E4 than E1, E2 and E3, respectively ($p < 0.05$).

Results from the peak distance covered in HI in a five-minute period (P5-min), in the next five-minute period (Next5-min), in the remaining five-minute periods (Av5-min), and the variation of distance covered from the P5-min to Next5-min (%Dec$_{P5-N5}$) are presented in Figure 3. Players covered a higher distance in HI running in the P5-min ($p < 0.05$) from E4 than in the remaining P5-min periods of the other time points. Furthermore, in all assessments points of the season the distance covered in HI was higher in P5-min ($p < 0.05$) than in Next5-min and Av5-min within each assessment period. Moreover, higher ($p < 0.05$) Av5-
min values were observed in E4 than in E2 and E3. No significant differences were observed between the distance covered in HI in the Next5-min and Av5-min within the different assessment periods.

*Table 1 about here
*Table 2 about here
*Figure 3 about here

Physical fitness results
Results of the different physical tests performed within the two-week period between video-filmed matches are presented in Table 3.

*Table 3 about here

Training status in relation to match analysis
Time motion variables showed that the TD and the distance covered in HI for the first and second halves were not significantly different (4585 ± 265-m vs. 4570 ± 301-m and 632 ± 75-m vs. 680 ± 150-m, respectively). The distance covered in HI during the first 15-minutes period of each half (1st and 4th 15-minutes periods of the game) was higher (p < 0.05) than in the last 15-minute period of each half (3rd and 6th periods of the game, respectively; Figure 4). Tables 4 and 5 show the relationship between time-motion variables and the different muscle power related tests, isokinetic parameters and tests composite scores, respectively. Performance in sprint tests (T5 and T30) and in the composite score of power related tests (CSPRT) were the physical-related parameters that showed the highest correlations with time-motion variables. T30 and CSPRT showed significant correlations (r = 0.622-0.726) with the distance covered in HI running in the five-minute period of the game after P5-min (Next5-min), with the decrement (%) in HI running from P5-min to Next5-min (%DecP5/N5) and with the distance in sprinting in the second half of the game (SP2ndhalf; Table 4).
Knee extension peak torque of non-dominant leg (KEND) showed significant correlations (r ranging from 0.56 to 0.73) with the following time motion parameters: decrement (%) in HI between the highest (4th) and the lowest (6th) intense 15-minutes periods of the second half (HI%Dec4/6), average decrement (%) in HI from the highest to the lowest intense 15-minutes periods (1st to 3rd and 4th to 6th) of both halves (HI%AvDec1/3rd/4th/6th) and decrement (%) in HI from the 1st to the last 15-minutes periods (HI%Dec1/6th) of the game (Table 5). The decrement (%) in HI from the highest to lowest intense 15-minutes periods (1st to 3rd and 4th to 6th) of both halves (HI%AvDec1/3rd/4th/6th) was also correlated (r = 0.59 to 0.73) with knee flexion peak torque of the non-dominant leg (KFND), and with the composite scores of knee extension (CSKE), knee flexion (CSKF) and knee extension and flexion (CSKEF). The distance covered in the YYIE2 was not correlated with any of the time-motion parameters analyzed.

*Table 4 about here
*Table 5 about here
*Figure 4 about here

**DISCUSSION**

The main findings of the present study were that alterations in game physical parameters of professional soccer players occur during the season and that their training status is related to a greater ability to maintain HI-related performance variables during the match. Time-motion analysis of the matches performed at different time points of the season showed that players covered greater total and HI running distances in the last quarter of the season (E4; Table1). Also, it was observed that players were more frequently engaged in HI activities (FHI) during the last quarter of the season (E4; Table1) than in the remaining season periods (E1, E2 and E3). Although differences in HI match running were only significant from E2 and E3 to E4, the analysis of each match half showed that players performed more HI running in the first half in E4 than in the first halves of the remaining periods.
A greater distance in HI was covered in the peak and in the lowest 15-minutes periods of the match in E4 than in the correspondent 15-minutes periods of the other time points of the season. Moreover, the amount of HI performed in the last 15-minutes period of each half, which is indicative of the ability to maintain performance during the game (26), was again higher in E4. In agreement with our findings, others observed that players covered a greater distance in HI at the end than in the middle of the competitive season (30,42). Although not controlled in the present study, an improved physical capacity in the last part of the season could explain, at least in part, our results. In fact, increases in match-related physical performance could be attributed to an improved physical capacity (42). Several studies observed an increase in different physical parameters towards the end of the season (13,21,25,35). Studies involving longitudinal analysis of soccer players physical capacity throughout the season observed increases in soccer specific endurance fitness (25,35), repeated sprint ability (21), speed (13), agility (13) and jump performance (35) towards the end of season. Nevertheless, it is important to note that these findings have not been corroborated by others (14,29).

The performance in a group of physical fitness measures and in a composite score of power-related tests (T5, T30, COD, CMJ and CSPRT) was related to certain time-motion game physical parameters (Table 3). In fact, players with better T5, T30, COD abilities and CSPRT showed an increased performance in Next5-min, a lower decrement from P5-min to the Next5-min, (%DecP5,N5) and performed a higher sprint distances during the second half of games. These findings suggest that soccer players with improved capacity to perform sport specific maximal dynamic activities have an increased ability to maintain performance during short periods of high intensity intermittent exercise and a greater fatigue resistance in the second half of the game. Also, some isokinetic strength parameters (Table 4) and composite scores of knee extension (CSKE), knee flexion (CSKF) and knee extension and flexion (CSKEF) were correlated with game-related physical performance (Table 5). Relationships were observed between KE and KF muscle strength of the non-dominant leg, CSKE, CSKF,
and CSKEF and the following fatigue parameters, namely: decrement in HI from the first and to the final 15-minutes periods of the game and decrement in HI from the highest to lowest intense 15-minutes periods of both halves. These results suggest that greater levels of lower limb strength are related to a higher ability to maintain performance during games (Table 5). This seems to suggest, that players with greater ability to rapidly produce force, allowing fast accelerations and decelerations, and to quickly and efficiently perform complex and coordiated movements, are able to perform at high level in certain game-related physical parameters.

Accordingly, some studies showed that strength (16) and sport specific muscle power evaluated by sprint ability (16) and agility (33) are influenced by the competitive level of the players. Also, high performance levels in jump ability, leg extension strength (3) and in intermittent exercise protocols (43) were observed in players from more successful teams compared with their less successful counterparts.

Rampinini et al. (41) did not observe any relationships between both the best time during a RSSA test (RSSA\textsubscript{best}) and jump ability (Squat Jump), with different match time-motion variables (TD, HI, very high intensity running, and sprinting). However, despite low to medium intensity running is the predominant activity during the match, power-based efforts such as sprints, changing of direction and speed, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power, are widely accepted as essential factors to success in soccer performance (16). In fact, a massive metabolic load is imposed on players not only during the maximal intensities phases of the game but every time acceleration occurs, even when speeds are low (36). These speed and direction of movement changes performed during games impose high levels of stress to the involved musculature, thereby affecting energy usage and resulting in a higher physiologic impact than habitual forward movements (18). Indeed, higher VO\textsubscript{2} (11), blood lactate (11,18), heart rate (18) and rate of perceived exertion (18) values were observed during high intensity intermittent exercise during shuttle mode than during in-line format. Some possible reasons could be the involvement of additional muscles, such as upper
body muscles (22) and of different neuromuscular activation patterns during COD activities (11). At high speed displacements and during intermittent shuttle running, turning technique becomes more important, and anaerobic power is essential because players might accelerate after turning to reach the desired speed (18). The physiological demands and functional characteristics that are typical of soccer specific activity patterns may explain the observed correlations found in the present study between power-related tests and game-physical parameters.

Fatigue is a complex phenomenon that cannot be simply explained by a single factor (19). The ability to resist to fatigue has been related to different functional and physiological features (6,12,19,31). Improvements in coordination specificities (12) and a greater ability to COD (19) are positively associated with performance and with an attenuated fatigue response. Indeed, an optimized performance during the game and particularly during the most intense periods would be expected in players with neuromuscular features tuned for the movements performed during the game (15).

Soccer players with greater CSPRT values may have a higher ability to accelerate, decelerate and change of direction than the players with lower values, leading to an improved physical capacity. This greater physical capacity may likely offset some of the mechanical and neuromuscular effects of repeated stretch-shortening cycle (SSC) fatigue (15). For instance, improved training status seems to be related to the higher ability to regulate joint stiffness more efficiently during exposure to maximal intensity repeated sprints (15).

As games progress, some examples of game–induced fatigue are the reductions in concentric (4,28,40) and eccentric muscle strength (40), as well as in EMG activity (39) of the major lower limb muscles. Game-induced performance decrements are also evident in players’ decreased ability to perform certain strength dependent actions such as sprint (4,28,32) and jump (4,28). In accordance, players’ fatigue reflected in reduced electrical activity of muscles and in compromised strength towards the final periods of the match may cause a lower work-rate towards the end of soccer match (39,40). Moreover, since each specific game action requires breaking and propulsive
forces, the importance of the strength and endurance capacities of leg muscles likely increases as the game progresses (10,11). Thus, strength decrements during the game could affect the performance of explosive actions such as jumping, sprinting and changings of direction, which require high quadriceps strength at the initiation of movement when the joint extension velocity is low (40). It is plausible to assume that soccer players with higher levels of strength have greater ability to maintain strength towards the final stages of each half of the game than players with less strength, and thus show a lower decrement in work rates.

Data suggest that athletes’ ability to exercise during longer periods of time is usually related to their endurance capability (e.g., anaerobic threshold, VO$_{2\text{max}}$). Nevertheless, improved endurance is also influenced by factors related to muscle recruitment and force production (37,38). It was recently observed that distance running performance and running economy of well-trained distance runners are related to the neuromuscular capacity to produce force (34). Also, improvements in soccer players’ maximal strength resulting from training and neural adaptations lead to improved running economy by 4.7% both at the lactate threshold and at a fixed velocity in a treadmill test (20). Rapid force production is considered essential for a wide range of athletes (20,34), with recent reports highlighting that an enhancement in neuromuscular function can be determinant in improving short and long term endurance capacity (1). In fact, there is a consensus that training-related improvements in motor unit recruitment and synchronization result in force potentiation, improving efficiency and coordination, which may delayed the onset of fatigue (17,37,38).

In summary, this study gives empirical support to the neuromuscular parameters measured by sprint, COD, jump ability, strength parameters and their composite scores as indicators of physical performance of soccer players during games. However, it is important to refer that moderate correlations do not affirm a direct cause and effect (41). Indeed, it should be considered that although data from laboratory and field tests are useful in providing information on players’ general physical profile and soccer-specific fitness, test results
should not be used to predict the overall performance during match-play because of the complex nature of the demands of the game (47).

**PRACTICAL APPLICATIONS**

Our results highlight the importance of strength and power for soccer players. In fact, this study report an association between muscle strength and power and performance decrements in game-related physical parameters. Thus, soccer players’ training should incorporate specific exercise programs to improve the athletes’ strength and power during the performance of soccer specific activities. Also, the regular evaluation of the capacity of players’ neuromuscular system to produce force and to perform powerful specific sport activities (e.g. sprint, COD) is advised. In fact, athletes need to successfully perform over competitive seasons of around 10-11 months and, according to our results, these functional qualities of players’ physical fitness seem to be associated with their physical performance during games. Interventional studies designed to analyze the relationship between improvement in these physical parameters (e.g. strength, power, COD) and enhanced game-related physical parameters are warranted.

**ACKNOWLEDGEMENTS**

We thank to the technical staff and to the soccer players of the professional team participating in the study. The results of the present study do not constitute endorsement by NSCA.

**REFERENCES**


Table 1. Rank of opposing team, frequency of activities and distance covered in different locomotion categories throughout the different evaluation moments of the season (means ± standard deviation).

<table>
<thead>
<tr>
<th>Rank opposite team (result)</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rank opposite team</td>
<td>10&lt;sup&gt;th&lt;/sup&gt; - 4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>7&lt;sup&gt;th&lt;/sup&gt; - 9&lt;sup&gt;th&lt;/sup&gt;</td>
<td>14&lt;sup&gt;th&lt;/sup&gt; - 15&lt;sup&gt;th&lt;/sup&gt;</td>
<td>5&lt;sup&gt;th&lt;/sup&gt; - 2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Frequencies (n)</td>
<td>V-V</td>
<td>T-V</td>
<td>V-V</td>
<td>V-T</td>
</tr>
<tr>
<td>Total</td>
<td>1526 ± 251</td>
<td>1577 ± 200</td>
<td>1583 ± 184</td>
<td>1515 ± 143</td>
</tr>
<tr>
<td>FLI</td>
<td>635 ± 88</td>
<td>711 ± 90</td>
<td>718 ± 124</td>
<td>650 ± 89</td>
</tr>
<tr>
<td>FHI</td>
<td>130 ± 27**</td>
<td>130 ± 24**</td>
<td>130 ± 26**</td>
<td>189 ± 41</td>
</tr>
</tbody>
</table>

Distance covered (km)

| TD                          | 9.15 ± 0.64 | 9.0 ± 0.41* | 9.35 ± 0.47 | 9.6 ± 0.31 |
| TD 1<sup>st</sup> half      | 4.5 ± 0.33 | 4.5 ± 0.24** | 4.7 ± 0.27 | 4.8 ± 0.23 |
| TD 2<sup>nd</sup> half      | 4.65 ± 0.39 | 4.5 ± 0.2* | 4.6 ± 0.19 | 4.8 ± 0.15 |
| LI                          | 4.2 ± 0.53 | 4.4 ± 0.44 | 4.7 ± 0.41 | 4.3 ± 0.39 |
| LI 1<sup>st</sup> half      | 2.1 ± 0.31 | 2.2 ± 0.24 | 2.4 ± 0.28† | 2.2 ± 0.30 |
| LI 2<sup>nd</sup> half      | 2.1 ± 0.27 | 2.2 ± 0.21 | 2.3 ± 0.23 | 2.1 ± 0.26 |
| HI                          | 1.35 ± 0.28 | 1.25 ± 0.1** | 1.27 ± 0.1** | 1.9 ± 0.55 |
| HI 1<sup>st</sup> half      | 0.64±0.25** | 0.6 ± 0.09** | 0.63±0.07** | 1.0 ± 0.31 |
| HI 2<sup>nd</sup> half      | 0.72 ± 0.06 | 0.65 ± 0.05 | 0.64 ± 0.06 | 0.9 ± 0.25 |

*significantly different from E3 and E4 (p<0.05); **significantly different from E4 (p<0.05); †significantly different from E1; E1 - Evaluation 1; E2 - Evaluation 2; E3 - Evaluation 3; E4 - Evaluation 4; FLI - frequency of low intensity activities; FHI - frequency of high intensity activities; LI - Low-intensity running; HI - High intensity running; TD - Total distance. V - Victory; T - Tie.
Table 2. Distance covered in different categories of high-intensity running throughout the season (mean ± standard deviation).

<table>
<thead>
<tr>
<th>High-intensity running (m)</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate speed running</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; half</td>
<td>399 + 49</td>
<td>365 + 52*</td>
<td>367 + 50*</td>
<td>552 + 163</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; half</td>
<td>437 + 156</td>
<td>397 + 23</td>
<td>361 + 67</td>
<td>490 + 160</td>
</tr>
<tr>
<td>Total</td>
<td>837 + 157</td>
<td>763 + 48</td>
<td>728 + 99*</td>
<td>1043 + 320</td>
</tr>
<tr>
<td>High speed running</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; half</td>
<td>190 + 34*</td>
<td>189 + 34*</td>
<td>210 + 43</td>
<td>338 + 104</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; half</td>
<td>229 + 77</td>
<td>200 + 30*</td>
<td>219 + 22</td>
<td>322 + 89</td>
</tr>
<tr>
<td>Total</td>
<td>420 + 107</td>
<td>380 + 56*</td>
<td>430 + 54</td>
<td>661 + 193</td>
</tr>
<tr>
<td>Sprint</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; half</td>
<td>46 + 12*</td>
<td>50 + 19*</td>
<td>53 + 26*</td>
<td>124 + 50</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; half</td>
<td>52 + 21</td>
<td>46 + 12</td>
<td>59 + 16</td>
<td>82 + 30</td>
</tr>
<tr>
<td>Total</td>
<td>98 + 28*</td>
<td>96 + 25*</td>
<td>111 + 33*</td>
<td>206 + 78</td>
</tr>
</tbody>
</table>

* significantly different from E4 (p < 0.05); E1 - Evaluation 1; E2 - Evaluation 2; E3 - Evaluation 3; E4 - Evaluation 4;
Table 3. Physiological and functional data of the professional soccer players (mean ± standard deviation; N=13).

<table>
<thead>
<tr>
<th>Muscle power related tests</th>
<th>Isokinetic knee strength (90°.s⁻¹; Nm)</th>
<th>Intermittent endurance test</th>
</tr>
</thead>
<tbody>
<tr>
<td>T5 (s)</td>
<td>T30 (s)</td>
<td>COD (s)</td>
</tr>
<tr>
<td>1.02±0.05</td>
<td>4.2±0.13</td>
<td>8.57±0.4</td>
</tr>
</tbody>
</table>

YYIE2- Yoyo intermittent endurance test level 2; T5- 5 meters sprint time; T30 – 30 meters sprint time; COD- change of direction test; CMJ – counter movement jump; KED – Peak torque of knee extensors dominant leg; KEND – Peak torque of knee extensors non-dominant leg; KFD – Peak torque of knee flexors dominant leg; KFND – Peak torque of knee flexors non-dominant leg; HR$_{\text{max}}$ – maximum heart rate; HR$_{\text{mean}}$ – mean heart rate;
Table 4. Correlations between time-motion variables and sprint, agility, CMJ, and Composite scores of power related tests (CSPRT).

<table>
<thead>
<tr>
<th></th>
<th>T5 (s)</th>
<th>T30 (s)</th>
<th>COD (s)</th>
<th>CMJ</th>
<th>CSPRT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Next5-min</strong></td>
<td>-0.578* (p=0.039)</td>
<td>-0.631* (p=0.021)</td>
<td>-0.545 (p=0.054)</td>
<td>0.400 (p=0.175)</td>
<td>0.677* (p=0.011)</td>
</tr>
<tr>
<td>%Dec&lt;sub&gt;P5/N5&lt;/sub&gt;</td>
<td>0.569* (p=0.042)</td>
<td>0.622* (p=0.023)</td>
<td>0.601* (p=0.030)</td>
<td>-0.516 (p=0.071)</td>
<td>-0.725** (p=0.005)</td>
</tr>
<tr>
<td>HI&lt;sub&gt;3rd&lt;/sub&gt;</td>
<td>-0.674* (p=0.012)</td>
<td>-0.473 (p=0.046)</td>
<td>-0.561* (p=0.022)</td>
<td>0.195 (p=0.031)</td>
<td>0.598* (p=0.034)</td>
</tr>
<tr>
<td>HI&lt;sub&gt;4th&lt;/sub&gt;</td>
<td>-0.623* (p=0.023)</td>
<td>-0.581* (p=0.013)</td>
<td>-0.473 (p=0.043)</td>
<td>0.220 (p=0.060)</td>
<td>0.534 (p=0.016)</td>
</tr>
<tr>
<td>HI&lt;sub&gt;3rd + 6th&lt;/sub&gt;</td>
<td>-0.404 (p=0.171)</td>
<td>-0.348 (p=0.044)</td>
<td>-0.013 (p=0.096)</td>
<td>0.555 (p=0.052)</td>
<td>0.413 (p=0.016)</td>
</tr>
<tr>
<td>SP&lt;sub&gt;1st half&lt;/sub&gt;</td>
<td>-0.589 (p=0.034)</td>
<td>-0.458 (p=0.015)</td>
<td>-0.147 (p=0.074)</td>
<td>0.099 (p=0.025)</td>
<td>0.344 (p=0.023)</td>
</tr>
<tr>
<td>SP&lt;sub&gt;2nd half&lt;/sub&gt;</td>
<td>-0.564 (p=0.045)</td>
<td>-0.726** (p=0.005)</td>
<td>-0.341 (p=0.024)</td>
<td>0.347 (p=0.045)</td>
<td>0.622* (p=0.005)</td>
</tr>
<tr>
<td>SP</td>
<td>-0.621* (p=0.023)</td>
<td>-0.650* (p=0.016)</td>
<td>-0.298 (p=0.032)</td>
<td>0.089 (p=0.072)</td>
<td>0.521 (p=0.023)</td>
</tr>
<tr>
<td>SP&lt;sub&gt;1st + 4th&lt;/sub&gt;</td>
<td>-0.590 (p=0.034)</td>
<td>-0.482 (p=0.006)</td>
<td>-0.194 (p=0.025)</td>
<td>0.101 (p=0.074)</td>
<td>0.430 (p=0.013)</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; T5 - 5-m sprint time; T30 - 30-m sprint time; COD - change of direction test; CMJ - countermovement jump; Next5-min - peak distance in HI in a five-minute period after peak five-minute period (P5-min) of the game; %Dec<sub>P5/N5</sub> - decrement from P5-min to Next5-min; HI<sub>3rd</sub> - distance in HI in the 3<sup>rd</sup> and 4<sup>th</sup> 15-minutes periods of the match; HI<sub>3rd + 6th</sub> - sum of distance in HI in the 3<sup>rd</sup> and 6<sup>th</sup> 15-minutes period of the match; SP<sub>1st half</sub> - distance in sprinting in the first half of the match; SP<sub>2nd half</sub> - distance in sprinting in the second half of the match; SP - distance in sprinting during all match; SP<sub>1st + 4th</sub> - sum of sprinting in the 1<sup>st</sup> and 4<sup>th</sup> 15-minutes periods of the match;
Table 5. Correlations between time-motion variables, isokinetic parameters and composite scores of isokinetic strength

<table>
<thead>
<tr>
<th></th>
<th>KED (Nm)</th>
<th>KEND (Nm)</th>
<th>KFD (Nm)</th>
<th>KFND (Nm)</th>
<th>CSKE (Nm)</th>
<th>CSKF (Nm)</th>
<th>CSKEF (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI%Dec1st/3rd</td>
<td>-0.312</td>
<td>-0.560</td>
<td>-0.346</td>
<td>-0.586*</td>
<td>-0.470</td>
<td>-0.485</td>
<td>-0.497</td>
</tr>
<tr>
<td></td>
<td>(p=0.323) (p=0.058) (p=0.270) (p=0.045) (p=0.123) (p=0.110) (p=0.100)</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>HI%Dec4th/6th</td>
<td>-0.493</td>
<td>-0.611*</td>
<td>-0.414</td>
<td>-0.449</td>
<td>-0.594*</td>
<td>-0.450</td>
<td>-0.542</td>
</tr>
<tr>
<td></td>
<td>(p=0.104) (p=0.035) (p=0.181) (p=0.143) (p=0.042) (p=0.142) (p=0.069)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI%Dec1st/6th</td>
<td>-0.328</td>
<td>-0.581*</td>
<td>-0.329</td>
<td>-0.429</td>
<td>-0.490</td>
<td>-0.395</td>
<td>-0.459</td>
</tr>
<tr>
<td></td>
<td>(p=0.297) (p=0.047) (p=0.297) (p=0.164) (p=0.106) (p=0.204) (p=0.133)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HI%AvDec 1st/3rd-4th/6th</td>
<td>-0.486</td>
<td>-0.733**</td>
<td>-0.470</td>
<td>-0.667*</td>
<td>-0.657*</td>
<td>-0.592*</td>
<td>-0.649*</td>
</tr>
<tr>
<td></td>
<td>(p=0.109) (p=0.007) (p=0.123) (p=0.018) (p=0.020) (p=0.042) (p=0.022)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*p < 0.05; KED – peak torque of knee extensors dominant leg; KEND – peak torque of knee extensors non-dominant leg; KFD – peak torque of knee flexors dominant leg; KFND – peak torque of knee flexors non-dominant leg; CSKE - composite scores of knee extensors; CSKF - composite scores of knee flexors; CSKEF - composite scores of knee extensors and flexors; HI%Dec1st/3rd – decrement (%) in HI from the highest (1st) to the lowest (3rd) intense 15-minutes period of first half; HI%Dec4th/6th – decrement (%) in HI from the highest (4th) to the lowest (6th) intense 15-minutes period of second half; HI%Dec1st/6th – decrement (%) in HI from the 1st to the last 15-minutes period; HI%AvDec1st/3rd-4th/6th – Average decrement (%) from the highest to the lowest intense 15-minutes period (1st to 3rd and 4th to 6th) of both halves (HI%AvDec1st/3rd-4th/6th).
FIGURES

Figure 1 – Season profile and measurement schedule.

Figure 2 - Layout of the T-test. Modified from Semenick (1990).
Figure 3 - Results of peak distance covered in High-intensity in a five-minute period (P5-min), in the next five-minute period (Next5-min), in the average distance covered in the remaining five-minute periods (Av5-min) and variation from peak period to next five-minute period (%DecP5-N5); * significantly lower than P5-min (p < 0.05); † significantly higher than P5-min in E1, E2 and E3 (p < 0.05); ‡ significantly lower than Av5-min in E4 (p < 0.05).

Figure 4 – Distance covered in HI in fifteen-minute periods (n=13). * Significantly different from 0 – 15-minutes period (p < 0.05); † significantly different from 45 – 60-minutes period (p < 0.05).
Title: Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters

Running Title: LIST and Football

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Abstract

The aim of the present study was to analyse the impact of Loughborough Intermittent Shuttle Test (LIST) vs. soccer match on heart rate (HR), muscle damage, redox status, blood leukocytes and neuromuscular function throughout 72h recovery. Sixteen male soccer players (21.3±1.1 years; 175.0±6.0 cm; 70.7±6.3 kg) completed LIST and performed a soccer match separated by 2wks and data were collected before, 30 min, 24, 48 and 72Hhours after LIST and match. HR, plasma creatine kinase (CK) activity, myoglobin (Mb), uric acid (UA), protein sulfhydryls (-SH), malondialdehyde (MDA) contents, total antioxidant status (TAS), blood leukocyte counts, delayed onset muscle soreness, 20m sprint and jump performances, and maximal isokinetic knee extension and flexion were analyzed. HR after LIST was significantly lower than after the match. Post-match TAS was lower and UA was higher than after LIST. Thirty minutes and 24h after soccer MDA was higher and –SH was lower than after LIST (p<0.05). LIST and soccer match induced elevation in total leukocytes and a reduction in lymphocytes at 30 min. This reduction in blood lymphocytes 30 min after match was lower than after LIST. In conclusion, the impact of both exercises did not differ regarding the observed muscle damage markers and some neuromuscular parameters, although soccer require higher cardiac demand and induced higher changes on redox status, adenine nucleotide metabolism and on lymphocyte counts than LIST, which should be taken into account when using LIST to simulate a match to study these type of physiological and biochemical-related endpoints.

Keywords: Intermittent exercise, muscle damage, oxidative damage, antioxidants, fatigue and recovery
Introduction

The physiological, biochemical and neuromuscular impact of intermittent multi-sprint sports, including soccer, has been studied through different methodologies. Time motion analysis of soccer match provides information regarding total distance covered and type of movement, number of physical contacts, tackles, headers and kicks performed by players. Moreover, specific field approaches during soccer matches (Krustrup et al., 2006), tentative replications of match demands in laboratory (Drust, Reilly, & Cable, 2000) or a combination of field and lab tests (Greig, McNaughton, & Lovell, 2006) have been designed to monitor heart rate, blood and muscle metabolite alterations, estimated energy expenditure and oxygen uptake of soccer players. These laboratory and field tests are used to uncover the lack of control of the activity pattern and exercise intensity in a game, resulting from the randomized order of players’ actions, thus allowing a standardized analysis of metabolic, biochemical and functional features of intermittent exercise. Among several intermittent field tests, the Loughborough Intermittent Shuttle Test (LIST) has been used to study the effects of the ingestion of carbohydrate-electrolyte solutions or meals (Foskett, Williams, Boobis, & Tsintzas, 2008; Nicholas, Tsintzas, Boobis, & Williams, 1999; Nicholas, Williams, Lakomy, Phillips, & Nowitz, 1995), fluid ingestion and gastric emptying (Leiper, Nicholas, Ali, Williams, & Maughan, 2005), muscle metabolism and temperature (Morris, Nevill, Boobis, Macdonald, & Williams, 2005), muscle soreness and damage (Thompson, Nicholas, & Williams, 1999), heat acclimatisation protocols (Sunderland, Morris, & Nevill, 2007), cryotherapy treatment against muscle damage (Bailey et al., 2007), as well as the influence of antioxidants (Kingsley, Wadsworth, Kilduff, McEneny, & Benton, 2005; Thompson, Williams, Kingsley, et al., 2001) on intermittent exercise performance and recovery with special reference to soccer. In fact, some authors have suggested that LIST is a field test that simulates the activity pattern and the workload imposed by soccer, i.e., was designed to mimic the activities performed and the distance covered in a typical soccer match (Bishop, Blannin, Robson, Walsh, & Gleeson, 1999;
Nicholas, Nuttall, & Williams, 2000). This was based on data from time-motion analysis and estimated indirect calorimetry variables (Nicholas, et al., 2000). However, the analysis of the distance covered by players during the match tends to underestimate the energy expended as many unorthodox modes of motion, such as running backwards and sideways, jumping, decelerating and changing direction, as well as the dribbling and contesting possession accentuate the mechanical and metabolic loading (Reilly, 1997). Moreover, even considering the pre-defined turns implying acceleration and decelerations during LIST, the great quantity and variety of soccer actions associated with eccentric contractions during a match are expected to cause additional muscle disturbances in soccer when compared to LIST.

Recent studies from our lab and others have analyzed the response of performance indices, muscle damage, inflammation as well as oxidative stress and damage markers of male and female soccer players during the recovery from a match (Andersson et al., 2008; Ascensao et al., 2008; Ispirlidis et al., 2008), as the recovery is thought to be influenced by changes in these functional and biochemical parameters. Some of these endpoints have also been measured during the recovery period after the LIST (Bailey, et al., 2007; Kingsley, et al., 2005; Thompson, et al., 1999; Thompson et al., 2003; Thompson, Williams, Kingsley, et al., 2001). In addition to the comparison of time-motion analysis and estimated indirect calorimetry variables between LIST vs. soccer match (Nicholas, et al., 2000), no data had been published comparing the impact of LIST vs. soccer on biochemical and neuromuscular parameters. Therefore, it would be of interest to examine whether, and to what extent, muscle damage, plasma oxidative stress and damage and blood inflammatory markers, as well as lower limb neuromuscular variables such as jump, 20m sprint ability and strength performance, are altered in response to LIST vs. soccer. Thus, the present study aimed to comparatively analyse the effect of LIST vs. soccer match on muscle damage, plasma antioxidant capacity, oxidative damage, blood leukocyte counts, and neuromuscular variables throughout 72h post-exercise recovery period. Our hypothesis is that soccer induces higher levels of muscle and oxidative damage than LIST.
Methods

Subjects
Sixteen male soccer players from 2nd and 3rd Portuguese divisions participated in this study after being informed about the aims, experimental protocol, procedures and after delivering writing consents. At the time of the experiments, the players were in the competitive period of the season, performing 4-5 training sessions per week. The experimental protocol was approved by the Ethical Committee of Faculty of Sport, University of Porto, Portugal, and followed the Declaration of Helsinki of the World Medical Association for research with humans.

Experimental design and procedures
The players performed the LIST and one soccer match separated by 2-wks (Figure 1). For 2 weeks prior to data collection and during the protocol period, soccer players were instructed not to change their normal eating habits and to refrain from additional vitamin, antioxidant dietary supplementation or any recovery treatment such as cryotherapy. Subjects were also instructed to abstain from exhaustive exercise during the 72-h pre- and post- LIST and match, with exception of the functional evaluation tests. The overall set up was performed during a 3-wks interruption of the competitive period. During the remaining days, with the exception of the 72h pre and post protocol periods, the players were engaged in normal training routines.

The temperature at the days of field exercise (LIST and soccer match) was around 16°C.

***Insert Figure 1***

Blood samples and functional data (jump and sprint performance, quadriceps and hamstrings muscle strength) were assessed pre LIST/match and at 30 min, 24, 48 and 72 h of the recovery period. On the day of the LIST/match, players arrived at the laboratory after an overnight fast between 10.00 and 12.00 h. A
resting blood sample was taken after subjects had been standing for at least 15 min, after which subjects consumed a light standardizing meal and drink and rested for 2 h. According to Thompson et al. (2003) the meal consisted of 1.7 g/kg white bread and 0.3 g/kg of low-fat spread. Rest muscle strength, jump and sprint performance were assessed during the 2 h period between the consumption of pre-exercise meal and the start of the LIST/match.

For 3 days after the LIST/match, subjects returned to the laboratory. A blood sample was taken from the forearm vein in the same conditions above described. Subsequently, the players performed the sprint and strength tests as outlined below.

**Preliminary measurements**

The players performed an incremental (0.1 m/sec increase each 1min step) treadmill (Quasar-Med, Nussdorf, Germany) test until voluntary exhaustion to determine maximal oxygen uptake (VO$_{2}$max) and maximal heart rate (HRmax, Vantage NV, Polar Electro, Finland). Expired respiratory gas fractions were measured using an open circuit breath-by-breath automated gas-analysis system (Cortex, Metalyzer, 3B, Germany). From the VO$_{2}$max, running speeds corresponding to 55% and 95% VO$_{2}$max were calculated. Thereafter, the subjects performed the LIST for 30 min to familiarize with the test. Jump and 20m sprint abilities and strength performance were also evaluated at baseline as described below.

**The Loughborough Intermittent Shuttle Test**

The 90 min shuttle run test was conducted according to Thompson et al. (1999) in a natural green soccer pitch. Briefly, the participants were required to run between two lines, 20m apart, at various speeds dictated by an audio signal and based on the velocities corresponding to their individual VO$_{2}$max. The exercise periods were designed as follows:

* 3 x 20 m walking
* 1 x 20 m maximal running sprint
* 4 sec recovery
* 3 x 20 m at a running speed corresponding to 55% VO$_{2\max}$
* 3 x 20 m at a running speed corresponding to 95% VO$_{2\max}$

This pattern was repeated for each 15 min exercise block followed by the corresponding 3 min recovery period for 5 times.

Heart rate (HR) during the LIST was measured and recorded every 5 s.

**Match time-motion analysis**

For time motion analysis each player was video-filmed close up during the entire match. The videotapes were later replayed for computerized time-motion analyses according to the procedures described by Mohr et al. (2003) The used motor pattern categories included standing (0 km.h$^{-1}$), walking (6 km.h$^{-1}$), jogging (8 km.h$^{-1}$), low-speed running (12 km.h$^{-1}$), moderate-speed running (15 km.h$^{-1}$), high-speed running (18 km.h$^{-1}$), sprinting (30 km.h$^{-1}$), sideways, and backwards (10 km.h$^{-1}$) running. The match activities were later analysed considering standing, walking, jogging, cruising, sprinting, backwards running, and sideways running.

Heart rate was also measured during the match as previously described.

**Delayed Onset muscle soreness (DOMS)**

After LIST/match and prior to blood sampling, each subject was asked to complete a leg muscle soreness questionnaire, in which they rated their perceived muscle soreness on a scale from 0 (normal absence of soreness) to 10 (very intense sore).

**Blood sampling and preparations**

All venous blood samples were taken by conventional clinical procedures as described previously (Magalhaes et al., 2007). An aliquot of the whole blood was used to perform leukocyte counts as indirect markers of muscle damage. The remaining freshly withdrawn blood was immediately centrifuged at 3000 rpm during 10 min to obtain plasma. Plasma was separated into aliquots and rapidly frozen at –80°C for later biochemical analysis of the muscle damage markers myoglobin (Mb) and creatine kinase (CK), as well as the redox state
using total antioxidant status (TAS), malondialdehyde (MDA), protein sulfhydryl groups (SH) and uric acid (UA).

**Biochemical Assays**

**Muscle damage**

Plasma CK activity was determined spectrophotometrically using a commercial kit (ABX A11A01632, Mompelier, FR). Plasma Mb concentration was assessed using a commercial kit (myoglobin bioMerieux 30446, Carnaxide, PT). Leukocyte count was assessed by an automatic cell counter (Horiba 60; ABX Diagnostics, France). Whole blood smears on glass slides (VBS 655/A Microscope - Biosigma) were used for white blood cell differential analysis. Smears were stained using Wright coloring (Merck) and air-dried. Cell differentials were performed using an Olympus microscope equipped with 1000X oil immersion lens.

**Redox state**

TAS was measured spectrophotometrically using a commercial kit (Randox NX2332 Crumlin, UK). Uric acid was determined by an enzymatic method using a commercial kit (Horiba ABX A11A01670, Montpellier, France). Plasma MDA was assayed according to Rohn et al. (1993) with some modifications and measured by the formation of thiobarbituric acid reactive substances at 535 nm. Plasma SH was spectrophotometrically evaluated at 414nm according to Hu (1990). Protein content was spectrophotometrically assayed using bovine serum albumin as standard according to Lowry et al. (1951). Samples were analysed in duplicate and the mean of the two values was used for statistical analysis.

**Jumping performance**

Vertical jumping was evaluated on a Bosco’s mat (Ergojump, Globus, Italy). In accordance with Hertogh et al., (2005) free counter-movement jumps with extension of both upper limbs were chosen to simulate spontaneous jumping movements. The depth of the counter-movement was self-selected and
represented each players’ optimal depth for maximal jump. Each athlete performed three jumps and the best result expressed as jump height was recorded.

**20m sprint ability**
Sprint ability measurements were carried out using telemetric photoelectric cells placed at 0 and 20m (Brower Timing System, IRD-T175, USA). The players stood 1m behind the starting line, started on a verbal signal being time activated when players cross the first pair of photocells, and then ran as fast as they could to complete the 20m distance. Players completed two runs interspersed by 1 min recovery period and the best time was registered.

**Strength assessment**
To evaluate muscle function subjects were familiarized with the muscle function test on at least two occasions during preliminary visits to the laboratory. Maximal gravity corrected concentric peak torque of quadriceps and hamstrings was measured during isokinetic knee joint movement of dominant leg at an angular velocity of 90 s⁻¹ (1.57 rad s⁻¹) using a isokinetic dynamometer (Biodex System 2, USA) as described previously by our group (Magalhães, Oliveira, Ascensao, & Soares, 2004).

**Fluid loss and intake**
To determine sweat loss the players were weighed wearing dry shorts immediately before and after the LIST and match using a digital weight (Tanita Scale BC533). The subjects were allowed to drink water *ad libitum* during both the LIST and the match, and their water intake was recorded.

**Statistics**
Mean, standard deviation and standard error mean were calculated for all variables. A Kolmogorov-Smirnov test was used to test whether physiological, biochemical and neuromuscular-related variables were normally distributed.
Two-way analysis of variance (ANOVA) for repeated measures followed by the Bonferroni post-hoc test was used to compare variables between LIST and soccer at the analyzed time points (before vs. 30 min vs. 24h vs. 48 h vs. 72 h). When there were only single comparisons, a paired sample t-test was used to determine whether any differences between LIST and soccer existed. All data analysis was performed using SPSS 17.0 package. The significance level was set at 5%.

Results
Physiological and anthropometric characteristics of the soccer players are presented in table 1.

*** Insert Table 1 ***

Time-motion analysis showed that players were around 80 minutes of match time involved in low intensity activities including standing, walking, jogging and cruising, and 8 minutes in high intensity activities including sprinting, backwards and sideways running, corresponding to approximately 92 and 8% of the total match time, respectively (table 2).

*** Insert Table 2 ***

The mean heart rate during the match was 173.0±8.8 bpm and the peak heart rate was 195.6±6.0 bpm, which corresponds to 87.1±3.2% and 99.7±7.0%, respectively, of the maximal heart rate previously determined. Mean heart rate during the match (including 1st and 2nd halves) was significantly higher than during the LIST (Figure 2).

*** Insert Figure 2 ***

Plasma Mb content increased 30 min after both LIST and match (p<0.05), returning to baseline at 24, 48 and 72 h recovery. No significant differences
were found between LIST and soccer in any analyzed time point (Fig 3A). Plasma CK activity and DOMS increased at 30 min, 24, 48 and 72 h after LIST and match when compared to pre-exercise values (p<0.05), but no significant differences were found between protocols in any time point (Fig 3B, 3C).

*** Insert Figure 3 ***

Plasma TAS (Fig 4A) increased significantly at 30 min, 24, 48 and 72 h recovery time points after both LIST and match. Plasma UA only increased at 30 min after both LIST and match (Fig 4B). The increases in TAS and UA at 30 min were higher after the match than after the LIST (p<0.05). Plasma MDA and SH levels (Fig 4C, 4D) respectively increased and decreased at 30 min, 24, 48 and 72 h both after LIST and soccer (p<0.05). The increase in MDA and the decrease in SH contents 30 min and 24 h after the match were significantly higher than after LIST.

*** Insert Figure 4 ***

LIST and match increased blood leukocytes counts (Fig 5A) at 30 min (p<0.05) which returned to baseline values at 24, 48 and 72h recovery. No differences were observed between LIST and match for any time point. After both LIST and match, lymphocytes counts at 30 min were significantly lower than pre-exercise values, returning to baseline values at 24 h. However, match induced a significantly higher lymphopenia than LIST at 30 min.

*** Insert Figure 5 ***

LIST and match induced significant reductions in jump (fig 6A) and sprint (fig 6B) abilities as well as in isokinetic peak torques for knee extension (fig 6C) and flexion (fig 6D) until 72 h recovery. No significant differences were found between LIST and match in the analyzed neuromuscular parameters with the
exception of sprint performance, which was less affected after LIST than after soccer at 30 min and 24 h recovery (p<0.05).

*** Insert Figure 6 ***

The fluid loss during LIST vs. match was 0.88 ± 0.17 L vs. 0.90 ± 0.2 L, or 1.2 ± 0.3 % vs. 1.2 ± 0.5 % of the body mass, respectively. The fluid intake was 0.74 ± 0.1 L vs. 0.65 ± 0.1 L. Thus, the total fluid loss was similar between the two conditions, respectively 1.62 ± 0.4 L vs. 1.55 ± 0.3 L, corresponding to 2.3 ± 0.3 % vs. 2.2 ± 0.4 % of the body mass.

Discussion

Intensity of the soccer match

The match examined in the present study was a friendly game played by secondary division players, and it should be thus considered how far the intensity is from games played at an elite level. Nevertheless, the mean and absolute HR values were similar to those reported for Danish soccer players from similar level (Krstrup, et al., 2006). Additionally, time-motion analysis showed that the frequency and the percentage of time both at low and high intensity activities were also similar to that described for players of the same level, although below to those observed in elite players (Mohr, et al., 2003). These observations may suggest that the analyzed match intensity was somewhat similar to other non-elite games, and probably lower than the intensity performed by elite soccer players.

Muscle damage

The tendency and magnitude of changes induced by both LIST and match in muscle damage-related parameters were somewhat expected and in the range of similar exercise protocols (Andersson, et al., 2008; Bailey, et al., 2007; Kingsley, et al., 2005; Thompson et al., 2004; Thompson, et al., 1999; Thompson, Williams, McGregor, et al., 2001), being the significant alterations observed after the match in plasma CK, Mb, DOMS and leg strength close to
some reported after LIST protocol (Kingsley, et al., 2005; Thompson, et al., 1999; Thompson, et al., 2003; Thompson, Williams, Kingsley, et al., 2001).

Although the activity pattern of LIST is representative of the typical activities of soccer, there are activities such as jumping, running backwards and time in possession of ball that are not included. Some of these unorthodox activities, together with tackles and sudden direction changes rely greatly on eccentric contractions, probably increasing the neuromuscular demands imposed by match when compared with LIST. The considerable amount of this type of lengthening-based contractions characteristic of soccer were initially expected to induce additional signs of muscle injury in match when compared to LIST. However, no differences in plasma CK and Mb, as well as in DOMS levels and lower limb strength were observed between the match and the LIST during recovery. One hypothetical reason to explain this absence of differences might be the number of turns, including accelerations and decelerations during LIST.

As reported after other types of exercise (Ascensao et al., 2007; Magalhaes, et al., 2007) and also after match (Ascensao, et al., 2008; Ispirlidis, et al., 2008), data showed that LIST and match induced a leukocytosis. This can be ascribed to the mobilization of blood cells from marginal pools by hemodynamic redistribution and augmentation that resulted from exercise-related metabolic conditions, such as enhanced catecholamine secretion (Bangsbo, 1994). Our results also reported a marked lymphocytopenia during the subsequent period after both exercise protocols. Nevertheless, lymphocyte counts differed significantly between LIST and match at 30 min recovery being lower in match than in LIST. In comparison to concentric exercise, eccentric activity has been shown to result in a greater release of immune system modulators such as proinflammatory cytokines, acute phase proteins, and the recruitment of phagocytic cells with the potential to release ROS (Malm et al., 2000). These signals may favor additional oxidative stress and damage, as well as apoptosis in several tissues and cells, including lymphocytes. However, the hypothesis that a higher apoptosis-induced lymphocytopenia observed after match than after LIST can be attributed to the referred unorthodox eccentric activities during match is unlikely, as Simpson et al.(2007) reported that the levels of blood
lymphocytopenia apoptosis observed after downhill running were not different compared with intensive running. The precise reasons for the lower lymphocyte counts after match should be further investigated.

**Redox status**

Match induced higher increases in UA (30 min) and MDA (30 min and 24 h) contents than LIST. Moreover, TAS (30 min) and SH (30 min and 24 h) were lower after match than after LIST. These results suggest that match may induce higher changes on redox status and on adenine nucleotide metabolism than LIST. Given that around 1-5% of the total oxygen uptake results in the generation of superoxide radical and given the elevated oxygen consumption accompanying both LIST and match, it is not surprising its impact on these biomarkers of oxidative stress and damage. Furthermore, other sources of free radicals can influence cellular and blood antioxidant status. For example, stress hormones undergoing autoxidation (Cooper, Vollaard, Choueiri, & Wilson, 2002) and circulating neutrophils-induced oxidative burst (Quindry, Stone, King, & Broeder, 2003) can contribute to blood oxidative stress and damage. The influence of eccentric exercise-mediating muscular damage-like events on the formation of free radicals has also been reported (Lee & Clarkson, 2003). Considering the specific physiological demands imposed by the intermittent exercise models used, none of these potential free radical sources should be ruled out, although we cannot conclusively demonstrate a causal link between any of those potential sources and the increased plasma oxidative stress and damage.

This study also shows that plasma TAS and UA increased at 30 min after both LIST and match. The observation that plasma UA levels increased in response to LIST and match is consistent with the findings from other studies (Ascensao, et al., 2007; Magalhaes, et al., 2007). However, match induced a higher increase in UA levels than LIST (at 30 min), which should probably due to an enhanced contribution of purine metabolism during match than during LIST. Recent data from Krustup et al. (2006) showed a significant decrease in muscle ATP levels after an intense exercise period in the second half and after
the entire soccer match, as well as significant increase in muscle inosine monophosphate content after an intense exercise period in the second half. Moreover, increased blood ammonia, plasma UA and hypoxanthine contents were earlier reported (Bangsbo, 1994). Therefore, it is likely that the observed increased oxidative stress and damage during the intense exercise periods comprised during the match might have a higher contribution from xanthine oxidase free radical generating system than during LIST.

The enhanced oxidative damage induced by the match compared to LIST can also be observed by the accumulation of lipid peroxidation by-products, measured as plasma MDA. Accordingly, the match also induced a significant decrease in plasma SH, suggesting increased disulphide linkages (-S-S-) from both proteins and reduced glutathione (GSH).

Since both LIST and match did not seem to represent sufficient severe muscular stimuli to cause leukocyte infiltration, as shown by the maintenance of blood leukocyte counts from 24 to 72 h recovery period compared to baseline, the possible effects of neutrophils-related oxidative burst on muscle damage induced by match should probably be ruled out. However, other immune cell-mediated free radical production during the post-exercise periods might be considered, such as monocyte and macrophage oxidative burst (MacIntyre, Reid, & McKenzie, 1995). It is possible that a delayed and continuous monocyte mobilization from bone narrow, thus compensating infiltration of these cells into muscle after damaging exercise (MacIntyre, et al., 1995) had occurred masking leukocyte count changes in blood.

Neuromuscular function
In accordance with previous reports (Andersson, et al., 2008; Bailey, et al., 2007; Kingsley, et al., 2005; Krstrup, et al., 2006; Nicholas, et al., 2000; Thompson, et al., 2004; Thompson, et al., 1999; Thompson, et al., 2003; Thompson, Williams, McGregor, et al., 2001), both LIST and match impaired neuromuscular parameters assessed through measurements of sprint, jump and strength performance. Interestingly, only sprint ability was more affected by match than by LIST. Accordingly, Mohr et al. (2005) observed that soccer
players decreased their ability to sprint after intense periods of the game, as well as after the end of the first and second halves. This temporary fatigue-related impairment should also be expected in jump and strength performance. In fact, considering that, at least in part, similar involvements of metabolic pathways should occur in energy turnover during these maximal power-elicited running and jumping tests, these distinct results were unexpected. An hypothetical explanation based on the presumable changes in the force-velocity relationship as a result of selective damage to type II muscle fibres induced by the great amount of eccentric exercise during match is truly appealing (Twist & Eston, 2005). In fact, a greater reliance on intra-muscular high-energy phosphates in the countermovement jump instead of glycogen was likely to occur. The relatively longer duration of the 2-3 seconds sprint test would rely more heavily on glycogen metabolism, which may be affected by the presumable eccentric exercise-induced muscle-damage and could, at least partially, explain differences between the two tests (Twist & Eston, 2005). In fact, both LIST and match induce significant glycogen depletion in skeletal muscle fibres (Krustrup, et al., 2006; Nicholas, et al., 1999), although the effects of glycogen depletion on high intensity/short duration performance should only occur below critical levels. Alternatively, considering the distinct levels of motor coordination involved in the actions of jumping and sprint running, the repeated eccentric actions performed during the match might cause disturbances in movement control (Bottas, Linnamo, Nicol, & Komi, 2005) affecting more pronouncedly the running performance, which likely rely to a greater extent on muscular coordination than a single jump or leg curl and extension.

In summary, the impact of LIST and match did not differ regarding the observed muscle damage markers and some neuromuscular parameters, although match require higher cardiac demand and induced higher changes on redox status, adenine nucleotide and on lymphocyte metabolism than LIST, which should be taken into account when using LIST to simulate a soccer match to study these type of physiological and biochemical-related endpoints.
Acknowledgments

We would like to thank to soccer players involved in the study for their committed participation. The excellent technical and practical assistance and skillful involvement of Sergio Ribeiro, Ricardo Ladeira, Laura Pereira, Bárbara Duarte, Henrique Reguengo, and camera operators as well as the friendly help of André Seabra in the statistics revision is also appreciated. The authors are grateful to City Council of Maia for providing the pitch where soccer match was carried out. António Ascensão is supported by a grant from the Portuguese Foundation for Science and Technology (SFRH/BPD/42525/2007).

References


Table 1. Anthropometric and physiological characteristics of the subjects before LIST and match

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before LIST</th>
<th>Before match</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>21.3 ± 1.1</td>
<td>21.3 ± 1.1</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>70.7 ± 6.3</td>
<td>69.8 ± 5.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.0 ± 6.0</td>
<td>175.0 ± 6.0</td>
</tr>
<tr>
<td>% Body fat</td>
<td>8.3 ± 1.9</td>
<td>7.9 ± 2.9</td>
</tr>
<tr>
<td>VO$_2$max (mL.kg$^{-1}$.min$^{-1}$)</td>
<td>55.1 ± 5.1</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean ± SD; VO$_2$ max, maximal oxygen uptake
Table 2. Frequency, mean duration and percent of match time spent on the considered motor categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Standing</th>
<th>Walking</th>
<th>Jogging</th>
<th>Cruising</th>
<th>Sprinting</th>
<th>Backwards running</th>
<th>Sideways running</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency ( (n) )</td>
<td>114 ± 44.8</td>
<td>408.6 ± 93.8</td>
<td>441.9 ± 96.2</td>
<td>69.6 ± 10.4</td>
<td>41.7 ± 18.0</td>
<td>122.1 ± 26.6</td>
<td>64.9 ± 4.8</td>
</tr>
<tr>
<td>Mean duration (min)</td>
<td>7.0 ± 2.5</td>
<td>39.5 ± 3.6</td>
<td>31.8 ± 7.4</td>
<td>2.9 ± 1.5</td>
<td>2.2 ± 1.5</td>
<td>4.3 ± 1.3</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>% total time</td>
<td>7.8 ± 3.4</td>
<td>43.8 ± 7.9</td>
<td>35.3 ± 5.6</td>
<td>5.8 ± 2.3</td>
<td>2.5 ± 1.3</td>
<td>4.8 ± 1.9</td>
<td>1.6 ± 0.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD
Figure 1 – Test schedule for the whole test period. Downward arrows denote the time points when sprint and jump performance, isokinetic strength and DOMS were measured. Drop marks denote the time points when blood samples were taken. HR – heart rate.
Figure 2 – Mean heart rate (bpm) values observed during LIST and soccer (1st and 2nd halves and overall). Values are mean and SD. * vs. LIST
Figure 3 – Plasma myoglobin content (A), creatine kinase activity (B) and perceived delayed onset muscle soreness (C) during the 72 h recovery following LIST (triangles) and soccer (squares). Values are mean and SEM. * vs. pre-exercise for both exercises (LIST and soccer); no significant differences were found between LIST and soccer.
Figure 4 – Plasma total antioxidant status (A), uric acid (B), malondialdehyde (C) and sulfhydryl (D) contents during the 72 h recovery following LIST (triangles) and soccer (squares). Values are mean and SEM. * vs. pre-exercise for both exercises (LIST and soccer); # LIST vs. soccer.
Figure 5 – Blood leukocytes (A) and lymphocytes (B) counts during the 72 h recovery following LIST (triangles) and soccer (squares). Values are mean and SEM. * vs. pre-exercise for both exercises (LIST and soccer); # LIST vs. soccer.
Figure 6 – Jump performance (A), sprint performance (B), isokinetic peak torque for knee extension (C) and flexion (D) during the 72 h recovery following LIST (triangles) and soccer (squares). Values are mean and SD. * vs. pre-exercise for both exercises (LIST and soccer); # LIST vs. soccer.
Experimental Work

Study V

TITLE: Neuromuscular function, hormonal and redox status, muscle damage of professional soccer players after a high-level competitive match

RUNNING HEAD: Impact of a soccer match

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Under review
Abstract

The main aim was to analyse the impact of an official high-level competitive match on hormonal and redox status, muscle damage and inflammation and neuromuscular function. Seven high-level male soccer players from the same team performed an official match and data was collected 72-h before, 24-h, 48-h and 72-h post-match. Plasma testosterone/cortisol ratio (T/C), creatine kinase (CK), superoxide dismutase (SOD), glutathione peroxidase (GPX) and reductase (GR) activities, myoglobin (Mb), C-reactive protein (CRP), uric acid (UA), protein sulfhydryls (-SH), malondialdehyde (MDA) contents and total antioxidant status (TAS) were measured. 5 and 30-m sprint, jump and change of direction performance, and maximal isokinetic knee extension and flexion were obtained as neuromuscular functional parameters. Cortisol increased and T/C decreased until 48-h recovery (P<0.05). Mb, CRP and -SH (P<0.05) increased at 24-h and CK, TAS, SOD and MDA (P<0.05) increased up to 48-h recovery. GR increased and GPX decreased at 24-h recovery (P<0.05). Jump performance decreased 24-h post-match (P<0.05). In conclusion, a high-level official match increased catabolic hormonal environment, muscle damage and inflammation as well as oxidative stress at 24-h and 48-h of recovery period. Regardless these alterations, neuromuscular performance of high-level players are affected to a smaller extent and only until 24-h of recovery period.

Keywords: soccer, muscle damage, oxidative stress, anabolic:catabolic balance, fatigue, recovery.
Soccer has been a topic of intense research in the last decade. Different studies showed that soccer competitive demands may impose strains to various physiological systems, including musculoskeletal, nervous, immune and metabolic, to a point where recovery strategies post-exercise became influential in the preparation of the next match (Reilly and Ekblom 2005). During the soccer season, players' physical performance is not only determined by appropriate conditioning but also by the ability to recover and regenerate following multiple stress stimuli (e.g. training and competition) (Kraemer et al. 2004). Moreover, match competition, in particular, seems to represent a major source of physical stress and physiological demands during the competitive season (Reilly and Ekblom 2005; Bangsbo et al. 2006; Reilly et al. 2008). Performance decrements (Ascensao et al. 2008; Ispirlidis et al. 2008; Rampinini et al. 2011; Magalhaes et al. 2010), increases in muscle damage markers (Ascensao et al. 2008; Ispirlidis et al. 2008; Magalhaes et al. 2010; Andersson et al. 2008), a robust pro- and anti-inflammatory cytokine response (Andersson et al. 2010a; Ispirlidis et al. 2008), increased catabolic (Ispirlidis et al. 2008; Kraemer et al. 2004), and pro-oxidant states (Ascensao et al. 2008; Magalhaes et al. 2010) have been observed immediately after “friendly” soccer matches (i.e., without value of any kind to any competitor regardless of the outcome of the competition) and throughout the post-match recovery. Although a considerable amount of studies have been using friendly match and soccer specific protocols performed in laboratory as a model to reproduce the overall game physical demands, data emerging from high-level competitive games are scarce. The analysis of high-level official soccer matches is interesting for both coaches and researchers as these truly represent the real context of a high-level competition. In fact, it is reasonable to assume that the physiological and psychological stress imposed by a friendly match does not mimic the real stress of an official soccer competition. Despite the scarcity of studies, Rodriguez et al., (2007) report that exercise intensity (absolute values and percentage of maximum heart rate) in friendly matches was lower than in official matches of a soccer competition. In addition, due to the increase in the frequency of match
commitments in professional soccer, a clear knowledge of the impact and time course of the physiological changes induced by an official soccer match will help the designing and development of more effective strategies to accelerate the recovery (Andersson et al. 2008).

When research protocols aim to study fatigue and recovery in sport, specific performance variables and biochemical and muscle-status markers should be included in the analysis to achieve a higher efficiency of the monitorization process (Bishop et al. 2008). In fact, performance measurements, hormonal and oxidative and muscle damage markers have been suggested as important stress biomarkers in the monitorization of fatigue and recovery (Finaud et al. 2006; Kraemer and Ratamess 2005; Bishop et al. 2008). Although associations of pro-oxidant redox status, inflammation, muscle damage and physical performance have been established in high-level male (Ispirlidis et al. 2008; Rampinini et al. 2011) and female (Andersson et al. 2010a; Andersson et al. 2010b) and low-level male soccer players (Magalhaes et al. 2010; Fatouros et al. 2010; Ascensao et al. 2008) after friendly matches, they have not been yet examined in the same cohort of male professional players during the post-match recovery period that follows a high-level competitive official match. Given the higher training background of professional players and previous reports from elite female players (Andersson et al. 2008), high-level junior players (Rampinini et al. 2011) and adult elite players (Odetoyinbo et al. 2009) it is expected that the performance impairments and biochemical responses will be less exuberant than those typically reported in lower-level soccer players (Ascensao et al. 2008; Magalhaes et al. 2010; Fatouros et al. 2010). The purpose of the present study is to examine, for the first time, whether and to what extent, muscle damage, plasma oxidative stress and damage, hormonal changes, plasma inflammatory markers, as well as lower limb neuromuscular variables such as jump, 5 and 30-m sprint, change of direction ability and strength performance, are altered in response to a high-level official soccer match.
Methods

Subjects
A group of 7 male professional outfield soccer players (age: 27.4± 4.3 yrs, height: 181.53 ± 8.1 cm, weight: 81.5 ± 8.2 kg, % body fat: 8.1 ± 1.6%) from a team competing in the Portuguese Professional Soccer League participated in this study. The total match duration was 94-min and finished with a draw (2-2). Only the players that played the entire match were used in the analyses. As so, given that one player from the first eleven was injured and substituted in first quarter of the second half and two other were substituted due to coach tactical-technical options in the beginning of the second quarter of the second half only seven players played the full time and thus were involved in the present study. Moreover, the goalkeeper was also excluded due to the obvious physical loading differences compared with the other players of distinct position roles. In each evaluation, only free of injury players involved in the full training schedules were tested. In accordance with the club policy, all soccer players underwent usual physical examination in the beginning and throughout the season (e.g. blood sample analyzes, rest ECG, lung X-Ray). The experimental protocol was approved by the local board, and followed the Declaration of Helsinki of the World Medical Associations for research with humans. All participants were fully informed about the aims, experimental protocol and procedures, and provided written informed consent.

Experimental design
The official match used in this study was the last official game of the championship. The last game of the season was chosen to allow the repeated examination needed for analysing the post-match recovery period. Therefore, the overall set up was performed during the last week of the Portuguese soccer competitive period and the first week after the last official match (off-season). Being the last official match, the players were not involved in any programed training activity and were asked to refrain from any form of physical exercise during the experimental period. Subjects were engaged in normal training
routines during the 72 hours before match, and abstained from exercise during the 72 hours post-match, with exception of the functional evaluation tests. No performance or biochemical variables were obtained at the end of the match due to logistical problems. Blood samples and functional data, namely countermovement jump (CMJ), 5 (T5) and 30 (T30) meters sprint times, change of direction ability (COD; T-test) and leg extensor and flexor isokinetic concentric maximal strength were assessed at different time points namely: pre-match (96-h post the previous official match and 72-h before the studied match), and at 24-h, 48-h and 72-h of the recovery period.

**Procedures**

The game started at 4 p.m. and ended at 6 p.m. with temperature and relative humidity around 20ºC and 45-50%, respectively. In order to match the circadian rhythms of the different variables, data collection (blood sample and functional data measurements) in all time points was always performed at 6 p.m. Procedures order were as follows: 1**(st)** - Blood samples collection; 2**(nd)** - CMJ, 3**(rd)** - T5 and T30, 4**(th)** - COD and 5**(th)** - Isokinetic strength. In the laboratory, a resting blood sample was taken after subjects have been standing for at least 15 min, after which subjects consumed a light standardized meal and drink and rested for 2 h. The meal consisted of 1.7 g white bread and 0.3 g of low-fat spread; both values are per kilogram of body mass (Thompson et al. 2003). CMJ, sprint ability and COD tests were performed in indoor facilities. Before the tests, all the players performed a 10 to 15-min warm-up consisting of light jogging, specific mobility exercises and stretching routine, and 10 meters (m) sprints. Players completed two rounds of each test in the same sequence and the best result was considered for analysis. Each subject was allowed to a minimum of 5-min rest between tests to ensure adequate recovery. Players were instructed to maintain normal routines for daily food and water intake, and followed the same dietary recommendations defined by the medical staff. In addition, during the days of physical tests, players were instructed to refrain from drinking beverages containing caffeine and/or alcohol and from consuming food during the 2 hours before testing.
Physical Performance Tests

Counter-movement Jump (CMJ)
The CMJ was performed using a platform, Ergojump (Digitime 1000, Digest Finland) according to (Bosco et al. 1983) whereby the highest vertical jump (cm) and the longest flying time (s) were registered. The best of the two trials was used for data analyses. Sprint ability

Sprint ability
Sprint measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA) mounted on tripods positioned approximately 0.75-m above the floor and situated 3-m apart facing each other on either side of the starting line (0 m), at 5 and at 30-m. The players stood 0.3-m behind the starting line, started at their own discretion, being time activated when players cross the first pair of photocells, and they ran as fast as they could to complete 30-m distance. The fastest trial was used for data analysis.

Change of direction ability (COD)
Change of direction ability (COD) was evaluated using an agility test (T-test). T-test does not include a perceptual or decision-making component and can be used to measure change of direction ability. An adapted version of the T-test by Semenick (Semenick 1990) was performed. Subjects began with both feet 0.3-m behind the starting point A. At their own discretion, each subject sprinted forward 9.14-m (10 yd) to point B and touched the base of the cone with the right hand. They than sprinted to the left 4.57-m (5 yd) and touched the base of a cone (C) with the left and. Subjects then sprinted to the right 9.14-m (10 yd) and touched the base of a cone (D) with the right hand. They then sprinted to the left 4.57-m back to point B and touched the base of a cone with the left hand. They turn 270º and then ran to point A, passing the finishing line. Two test trials were performed, and times were recorded to the nearest one-hundredth of a second. As described for sprint ability, measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175, Utah, USA). The players stood 0.3-m behind the starting line, being time
activated when they passed the electronic sensors, and the clock stopped the instant players again crossed the Point A. The fastest trial was used for data analyses.

**Strength**

In order to evaluate muscle function, subjects were familiarized with the muscle function test on at least two occasions during preliminary visits to the laboratory. Maximal gravity corrected concentric peak torque of quadriceps and hamstring and ratio between concentric hamstring (H) and quadriceps (Q) peak torque values (H/Q) were measured during isokinetic knee joint movement (Biodex System 2, NY, USA) of the dominant and non-dominant leg at the angular velocity of $90^\circ \cdot s^{-1} \ (1.57 \ rad.s^{-1})$, according to Magalhães et al., (2004). After individual self-report, the dominant leg was determined by a routine visual inspection in a simple target-kicking test requiring accuracy. Prior to muscle function measurements, subjects perform a standardized warm-up consisting of 5-min period on a cycle ergometer (Monark E-824) with a fixed load corresponding to 2% of body weight. Players were then seated on the dynamometer chair at $85^\circ$ inclination (external angle from the horizontal) with stabilization straps and the knee to be tested was positioned at $90^\circ$ of flexion ($0^\circ$= fully extended knee). The subjects were instructed to kick and also to bend the tested leg as hard and as fast as they could through a complete range of motion (from $90^\circ$ to $0^\circ$). All subjects also performed a specific sub-maximal warm-up protocol on the Biodex device in order to familiarize with the isokinetic device and test procedure. Three maximal repetitions at angular velocity $90^\circ \cdot s^{-1} \ (1.57 \ rad.s^{-1})$ were therefore carried out. The ratio between concentric hamstring (H) and quadriceps (Q) peak torque values (H/Q) was also determined and expressed as percentage.

**Blood sampling and preparations**

All the venous blood samples were taken by conventional clinical procedures using EDTA as anticoagulant. Nevertheless, no tourniquet was used in order to minimize potentially oxidative stress induced by an ischemia-reperfusion maneuver.
The freshly withdrawn blood (~10ml) was immediately centrifuged at 3000 rpm during 10 minutes for careful removal of the plasma. Plasma was separated into several aliquots and rapidly frozen at – 80ºC for later biochemical analysis of plasma total testosterone (T), cortisol (C), myoglobin (Mg), creatine kinase (CK), uric acid (UA), C-reactive protein (CRP), total antioxidant status (TAS), superoxide dismutase (SOD) glutathione peroxidase (GPX) and glutathione reductase (GR), sulfhydryl groups (-SH) and malondialdehyde (MDA).

**Biochemical Assays**

**Anabolic/catabolic Status**
Plasma testosterone (T) and cortisol (C) were measured immune-enzymatically using commercial test kits VIDAS® testosterone (REF. 30418) and VIDAS® cortisol S (REF. 30451).

**Muscle Damage**
Plasma creatine kinase (CK) activity was determined spectrophotometrically using a commercial test kit (ABX A11A01632, Mompelier, FR).
Plasma myoglobin concentration was assessed using a commercial test kit (myoglobin bioMerieux 30446, Carnaxide, PT).

**Inflammatory Marker**
C-reactive protein (CRP) was measured using an enzyme-linked immune sorbent assay (ELISA) system (PENTRA 400, Horiba)

**Redox Status**
Total antioxidant status (TAS) was measured spectrophotometrically using a commercial kit (Randox NX2332 Crumlin, UK). Uric acid (UA) was determined by an enzymatic method using a commercial kit (Horiba ABX A11A01670, Montpellier, France).
Regarding enzyme activities in plasma, superoxide dismutase (SOD) activity was measured spectrophotometrically at 550 nm using a commercial Ransod kit from Randox (catalogue no. SD 125, Crumlin, UK). The activity of glutathione peroxidase (GPX) was assayed by a spectrophotometric technique at 340 nm using a commercial Ransel kit from Randox (catalogue no. RS 505). The activity of glutathione reductase (GR) was measured with a spectrophotometric
procedure at 340 nm using a commercial GR kit from Randox (catalogue no. GR 2368).

Plasma MDA was assayed according to Rohn et al., (Rohn et al. 1993) with some modifications and measured by the formation of thiobarbituric acid reactive substances at 535 nm. Plasma SH was spectrophotometrically evaluated at 414 nm according to Hu (Hu 1990). Protein content was spectrophotometrically assayed using bovine serum albumin as standard according to Lowry et al., (Lowry et al. 1951). Samples were analyzed in duplicate and the mean of the two values was used for statistical analysis.

**Statistical Analysis**
Mean, standard deviation (SD) and/or standard error mean (SEM) were calculated. Normality was tested with the Shapiro-Wilks test. After this assumption, One-way analysis of variance (ANOVA) with repeated measures was used to establish whether any of the subsequent test results were significantly different from pre-match results. All data analysis was performed using SPSS 18.0 package. A significance level of 0.05 was chosen.

**Results**
Results of performance in the different muscle power-related tests and isokinetic strength are present in Table 1 and Table 2, respectively. CMJ performance was significantly reduced \( (P < 0.05) \) at 24-h of the post-match recovery period. No changes were observed in the other evaluated parameters.

No significant alterations were observed in plasma total testosterone (T; Table 3) concentration during the recovery period, however plasma cortisol (C) concentration significantly increased at 24-h and 48-h, returning to baseline levels at 72-h \( (P < 0.05; \) Table 3). The T/C ratio decreased significantly at 24-h and 48-h, returning baseline levels at 72-h \( (P < 0.05; \) Table 3).

As can be observed in Table 3, plasma myoglobin (Mb) content was significantly higher \( (P < 0.05) \) at 24-h than in the other assessed moments. Plasma creatine kinase (CK) activity increased at 24-h and 48-h after the match and returned pre-match values at 72-h of the recovery period \( (P < 0.05) \).
A significant increase in plasma C-reactive protein (CRP) content was observed at 24-h post-match (P < 0.05; Table 3) when compared to baseline.

Data concerning the variation on the different redox state parameters throughout the post-match recovery period are described in Table 4. Plasma total antioxidant status (TAS) significantly increased at 24-h and 48-h and returned to control values at 72-h (P < 0.05) of the recovery period. No significant alterations were observed in plasma uric acid (UA) content during the recovery period. Plasma superoxide dismutase (SOD) activity increased at 24-h and 48-h after the match and returned to baseline values at 72-h of the recovery period (P < 0.05). Plasma glutathione peroxidase (GPX) activity decreased at 24-h post-match (P < 0.05) and recovered to control values thereafter. The activity of glutathione reductase (GR; P < 0.05) and protein sulfhydryl content (-SH; P < 0.05) in plasma was significantly higher at 24-h of the recovery period. A significantly higher level of plasma malondialdehyde (MDA) was analysed at 24-h and 48-h of the recovery period (P < 0.05).

**Discussion**

The overall data demonstrate, for the first time, that an official soccer match of elite players favours the hormonal catabolic environment during the recovery period. Also, increased levels of oxidative stress and muscle damage throughout 48-h of the recovery period were observed. However, despite these biochemical alterations, the functional performance pattern of high level soccer players seem to be only slightly affected during the 24-h to 72-h of the post-match recovery period after a competitive match.

Although performance decrements were noted in the muscle-related power and strength tests from pre-match to 24-h post-match, only CMJ significantly decreased. Recently, Rampinini et al., (2011) observed that high-level young players (U-20) partially recover performance at 24-h post-match, been completed at the 48-h of the recovery period. In contrast, data with semi-professional players (Ascensao et al. 2008; Magalhaes et al. 2010; Fatouros et al. 2010), reported longer recovery periods (48-h to 72-h). The reported greater
neuromuscular capabilities of high standard players (Cometti et al. 2001), may explain, at least in part, the observed differences between studies. Knowing that the mechanical stress induced by the eccentric component of the stretch-shortening cycle (SSC) activities may result in ultra-structural muscle damage (Malm 2001), it may be expected that these high level players may possess a high capability to cope with the match demands, and therefore be less susceptible to exercise-induce muscle damage (EIMD) and subsequent performance impairments. Recently, Odetoyinbo et al., (2009) observed that game-physical performances of professional players were not affected when two matches were played interspersed by two days of recovery. In this regard, players of higher standard have shown to be able to sustain a higher level of physical work throughout the match (Mohr et al. 2003), to more quickly recover from high intensity intermittent exercise (Edwards et al. 2003), as well as to regulate core body temperature more effectively at a sustained level (Edwards and Clark 2006). Probably, the higher training background may also explain the faster recovery. In fact, muscle adapts to training regimens that engage them in SSC and are less impaired with repeated exposure (Reilly et al. 2008). Nevertheless, in contrast with ours and others reports (Rampinini et al. 2011; Andersson et al. 2008; Krstrup et al. 2011), longer periods of residual fatigue were observed in professional male soccer players after a friendly match (Ispirlidis et al. 2008). Since the later study did not report the phase of season of the match, the effect of seasonal variations in physical fitness and, consequently, in the results can not be excluded. Moreover, in a recent report (Silva et al. 2011), we observed that the systematic participation of professional players in soccer matches favours the increase and maintenance of athletes knee extensor (KE) and knee flexor (KF) isokinetic muscle strength and short-sprint ability.

In the present study, CMJ was the only neuromuscular parameter impaired at 24-h recovery. Accordingly, Andersson et al., (2008) reported a longer impairment of CMJ, when compared to other neuromuscular parameters, after a friendly elite female soccer match. During recovery, the neuromuscular system can adjust to the contractile failure in a very flexible way (Nicol et al. 2006) that
turns it difficult to establish a direct link to which mechanism can be related to the distinct recovery pattern observed. Moreover, the different neuromuscular parameters assessed (CMJ, sprint and COD) have been described as independent motor abilities (Salaj and Markovic 2011). CMJ has been characterized as a concentric and slow SSC-related action (Salaj and Markovic 2011), that in contrast with other activities (e.g. running), rely in slower transition between stretch to shortening phases, as well as by a lower stretch velocity, which limits stretch reflex activation (Wilson and Flanagan 2008). Certain specific components of the SSC might attenuate the detrimental performance effects associated with EIMD i.e., increases the pre-activation of the muscle (Byrne et al. 2004). Nevertheless, studies investigating SSC fatigue reported greater performance impairments in SSC actions involving higher (e.g. sprint, drop jump) than lower (e.g. CMJ and Squat jump) ground impact forces (Nicol et al. 2006). However, others did not observe impairments in sprint performance (5-10-20-30-m) after EIMD (Semark et al. 1999). Despite the preservation of post-match muscle peak torque that may indirectly indicate absence of muscle damage we examine increases in other indirect markers of EIMD (e.g. CK). Moreover, it cannot be excluded, amongst other factors, a decrease in work capacity resulting of a lower force capability in all joint range of motion (e.g. force decrements or damage at specific muscle lengths). If this fact occurred, it may had influence distinctly the recovery pattern of the different motor abilities due to their kinetics and kinematics characteristics. Therefore, dissimilarities in the recovery pattern of the parameters measured in this study seem to highlight the complexity of the mechanisms of the neuromuscular SSC fatigue (Nicol et al. 2006). In accordance, Andersson et al., (2008) suggested that the greater delay in CMJ recovery compared to other neuromuscular parameters were not solely dependent of the recovery of the capacity of force production from the extensors and flexors muscles but also from additional mechanisms related with SSC fatigue. Results of H/QND ratio showed that in the non-dominant limb the extensors were more affected than the flexors, which may be related, at least in part, with different intramuscular motor pattern activation between both limbs.
According with our results, the official soccer match altered the catabolic/anabolic-related hormonal homeostasis towards a predominant catabolic response during the first 48 hours of the recovery period. Ispiridis et al., (2008) observed that a friendly soccer match induced significant increases in cortisol concentration only immediately after. Also in line with our findings, the authors did not observe alterations in plasma testosterone (free testosterone) concentration through the 144 hours of the recovery period of the study (Ispiridis et al. 2008). To our knowledge, no reports on T/C during the recovery period after a soccer match have yet been described. Although a decrease in anabolic/catabolic ratio have been suggested to decrease performance (Adlercreutz et al. 1986), different studies observed that soccer players performance increments can be related with both decrements (Gorostiaga et al. 2004) and increments in T/C (Kraemer et al. 2004).

The increase in plasma concentration of certain intracellular proteins (e.g. Mb, CK, LDH) has been widely used as indirect markers of tissue damage [for refs see (Powers and Jackson 2008; Malm 2001)]. Our data corroborate previous experiments carried out with semi-professional and professional players that show increases in plasma concentrations of certain intracellular proteins throughout recovery period. In this regard, increased Mb contents immediately after (Ascensao et al. 2008; Magalhaes et al. 2010) and in CK activity throughout the 72-h to 96-h of the recovery period of a friendly match (CK) of semi-professional (Ascensao et al. 2008; Fatouros et al. 2010; Magalhaes et al. 2010) and professional players (Ispiridis et al. 2008), have been reported. These previous studies with semi-professional (Ascensao et al. 2008; Magalhaes et al. 2010; Fatouros et al. 2010) and professional male players (Ispiridis et al. 2008), observed lower baselines values of CK (approximately ranging from 80 to 200 U.L⁻¹) than those observed in the present study (300.6 U.L⁻¹) and with other professional players (310 U.L⁻¹) (Rampinini et al. 2011). Considering that our baseline values were obtained 96-h after the previous official game of the regular championship, probably the higher pre-match CK values in the professional players involved in our study may be associated to "residual" levels of muscle damage because of the regular training that soccer
players undergone (McLellan et al. 2010). The longer responses (72-h to 96-h post-match) and/or higher variations (from baseline to 24 and 48-h of recovery) have been reported in semi-professional (Ascensao et al. 2008; Fatouros et al. 2010; Magalhaes et al. 2010) and professional players (Ispirlidis et al. 2008) (ranging from 400 to 800%) than the observed in our (85 to 201%) and other studies with players of the same standard (Krstrup et al. 2011; Andersson et al. 2008; Rampinini et al. 2011) can be related with the training status in the moment of the match. In fact, training status has shown to be a determinant factor in the magnitude of the post-exercise CK changes since less trained subjects have been shown to exhibit the higher post-exercise increases (Maxwell and Bloor 1981; Garry and McShane 2000). Nevertheless, given the high number of factors that are associated with plasma CK inter-individual variability (e.g. age, muscle mass, physical activity) both in rest and in response to exercise (low and high responders) (Brancaccio et al. 2007) it is difficult to compare the magnitude of the changes between studies. Despite some controversy, CK has been widely used as a stress bio-marker that reflects damage of the muscle tissue (Brancaccio et al. 2007). Given the small degree of neuromuscular impairments and the faster recovery period (only CMJ performance was impaired at 24-h) it is possible to assume that if any muscle damage occurred it was of minor magnitude allowing the preservation of the functionality of the contractile machinery and, concomitantly, of performance. In fact, some studies evidence that muscle enzyme release may not clearly reflect the size of the injury (e.g. evaluated by histological analysis), being the efflux of intracellular proteins a possible result of both damage and transient changes in membrane permeability (Malm 2001; Brancaccio et al. 2007).

An acute phase inflammatory response has been described after friendly-match performed by professional and semi-professional soccer players, suggested by an increase in blood leukocyte counts (Ascensao et al. 2008; Ispirlidis et al. 2008; Andersson et al. 2010a; Magalhaes et al. 2010), inflammatory mediators (Ispirlidis et al. 2008; Andersson et al. 2010a) and in cortisol levels (Ispirlidis et al. 2008). In accordance with our results, Ispirlidis et al., (2008) observed in
professional players that CRP content increased at the same time-point (24-h) of the recovery period and with the same magnitude after a friendly match. The high absolute levels of mitochondrial oxygen consumption, the increased oxidation of circulating catecholamines, the intermittent and repeated sprint actions-causing temporary ischemia-reperfusion events in skeletal muscle are plausible factors that may induce reactive oxygen and nitrogen species (RONS) production during and after a soccer match (Ascensao et al. 2008). However, an increase in RONS during and following acute exercise is believed to serve the necessary “signal” for the hormetic-associated up regulation in antioxidant defence commonly observed with chronic exercise training (Fisher-Wellman and Bloomer 2009). Although evidences suggest that soccer players may show higher plasma activity of certain endogenous antioxidants than untrained individuals, there are some contradictory reports regarding TAS (Brites et al. 1999; Banfi et al. 2006; Cazzola et al. 2003). We observed an increase in TAS at 24 and 48-h post-match recovery period. Our data, together with other reports examining semi-professional players TAS responses to other match models (e.g. friendly match and LIST), seems to support the idea that a soccer match induces a pro-oxidant insult resulting in a compensatory response in plasma TAS immediately after (Ascensao et al. 2008; Magalhaes et al. 2010) and during the first 48-h of post-match recovery period (Ascensao et al. 2008; Fatouros et al. 2010; Magalhaes et al. 2010). Consistently, Andersson et al., (2008) observed increases in specific endogenous (non-enzymatic) compounds and in certain dietary antioxidants immediately and during the 72-h post-match recovery period, respectively, being this delayed increase in dietary antioxidants during recovery a putative mechanism to strengthen the endogenous antioxidant defence and restore the redox balance (Andersson et al. 2010b).

Although we did not observe variations in uric acid (UA), recent studies have shown that plasma UA levels of semi-professional players increase immediately after a friendly match (Ascensao et al. 2008; Magalhaes et al. 2010) and during the 48-h (Fatouros et al. 2010) of the recovery period. Also, an increase in UA until 96-h of the recovery period of professional players has been reported (Ispirlidis et al. 2008). Nevertheless, Andersson et al., (2010b) observed that
after a friendly match, UA levels of elite female soccer players increased immediately after the game but return to pre-match levels at 21-h of the recovery period. All together, data seems to suggest that lower-level compared to high-level players rely more strongly on the degradation of purine nucleotides as reflected by the higher increments in UA contents throughout recovery. In addition, the longer post-match recovery pattern reported by Ispirilidis et al., (2008) e.g. physical parameters, CK and UA responses, in contrast to our and others (Andersson et al. 2008; Rampinini et al. 2011) involving players of the same standard could be related with players fitness levels in the moment of the match i.e., with seasonal variations in physical fitness. In line with previous antioxidant markers, plasma superoxide dismutase (SOD) activity was significantly higher during the 48-h of the post-match recovery period. Despite no reports of SOD kinetic after a soccer match have been published, studies with untrained individuals and other athletic populations suggest that physical exercise can result in increments or maintenance of SOD activity (Fisher-Wellman and Bloomer 2009). Given that SOD is considered the first enzymatic defence line against oxidative stress (Finaud et al. 2006; Powers and Jackson 2008) and that the magnitude of exercise-mediated changes in skeletal muscle SOD activity increases in function of the intensity and duration of the exercise (Powers and Jackson 2008), alterations may be expected due to the high physiologic demands of an official soccer match.

With respect to the antioxidant glutathione cycle-related enzymes, although we observed a decrease in GPX only at 24-h of recovery period, others (Fatouros et al. 2010), examining semi-professional players, observed an increased activity during the 24 to 72-h post-match recovery period. To our knowledge, this is the first study that analysed the effect of a soccer match in plasma GR activity. We observed an up-regulation of plasma GR activity at 24-h post-match. Taking into account the critical physiological role of the balance between the first (SOD) and second step (GPX and/or catalase) antioxidant enzymes, the significant increase in plasma SOD/GPX ratio observed after the game, mainly due to a decreased in GPX activity as referred to previously, seems to favour $H_2O_2$ accumulation. This fact, could be indicative of a low plasma
scavenging efficiency contributing to enhanced oxidative stress (Gaeta et al. 2002), which is consistent with the increased levels of MDA found in present study and others (Magalhaes et al. 2010; Ispirlidis et al. 2008; Ascensao et al. 2008; Fatouros et al. 2010).

Regarding plasma sulfhydryl residues (-SH) we observed an increase at 24-h of the recovery period. These results can indicate decreased disulphide linkages (-S-S-) from both proteins and reduced glutathione. These findings initially were not expected due to previous reports of decreases in plasma –SH levels of semi-professional soccer players immediately after and through the first 48-h of the recovery period (Ascensao et al. 2008; Magalhaes et al. 2010). Moreover, a decrease in GSH and GSH/GSSG and increases in GSSG during the post-match recovery period have been observed in semi-professional soccer players (Fatouros et al. 2010). Nevertheless, these findings were not confirmed in elite female players. Andersson et al., (2010b) observed that the immediately post-match increase in GSSG and decrease in GSH/GSSG returned to baseline at 21-h of the recovery period. Moreover, GSH content was unaltered during the observation period (Andersson et al. 2010b). The moment of the season when the investigation took place might contribute to the different results between studies. In fact, some reports suggest that the antioxidant status (e.g. TAS) may change within the season (Teixeira et al. 2009) influencing the capacity of the antioxidant defence system and the ability to restore the redox balance. Players training background (Martinovic et al. 2009) (e.g. years of practice) and standard (Nakagami et al. 2009) cannot be ruled out as factors that may influence the different results between studies, since they have been associated with higher concentrations of sulfhydryl groups. Probably the vigorous training and competition resulting in oxidative stress may lead to an up regulation in antioxidant defences and associated shift in redox balance in favour of a more reducing environment, thereby protecting athlete from excessive oxidative damage during subsequent training sessions (Fisher-Wellman and Bloomer 2009).

In summary, a high-level official match led to increased catabolic hormonal environment, markers of muscle damage, inflammation and oxidative stress
until 48-h of recovery period. Although match induced certain biochemical alterations, the performance of these high-level adult soccer players was affected only to a smaller extent and until 24-h of recovery period. This suggests that high-level players possess a high capability to cope with the game demands as shown by the fast recovery pattern and lower performance impairments, at least in the examined neuromuscular parameters. Nevertheless, together with previous studies conducted with players of different competitive level and gender, data confirm that soccer match induces temporary disturbances in the homeostasis of several physiological systems. Therefore, studies concerning recovery strategies in soccer are needed.

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Ethical standards
The authors declare that all the procedures of the experiments comply with the current laws of the country in which they were performed.

Conflict of interest
The authors declare that they have no conflict of interest.

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Andersson H, Bohn SK, Raastad T, Paulsen G, Blomhoff R, Kadi F (2010a) Differences in the inflammatory plasma cytokine response following two


Teixeira V, Valente H, Casal S, Pereira L, Marques F, Moreira P (2009) Antioxidant status, oxidative stress, and damage in elite kayakers after 1


Table 1: Variation of functional performance measurements in the four evaluation moments

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-match</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>T5-m (s)</td>
<td>1.013 ± 0.1</td>
<td>1.063 ± 0.51</td>
<td>1.01 ± 0.52</td>
<td>1.015 ± 0.17</td>
</tr>
<tr>
<td>T30-m (s)</td>
<td>4.204 ± 0.19</td>
<td>4.232 ± 0.2</td>
<td>4.202 ± 0.18</td>
<td>4.207 ± 0.22</td>
</tr>
<tr>
<td>CMJ (cm)</td>
<td>43.83 ± 4.89</td>
<td>40.75 ± 4.68*</td>
<td>43.15 ± 4.52</td>
<td>43.60 ± 4.61</td>
</tr>
<tr>
<td>COD (s)</td>
<td>8.67 ± 0.20</td>
<td>8.85 ± 0.41</td>
<td>8.57 ± 0.22</td>
<td>8.70 ± 0.25</td>
</tr>
</tbody>
</table>

Data are mean ± SD (n=7). T5-m – 5 meters sprint time; T30-m – 30 meters sprint time; CMJ – countermovement jump; COD – change of direction; * significantly different from pre-match and 72-h (P < 0.05)
Table 2 Variation of isokinetic strength measurements in the four evaluation moments.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-match</th>
<th>24-h post</th>
<th>48-h post</th>
<th>72-h post</th>
</tr>
</thead>
<tbody>
<tr>
<td>KED 90°.s⁻¹ (Nm)</td>
<td>279.8 ± 50.1</td>
<td>263.5 ± 59.3</td>
<td>272.9 ± 38.9</td>
<td>274.6 ± 51.1</td>
</tr>
<tr>
<td>KFD 90°.s⁻¹ (Nm)</td>
<td>155.2 ± 22.9</td>
<td>147.6 ± 22.1</td>
<td>150.9 ± 13.2</td>
<td>151.5 ± 23.6</td>
</tr>
<tr>
<td>H/Q D (%)</td>
<td>55.5 ± 3.1</td>
<td>56.7 ± 3.9</td>
<td>55.5 ± 3.7</td>
<td>55.5 ± 3.8</td>
</tr>
<tr>
<td>KEND 90°.s⁻¹ (Nm)</td>
<td>287.1 ± 60.1</td>
<td>258.9 ± 53.3</td>
<td>273.7 ± 36</td>
<td>282.2 ± 62.1</td>
</tr>
<tr>
<td>KFND 90°.s⁻¹ (Nm)</td>
<td>156.9 ± 25.1</td>
<td>145.7 ± 23</td>
<td>150.4 ± 15.5</td>
<td>151.4 ± 26.3</td>
</tr>
<tr>
<td>H/Q ND (%)</td>
<td>54.6 ± 4.8</td>
<td>56.8 ± 3.3</td>
<td>54.1 ± 2.8</td>
<td>54.3 ± 5</td>
</tr>
</tbody>
</table>

Data are mean ± SD (n=7). KED- peak torque in knee extension dominant legs; KEND- peak torque in knee extension non-dominant legs; KFD- peak torque in knee flexion dominant legs; KFND- peak torque in knee flexion non-dominant legs; H/Q D- concentric hamstrings/quadriceps strength ratio dominant leg; H/Q ND- concentric hamstrings quadriceps strength ratio non-dominant leg;
Table 3 Hormonal, muscle damage and inflammatory responses induced by an official soccer match.

<table>
<thead>
<tr>
<th></th>
<th>T (ng/ml)</th>
<th>C (ng/ml)</th>
<th>T/C (%)</th>
<th>Mb (µg/L)</th>
<th>CK (U/L)</th>
<th>CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-match</td>
<td>5.94 ± 0.4</td>
<td>50.5 ± 2.6</td>
<td>12.5 ± 0.6</td>
<td>17 ± 1.7</td>
<td>300.6 ± 34</td>
<td>1.04 ± 0.2</td>
</tr>
<tr>
<td>24-h post</td>
<td>6 ± 0.5 (+1)</td>
<td>64 ± 3 (+27)</td>
<td>10 ± 0.9 (-20)</td>
<td>40 ± 3.5 (+136)</td>
<td>900 ± 96 (+201)</td>
<td>2.4 ± 0.5 (+131)</td>
</tr>
<tr>
<td>48-h post</td>
<td>5.93 ± 0.6 (+0.2)</td>
<td>81 ± 9.1 (+60)</td>
<td>8.3 ± 1.5 (-34)</td>
<td>20.5 ± 1.9 (+21)</td>
<td>560 ± 62 (+86)</td>
<td>1.6 ± 0.4 (+54)</td>
</tr>
<tr>
<td>72-h post</td>
<td>5.94 ± 0.4 (+0.1)</td>
<td>52 ± 3.2 (+3)</td>
<td>11.5 ± 0.8 (-8)</td>
<td>17.5 ± 1.6 (+3)</td>
<td>320 ± 33 (+7)</td>
<td>1.07 ± 0.2 (+3)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM (percentage of variation - %). * significantly different from pre-match (P < 0.05); T- total testosterone; C- cortisol; T/C- testosterone cortisol ratio; CK- Creatine kinase; CRP- C-reactive protein;
Table 4 Oxidative stress and damage induced by an official soccer match

<table>
<thead>
<tr>
<th></th>
<th>TAS (nmol)</th>
<th>UA (mg/dl)</th>
<th>SOD (U/L)</th>
<th>GPX (U/L)</th>
<th>GR (U/L)</th>
<th>-SH (µmol/g)</th>
<th>MDA (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-match</td>
<td>1.06 ± 0.01</td>
<td>4.8 ± 0.11</td>
<td>711 ± 7.7</td>
<td>1006 ± 19</td>
<td>65 ± 0.7</td>
<td>1.2 ± 0.05</td>
<td>13 ± 1.2</td>
</tr>
<tr>
<td>24-h post</td>
<td>1.2 ± 0.04 (+13)*</td>
<td>4.9 ± 0.5 (+2.1)</td>
<td>781 ± 8 (+9.8)*</td>
<td>921 ± 32 (-8.4)*</td>
<td>72 ± 1.7 (+11)*</td>
<td>2.2 ± 0.1 (+83)*</td>
<td>22 ± 2.6 (+70)*</td>
</tr>
<tr>
<td>48-h post</td>
<td>1.15 ± 0.03 (+8.5)*</td>
<td>5.7 ± 0.3 (+19)</td>
<td>745 ± 5 (+4.8)*</td>
<td>964 ± 31 (-4.2)</td>
<td>67 ± 2 (+3.1)</td>
<td>1.1 ± 0.05 (-8.3)</td>
<td>17 ± 0.3 (+31)*</td>
</tr>
<tr>
<td>72-h post</td>
<td>1.07 ± 0.01 (+2.8)</td>
<td>4.7 ± 0.2 (+2.1)</td>
<td>712 ± 7.8 (+1.2)</td>
<td>1000 ± 18 (-0.6)</td>
<td>64.9 ± 0.8 (-0.2)</td>
<td>1.3 ± 0.05 (+8)</td>
<td>14 ± 1.3 (+7.7)</td>
</tr>
</tbody>
</table>

Data are mean ± SEM (percentage of variation - %). *significantly different from pre-match (P < 0.05); TAS- total antioxidant status; UA- uric acid; SOD- superoxide dismutase; GPX- glutathione peroxidase; GR- glutathione reductase; -SH- sulfhydryls groups; MDA- malondialdehyde;
General Discussion

The overall picture of the studies comprised in this dissertation is that even though soccer players are submitted to an increase in certain markers of physiological stress during practices and competitions, professional soccer players seem to possess a good capability to cope with the soccer physiological demands.

Data from this dissertation complemented with data from other studies conducted with soccer players of different standards, showed that both soccer specific endurance performance (e.g. game-physical performance) and neuromuscular function improve during the season (S-I and S-III) in spite of the increase in certain bio-markers of physiological stress observed during practices and competition (S-II, S-V). The recovery period between seasons (off-season) resulted in certain biochemical alterations such as a decrease in muscle damage and oxidative stress markers (S-II). Additionally, as already observed in other intermittent team sports such as handball (Gorostiaga et al., 2006; Granados et al., 2008), studies I and II showed that the individual match playing time (IMPT) throughout the season may influence players’ physical fitness. Moreover, associations between IMPT, physical performance in the match and in functional tests, and biochemical parameters were observed (S-I, S-II and S-III).

In line with previous data (Andersson et al., 2010a; Andersson et al., 2010c; Andersson et al., 2008; Ascensao et al., 2008; Greig, 2008; Krustrup et al., 2011; Rampinini et al., 2011; Reilly & Rigby, 2002; Small et al., 2008; Small et al., 2009; Thompson et al., 1999), our studies showed that soccer matches examined by distinct models such as intermittent shuttle exercise (S-IV), friendly match (S-IV) and/or official match (S-V) resulted in performance impairments and increased stress markers throughout the recovery period of semi-professional (S-IV) and professional players (S-V). Nevertheless, distinct recovery patterns and biochemical responses were observed in distinct standards of players and models of investigation (e.g., friendly match vs. soccer specific field test). In fact, a friendly-match (S-IV) when compared to a specific
protocol (LIST; S-IV) resulted in higher cardiac demands, changes on redox status, adenine nucleotide metabolism and on lymphocyte counts in semi-professional players. Moreover, in a high-level official match (S-V), increased catabolic hormonal environment, muscle damage and inflammation, as well as oxidative stress throughout recovery period were observed. However, in spite of these biochemical alterations, high-level players showed lower performance impairments and faster recovery kinetics of distinct neuromuscular capabilities. In addition, data from study III highlighted that professional players’ physical fitness and training status (e.g., neuromuscular capabilities) can be associated with physical performance in the match.

The knowledge of players’ physiological and functional characteristics is not only a matter of undeniable interest, but also the understanding of how these characteristics are altered throughout the training process is important for coaches, medical departments and researchers. In this regard, review I and II examined the different anthropometric, physiological and neuromuscular alterations that occur during the annual soccer training cycle (preseason, in-season and off season). Distinct patterns are presented in relation to the physiological and functional adaptations during preseason and in-season training. Most of the studies showed that VO$_2$max increases (average increments ranging 5 to 11%) during preseason (Aziz et al., 2005; Bogdanis et al., 2009; Caldwell & Peters, 2009; Kalapotharakos et al., 2011; Mercer et al., 1997; Metaxas et al., 2006) and is maintained towards in-season (Haritodinis et al., 2004; Kalapotharakos et al., 2011; Mercer et al., 1997; Metaxas et al., 2006; Mohr et al., 2002b). In addition, literature shows that above a threshold of 62 ml.kg$^{-1}$.min$^{-1}$, no significant increments are observed (Clark et al., 2008; Edwards et al., 2003a). In fact, the referred increases in VO$_2$max found in different standard of players during in-season (Caldwell & Peters, 2009; Ferrari Bravo et al., 2008; Jensen et al., 2009; Magal et al., 2009) occurred under this threshold. Maximal aerobic speed (MAS) improves during preseason (3.1-9.2%) (Arent et al., 2010; Haritodinis et al., 2004; Kalapotharakos et al., 2011; Metaxas et al., 2006; Wong et al., 2010), and improvements of different magnitude (4.5-21.6%) were also observed in different physiological indices.
measured during exercise at sub-maximal intensities (Helgerud et al., 2001; Impellizzeri et al., 2006; Kalapotharakos et al., 2011; Zoppi et al., 2006). Additionally, in line with other researches (Laia et al., 2009b; Krstrup et al., 2003a; Krstrup et al., 2006a; Rebelo & Soares, 1997), in study I we observed faster and greater soccer specific endurance (SSE; e.g. Yo-Yo tests) improvements during preseason than during the other periods of the season. In addition, field-tests showed great average increments in SSE (e.g., Yo-Yo tests, R-I and R-II) than physiological endurance-related parameters analysed in laboratory. Regarding in-season alterations, although the majority of the studies did not report in-season VO\textsubscript{2}\text{max} improvements, other endurance parameters, such as MAS and physiological adaptations during submaximal exercise performance (e.g., velocity at anaerobic threshold) seem to increase during this period (Casajus, 2001; Dupont et al., 2004; Impellizzeri et al., 2006; Jensen et al., 2009). In addition, albeit some evidence of improvements in time to exhaustion during maximal incremental tests (MIT) occur in parallel to increments in VO\textsubscript{2}\text{max}, there is evidence that professional soccer players increase MIT without increments in VO\textsubscript{2}\text{max} (Edwards et al., 2003a). It seems that highly trained players are able to increase exercise performance through enhanced anaerobic energy systems (Edwards et al., 2003a). As observed in study III, increases in soccer specific endurance performance (e.g., game-physical performance) occurred during in-season. Nevertheless, data reported in review I did not suggest significant changes in VO\textsubscript{2}\text{max} (Aziz et al., 2005; Casajus, 2001; Clark et al., 2008; Haritodinis et al., 2004; Heller et al., 1992; Kalapotharakos et al., 2011; Metaxas et al., 2006; Mohr et al., 2002b) and only smaller physiological adaptations during sub-maximal exercise occurred during in-season of professional players (Casajus, 2001; Edwards et al., 2003a; Impellizzeri et al., 2006; McMillan et al., 2005b). These facts highlight the high sensitivity of field assessments for monitoring the endurance ability of soccer players (Drust et al., 2007). Different investigations (Aziz et al., 2005; Carling et al., 2011; Faude et al., 2011; Meister 2011; Mohr et al., 2003; Nunez et al., 2008; Rampinini et al., 2007) using distinct methodological designs to analyse longitudinal changes in soccer players physical fitness challenged the common
concept amongst the soccer population (e.g. technical and medical staff) that players may accumulate “fatigue” during periods of heavy match commitments and/or as the season progresses (Reilly & Ekblom, 2005). Although studies generally report the maintenance or improvement in distinct measures of physical fitness during in-season (R-I), we observed that soccer SSE evaluated by YYIE2 performance decreases from the start to the end of the in-season (S-I). However, an in-season decrease in maximal performance capacity of professional players during an incremental exercise test, have been reported between these two periods (Faude et al., 2011). Additionally, lower values of SSE evaluated by the YYIR1 (Krustrup et al., 2003a) and YYIE2 (Bradley et al., 2010) that were not statistically significant have also been reported between these two time-points. Although without corroboration of other studies, Clarck et al., (2008) observed a decrease in AT from middle to the end-of-season (-6%) without significant changes in VO2max being reported. Nevertheless, in agreement with different reports dealing with high-level junior and senior players (Helgerud et al., 2001; Impellizzeri et al., 2006; Mohr et al., 2003; Rampinini et al., 2007b) certain game-related high-intensity categories were shown to progress throughout the season, particularly during in-season (S-III). In study III, we also observed that players were more frequently engaged in high-intensity activities during the last quarter of the season than in the previous season periods. Moreover, in this study (S-III), the amount of high-intensity running (HIR) performed in the last fifteen-minutes period of each half, which is indicative of the ability to maintain performance during the game (Krustrup et al., 2005), was again higher towards the end-of-season. In addition, at the end-of-season, a greater distance in HIR was covered in the peak and in the lowest fifteen-minute periods of the match than in the correspondent fifteen-minute periods of the other time points of the season (S-III). Study III seems to confirm evidence of others (Bradley et al., 2009; Mohr et al., 2003, 2005; Randers et al., 2010) of a decrease in the ability of maintain physical activity in the second half and towards the last 15-min period of the match as well as temporarily during the game. This fact has largely been suggested to occur as result of players experiencing accumulative fatigue during the match (Edwards & Noakes, 2009;
Mohr et al., 2005; Reilly et al., 2008). This has been proposed to be related to the fact that each player may employ a subconscious pacing strategy to ensure that they reach the end of the game without an excessive threat to their homeostasis, i.e., having physically working at a vigorous, yet sustainable level of performance (Edwards & Noakes, 2009). Edwards et al., (2009) proposed a pacing model to explain the self-regulation of elite soccer performance, which is based on both pre-match (intrinsic and extrinsic factors) and dynamic considerations during the game (e.g., accumulation of metabolites in the muscles, substrate availability). We can suggest that the increase in game-physical activity towards the end-of-season (S-III) can result, at least in part, from an improvement in some form of conscious (Carling & Bloomfield, 2010a) and/or subconscious pacing strategies by professional players (Edwards et al., 2009). Therefore, the development and improvement of pacing strategies allowing increased ability to preserve high intensity activity later in matches or high-intensity performance through the season cannot be excluded. This fact, seems consistent with the higher game physical performance towards end-of-season (S-III) without improvements in the majority of physiological and function parameters (R-I and S-I) and evidence of increases in certain stress biomarkers (S-II) being reported. Nevertheless, there are contradictions regarding the concept of team sport players pacing during matches (Aughey, 2010).

Endurance physiological determinants (e.g., VO\textsubscript{2} kinetics and ability to maintain acid-base balance) that have been demonstrated to be associated with the ability to increase performance (Christensen et al., 2011) and carried out via high-intensity intermittent exercise (Dupont et al., 2005; Rampinini et al., 2009a; Rampinini et al., 2009b) can occur during the season contributing to the improved game physical performance observed in study III and others (Mohr et al., 2003; Rampinini et al., 2007b). An improvement in running economy as the season progresses cannot be excluded. In fact, when high level athletes already have, either through training or selection, high values for VO\textsubscript{2max} and the ability to sustain VO\textsubscript{2max}, it is possible that further improvements in performance will depend on improved economy (Foster & Lucia, 2007). Although positive adaptations in running economy (RE) have mainly been
reported and investigated during preseason (Bogdanis et al., 2011; Helgerud et al., 2001; Helgerud et al., 2003; Impellizzeri et al., 2006), there are recent reports of increased RE (75% of MAS) in professional players after 2 weeks of high intensity training performed in the end of the competitive season (Christensen et al., 2011). Nevertheless, Macmillan et al., (2005b) did not found any change in RE after 10-wks of high-intensity aerobic training (6-wks in preseason plus 4-wks in in-season). However, given that RE has been assessed by treadmill running and significant differences exist between this assessment mode and soccer specific activity during training and matches, there is some conviction that soccer specific work economy may improve somewhat during in season, with relevant gains not detectable by conventional treadmill testing (Helgerud et al., 2001; McMillan et al., 2005b).

Some studies reported significant correlations between soccer players’ performance on distinct Yo-Yo tests and other performance measurements, such as players’ high-intensity running in the match (HIR) (Bradley et al., 2010; Krstrup et al., 2005; Krstrup et al., 2003b), total distance covered during match (Bradley et al., 2010; Krstrup et al., 2005), VO$_2$max (Castagna et al., 2006; Krstrup et al., 2006a; Rampinini et al., 2009a), incremental treadmill test performance (ITT) (Krustrup et al., 2006a) and peak power in counter movement jump (Castagna et al., 2006). Others have found correlations between performance in repeated sprint ability (RSA) and VO$_2$max (Rampinini et al., 2009a). However, some contradictory findings were found when correlations of HIR with ITT and VO$_2$max were analysed (Krustrup et al., 2005; Krstrup et al., 2003b). In addition, recent reports showed correlations between changes in SSE field tests (e.g., YYIE2) and changes in physical game performance (e.g., high-intensity running) during the season which were not evident for VO$_2$max (Bradley et al., 2011). However, it is important to note that most of the studies only detected moderate correlations, and so, do not establish a direct cause effect relationship (Rampinini et al., 2007a). We did not observe any correlation between YYIE2 performance and game-physical parameters (S-III). Thus, it is important to consider that even though data from laboratory and field tests are useful in providing information on players’ general
physical profile and soccer-specific fitness, results should not be used to predict the overall performance during match-play because of the complex nature of the demands of the game (Svensson & Drust, 2005). Literature have been showed (review I and II) positive adaptations on players’ neuromuscular system measured through distinct performance tests, e.g. sprint, change of direction ability (COD) and jump tests. In addition, this evidence seems to be more pronounced when jumps involve stretch-shortening cycle activities (SSC) and all body movement interactions (jumps with arm swing) (Aziz et al., 2005; Caldwell & Peters, 2009; Mercer et al., 1997; Ostojic, 2003; Sedano et al., 2011; Thomas & Reilly, 1979). Generally, data suggests that when early improvements are obtained in SSC activities (from PPS to BCP), they can be maintained and even further improved during in-season (Aziz et al., 2005; Chelly et al., 2009; Gorostiaga et al., 2004; Mujika et al., 2009; Ostojic, 2003; Sedano et al., 2011; Thomas & Reilly, 1979). Nevertheless, CMJ was unaltered from middle of season to the beginning of season, while performance in other SSC based activities showed the better scores, e.g. COD and sprint tests (S-I). These results suggest that performance in activities depending on stretch-shortening cycle may improve in these season time-points. This may suggest that neuromuscular adaptations affecting SSC mechanisms occur during in-season. It could be asked why this fact was not observed in CMJ tests. One possible reason may be that CMJ might not fulfil the criteria of SSC as would running-based exercises, which have very fast transitions from stretch to shortening phases, as well greater stretch velocity, both critical for stretch reflex activation (Wilson & Flanagan, 2008).

Regarding strength variations, our results (S-I) are in line with other studies suggesting that isoinertial assessment seems to be more effective than isokinetic evaluations to detect players’ performance improvements (Bogdanis et al 2009, 2011; Harris et al., 2007; Kotzamanidis et al., 2005; Malliou et al., 2003; Mercer et al., 1997; Nunez et al., 2008; Ronnestad et al., 2008; Ronnestad et al., 2011). The higher sensitivity of the isoinertial testing modes may result from of the neural and mechanical differences between isometric and isokinetic tests to sport-specific functional movements (Duchateau et al.,
It appears that greatest changes are observed when the mode of training matched that of testing (Harris et al., 2007). In fact, data from our study showed that players maintain the same levels of strength, evaluated by an isokinetic device, both on knee extension and flexor muscles, as well as bilateral strength differences throughout the soccer season. This stability of isokinetic strength parameters (e.g., KE) of professional soccer players throughout the season was also observed by other investigators (Malliou et al., 2003). In contrast, academic players showed a significant decrease of KE during an 11-week competitive soccer season (Kraemer et al., 2004). Possible differences in training background (professional vs. academic) and time devoted to training (e.g., strength training) may contribute to explain, at least partially, the referred differences between studies. Curiously, the KE peak torques observed in the present study (I) during the season were higher than those reported in other professional soccer players even at lower angular velocities (60º/s) (Malliou et al., 2003; Mercer et al., 1997).

Despite the multi-factorial aetiology of muscle injury, some evidence suggest that strength imbalances between agonistic and antagonistic muscles (Arnason et al., 2008; Croisier et al., 2008), as well as bilateral differences (BD) (Arnason et al., 2008; Croisier et al., 2008; Magalhães et al., 2004), previous injury (Arnason et al., 2008; Dauty et al., 2003) and lower resistance to fatigue (e.g. hamstrings) (Kawabata et al., 2000; Sangnier & Tourny-Chollet, 2008), are major risk factors for soft tissues injuries of soccer players. Interestingly, the H/Q ratio of the soccer players increased throughout the season (middle and end of season) and no changes were examined in BD (study I). As such, the increase and maintenance of lower limb muscle balances throughout the season may help, at least in part, to decrease the risk of muscle injury. In this regard, Croisier et al., (2008; 2003) examined that soccer players with lower limb muscle imbalances appear 4 to 5 times more likely to sustain a hamstring strain than those without the imbalance. Although we did not intend to compare or evaluate the efficiency of an intervention program in the prevention of muscle injuries, it’s important to highlight that when values of the ratio of concentric
hamstrings to concentric quadriceps peak torque ($H_{\text{conc}}/Q_{\text{conc}}$) of the players evaluated (study I) were under 0.55 (55%), players performed some specific strength exercises in order to restore a balance above this threshold. In addition, significant increases in H/Q ratio during in season phase were observed. Nevertheless, we calculated the “conventional” strength ratio ($H_{\text{conc}}/Q_{\text{conc}}$) and not the “dynamic” strength ratio (eccentric hamstring:concentric quadriceps peak torque). This last ratio has been shown to possess a higher discriminating character when assessing the risk of hamstring injury in professional soccer players (Croisier et al., 2008).

During the 90 min of the match, soccer players can perform 10-20 sprints, a high intensity running every 70s, about 15 tackles, 10 headings, 50 involvements with the ball, and 30 passes, as well as changing pace and sustaining forceful contractions to maintain balance and control of the ball against defensive pressure (Stolen et al., 2005). Therefore, it is agreed that high stress levels are imposed on the neuromuscular system in order to cope with these muscle power based efforts. In study I, we observed that the systematic participation of players in soccer matches favors the increase and maintenance of soccer players KE and KF muscle strength and sprint ability. This suggests that the short bursts of acceleration performed during games could have a positive effect in developing players’ ability to accelerate (Cometti et al., 2001; Wisloff et al., 2004). In addition, the better sprint and CMJ performance were observed in players with high IMPT in the middle of the season (S-II). In accordance with our data, Kraemer et al., (2004) observed that during and after an 11-week competitive soccer season, collegiate soccer players’ KF strength did not significantly decrease as much has KE. Moreover, the authors observed that starter players showed higher values of KF in the different evaluation points. The observed relationship between IMPT and these neuromuscular qualities suggests, at least in part, that male professional soccer players with higher IMPT are more prone to increase or maintain these physical qualities throughout the season, i.e., competition time may possibly contribute to influence certain physical characteristics of professional soccer players. Recently, Sporis et al., (2011) observed that starter players maintained and
improved agility parameters, as well as overall power performance (sprinting, jumping and kicking the ball) better than non-starters. These facts raised the question as whether “Can the official soccer game be considered the most important contribution to player's physical fitness level?” (Sporis et al., 2011). As confirmed in study IV and V, there is evidence that knee strength and SSC activities are affected by fatigue and muscle damage after the game (Ascensao et al., 2011; Ascensao et al., 2008; Ispirlidis et al., 2008). In addition, KF has been referred as a muscle group less resistant to fatigue than KE (Sangnier & Tourny-Chollet, 2008). In fact, after a match, higher force decrements (Andersson et al., 2008; Greig, 2008) and longer period of muscle soreness (Thompson et al., 1999) were found in knee flexors than in extensor muscles. This may explain, at least partially, the higher increases in KF found in players with more IMPT. In fact, muscle adapts to training regimens that rely in stretch-shortening cycles and are less impaired with repeated exposure (McHugh, 2003; Reilly et al., 2008).

Study I showed that improvements in KE and CMJ from start to end of preseason were significantly correlated with the improvement in sprint performance. KE strength also showed significant correlations with the CMJ and T5 performance. Moreover, strong correlations between CMJ and T30 and T5 were observed in distinct season time-points. Previous studies (Gorostiaga et al., 2004; Wisloff et al., 2004) showed that players with improvements in CMJ and in lower limb strength experienced improvements in short-sprint performance. As proposed by other researchers (Gorostiaga et al., 2004), this may suggest a possible transfer from leg power strength gains into enhanced sprint performance. In fact, there were significant associations between the different functional tests that rely in power activities (CMJ, T5, T30 and COD) in the different evaluation time points. Moreover, from start to end of preseason we also observed that improvement in the distance covered in YYIE2 was parallel to the performance increase in a muscle power test (CMJ). As highlighted in review II, it has been reported that both strength and endurance training programs during preseason and in-season could lead both to improvements in muscle power (e.g., 10 and 30-m sprint, vertical jump) and in
soccer specific endurance performance (e.g., YYIR1) (Bogdanis et al., 2011; Nunez et al., 2008; Wong et al., 2010).

Data from study III seems to corroborate other investigations reporting that game-related physical performance changes throughout the season (Mohr et al., 2003; Rampinini et al., 2007b) and is related with players' training status (Krustrup et al., 2003a; Krustrup et al., 2005; Rampinini et al., 2007a). In fact, some studies showed that soccer players' performance in the Yo-Yo intermittent recovery test both in males (Krustrup et al., 2003a) and females (Krustrup et al., 2005), in incremental field tests (shorter version of the University Montreal Track Test) (Rampinini et al., 2007a), and in repeated shuttle sprint ability test (Rampinini et al., 2007a) could be an indicator of game-related physical performance. In this regard, we examined (S-III) that different neuromuscular parameters (T5, T30, COD, CMJ, KE and KF), composites scores of power-related (CSPRT; T5, T30, COD and CMJ) and isokinetic strength tests (KE and KF), were correlated with certain game physical parameters (GPPs) analysed by time-motion, particularly in high-intensity locomotor categories. In fact, players with better performance in T5, T30, COD test and CSPRT showed more high-intensity running (HIR) in the five-minute period (Next5-min) after the peak five-minute period (P5-min) of during the match, a lower decrement in HIR from P5-min to the Next5-min (%DecP5-NS) and covered more distance in sprint during the second half of the match. These findings suggest that soccer players with improved capacity to perform sport specific maximal dynamic activities have an increased ability to maintain performance during repeated short periods of high intensity exercise and a high fatigue resistance in the second half of the game. Also, some isokinetic strength parameters (S-III) and composite scores of knee extension (CSKE), knee flexion (CSKF) and knee extension and flexion (CSKEF) were correlated with game-related physical performance (S-III). Relationships were observed between KE and KF muscle strength of the non-dominant leg, CSKE, CSKF, and CSKEF and the following fatigue parameters: decrement in HI running from the first to the last fifteen-minute period of the game and decrement in HI running from the first to last intense fifteen-minute periods of both halves (S-III). These data suggest that greater levels of lower
limb strength are related to a higher ability to maintain performance during games. Given the high stress upon the neuromuscular system during match, it seems that players with greater ability to produce force, fast accelerations and decelerations, and to quickly and efficiently perform complex and coordinated movements, can perform better in certain game-related physical parameters. In this regard, rapid force production is considered essential for a wide range of athletes (Hoff & Helgerud, 2004; Nummela et al., 2006). Recent reports highlighted that an enhancement in neuromuscular function can be determinant to improve short and long term endurance capacity (Aagaard & Andersen, 2010). In accordance with these authors, the possible contributing mechanisms may be related with an increase in the proportion of type IIA muscle fibers (less fatigable and yet highly capable of producing high contractile power) and the increase in rapid force capacity (RFD) (allowing a shorter propulsion phase, reducing the time of contraction-induced muscle occlusion, and hence increasing the time of muscle perfusion thereby increasing the mean capillary transit time (MTT)). Thus, a prolonged muscle relaxation phase potentially would allow for an increased MTT, enabling an increased diffusion of FFA into the muscle cells and an enhanced removal of metabolites produced by the contracting muscle (Aagaard & Andersen, 2010). Moreover, there is an agreement that training-related improvements in motor unit recruitment and synchronization seem to result in force potentiation, improving efficiency and coordination, which may delay the onset of fatigue (Aagaard & Andersen, 2010; Creer et al., 2004; Paavolainen et al., 1999a; Paavolainen et al., 1999b).

Even though low to medium intensity running are the predominant running activities during the match, it seems consensual that power-based efforts such as sprints, changing of direction and speed, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power of the neuromuscular system, are essential factors to successfully perform in soccer (Cometti et al., 2001). These speed and movement direction changes performed during game impose higher levels of stress on the involved musculature, thereby affecting energy usage and could result in a higher physiological impact than habitual forward movements (Dellal et al., 2010). In
fact, a massive metabolic load is imposed on players not only during the maximal intensities phases of the game but every time acceleration occurs, even when speeds are low (Osgnach et al., 2010). Indeed, higher VO$_2$ (Buchheit et al., 2010a), blood lactate concentration (Buchheit et al., 2010a; Dellal et al., 2010), heart rate (Dellal et al., 2010) and rate of perceived exertion (Dellal et al., 2010) values were observed in high intensity intermittent exercise performed in shuttle mode than during in-line format. Some possible reasons could be the involvement of additional muscles, such as upper body muscles (Jacobs et al., 1993), and different neuromuscular activation patterns during COD activities (Buchheit et al., 2010a). At high speed displacements and during intermittent shuttle running, turning techniques become more important, and anaerobic power is essential because players might accelerate after turning to reach the desired speed (Dellal et al., 2010). The physiological demands and functional characteristics that are typical of soccer specific activity may, at least in part, explain the observed correlations found in the present study (S-III) between power-related tests and game-physical parameters.

Fatigue is a complex phenomenon that cannot be simply explained by a single factor (Durandt et al., 2006). The ability to resist to fatigue has been related to different functional and physiological features (Bangsbo et al., 2006; Buchheit et al., 2010b; Durandt et al., 2006; Mohr et al., 2005). Improvements in coordination specificities (Buchheit et al., 2010b) and a greater ability to COD movements (Durandt et al., 2006) are positively associated with performance and with an attenuated fatigue response. Indeed, an optimized performance during game and particularly during the most intense periods would be expected in players with neuromuscular features tuned for the movements performed during game (Clark, 2009). Soccer players with greater CSPRT values may have a higher ability to accelerate, decelerate and change direction than players with lower values, leading to an improved physical capacity. This greater physical capacity may likely offset some of the mechanical and neuromuscular effects of repeated stretch-shortening cycle (SSC) fatigue (Clark, 2009). For instance, improved training status is related to higher ability to regulate joint stiffness more efficiently during exposure to maximal intensity
repeated sprints (Clark, 2009). The reported reductions in isometric (Ascensao 2011; Silva 2007), concentric (Ascensao et al., 2008; Rahnama et al., 2003) and eccentric muscle strength (Greig, 2008; Rahnama et al., 2003; Small et al., 2008) as well as in EMG activity (Rahnama et al., 2005) of the major lower limb muscles during the game are clear manifestations of game-induced fatigue. In study IV and V, in agreement with other observations, it was examined that game-induced performance decreases in strength and certain strength dependent actions such as sprint (S-IV; Andersson et al., 2008: Ascensao et al., 2008; Mohr et al., 2004; Small et al., 2009) and jump (S-IV and S-V; Andersson et al., 2008; Ascensao et al., 2008). According to literature, players’ fatigue reflected by decreases in EMG activity of major lower limb muscles and by compromised strength towards the final periods of the match may cause a lower work-rate towards the end of soccer match (Rahnama et al., 2005; Rahnama et al., 2003). In fact, both high intensity running and maximal accelerations seem to be reduced later in games, which are manifestations of game-induced fatigue (S-III; Bangsbo et al., 2006; Mohr et al., 2005). Moreover, since each specific game action requires breaking and propulsive forces, the importance of strength and endurance capacities of leg muscles likely increases as the game progresses (Brughelli et al., 2008; Buchheit et al., 2010a). Thus, strength decrements during the game could affect the performance of explosive actions such as jumping, sprinting and changing of direction movements, which require high quadriceps strength at the initiation of movement when the joint extension velocity is low (Rahnama et al., 2003). It is plausible to assume that soccer players with higher levels of strength will be more able to maintain that strength towards the final stages of each half of the game than players with less strength, and thus show a lower decrement in work rates. Generally, athletes’ ability to exercise during longer periods of time is usually related to their endurance capability (e.g., anaerobic threshold, VO₂ max). Nevertheless, as already stated, improved endurance is also influenced by factors related to muscle recruitment and force production (Aagaard & Andersen, 2010; Paavolainen et al., 1999a; Paavolainen et al., 1999b). It was recently observed that performance and running economy of well-trained distance runners are
related to the neuromuscular capacity to produce force (Nummela et al., 2006). In fact, although the causes of variability in RE are not well understood, it seems likely that anatomical traits, mechanical skill, neuromuscular skills, and storage of elastic energy are important factors (Hoff & Helgerud, 2004). Professional soccer players with higher improvements in different neuromuscular parameters (e.g., 1RM, maximal strength by lean leg volume-1RM/LLV) also showed high improvements in RE, SSE and fatigue resistance during a repeated sprint ability tests (Bogdanis et al., 2011).

Performance measurements and hormonal, oxidative and muscle damage markers have been suggested as important stress bio-markers in the monitoring of fatigue and recovery (Adlercreutz et al., 1986; Bishop et al., 2008; Clarkson & Tremblay, 1988; Finaud et al., 2006; Kraemer & Ratamess, 2005; Margonis et al., 2007; Urhausen & Kindermann, 2002). Interestingly, in study V we found increased levels of catabolic hormonal environment, muscle damage and inflammation, as well as oxidative stress at 24-h and 48-h of the recovery period after an official match of the competition phase in professional players. Although performance decrements were evident in the muscle-related power and strength tests from pre-match to 24-h post-match, only CMJ significantly decreased (S-V). With different match models (e.g. intermittent shuttle exercise vs. friendly match vs. official match), studies IV and V, reinforce the evidence observed by others that high standard players might show smaller post-match performance impairments and faster recovery kinetics (Andersson et al., 2008; Krstrup et al., 2011; Odetoyinbo et al., 2009; Rampinini et al., 2011). Recently, Rampinini et al., (2011) observed that performance of high-level players was partially recovered 24-h post-match and fully recovered 48-h post-match. In contrast, data from our study IV and from other studies with semi-professional players (Ascensao et al., 2008; Fatouros et al., 2010), reported longer recovery periods (48-h to 72-h). Recently, Odetoyinbo et al., (2009) observed that game-physical performance of professional players was not affected when two matches were played interspersed by two days of recovery. Additionally, Meister et al., (2011) observed that a 3-week period of high match exposure in elite football players did not affect laboratory, psychometric and performance
parameters. These differences in time to recover may be related, amongst other factors, to players’ physical fitness. The better physiological responses to match play (regulation of core body temperature) (Edwards and Clark 2006) and to high-intensity intermittent exercise (HIT) of high standard players (VO$_2$ Kinetics, blood La$^-$, H$^+$, HCO$_3^-$ - and RPE measured after HIT and the rate of La-accumulation during HIT) (Rampinini 2009a, 2009b), may explain, at least in part, the observed differences between studies. In this regard, players of higher standard seem to more quickly recover from high intensity intermittent exercise (Edwards et al., 2003) and to be able to sustain a higher level of physical work throughout the match (Mohr et al., 2003). In addition, higher standard players have a greater neuromuscular capability to perform powerful contractions during isokinetic force production tasks, as well as during and throughout the repetition of stretch-shortening cycle activities (SSC) (Cometti et al., 2001; Dauty & Potiron Josse, 2004; Impellizzeri et al., 2008a; Mujika et al., 2008; Rampinini et al., 2009b). Knowing that the mechanical stress induced by the eccentric component of the SSC activities may result in ultra-structural muscle damage (Armstrong, 1984; Clarkson & Tremblay, 1988; Friden & Lieber, 2001; Malm, 2001; Newham et al., 1987; Yu et al., 2004), it may be expected that these high level players have a higher capacity to cope with the match demands and therefore be less susceptible to exercise-induce muscle damage and subsequent performance impairments. In agreement, data from study III highlighted the relevance of players’ neuromuscular function for game physical performance. Nevertheless, in contrast with data provided by study V and others (Andersson et al., 2008; Krstrup et al., 2011; Rampinini et al., 2011), longer periods of residual fatigue was observed in professional male soccer players after a friendly match (Ispirlidis et al., 2008). Considering that an official match is expected to be more intense than a friendly match, the different recovery patterns between the former studies involving professional players may be associated with seasonal-related differences in physical fitness. Unfortunately, previous authors did not report the phase of season in which the match was performed. Differences in physical fitness levels that can be found during season (Carling & Orhant, 2010; Casajus, 2001; Dupont et al., 2004;
Edwards et al., 2003a; Gorostiaga et al., 2004; laia et al., 2009b; Kalapotharakos et al., 2011; Mohr et al., 2002b; Ostojic, 2003; Reinke et al., 2009; Sporis et al., 2008a) may affect the results and so their influence cannot be excluded. This fact highlights that studies on match fatigue and recovery should provide information regarding the time-point of the season where the match took place (pre-season vs. in-season vs. off-season) in order to allow a better interpretation and comparison of the results. In study I, we observed that the systematic participation of professional players in soccer matches favours the increase and maintenance of athlete’s knee extensor (KE) and knee flexor (KF) isokinetic muscle strength and short-sprint ability. In addition, in study II, in line with previous evidence and with research involving other team sport (Hoffman et al., 2005), we examined that the plasma kinetics of certain intracellular proteins and enzymes (e.g. decrease of plasma myoglobin (Mb) and creatine kinase trend to be lower towards end-of-season), may suggest a degree of sensitization (contact adaptation) of the muscles relating to the repeated traumas (mechanical and/or metabolic) occurring during the season (Hoffman et al., 2005). Accordingly, we examined that although C-reactive protein (CRP) increased from start to middle- and end-of-season, peak values of this bio-marker as well as from CK and Mb were observed in the middle of the competitive season. Curiously, in agreement with others studies (Mohr et al., 2003; Rampinini et al., 2007b) we observed (S-III) that physical match performance was higher towards de end-of-season. Knowing that the mechanical stress induced by the eccentric component of the SSC activities, typical in soccer practice and competition, may result in ultra-structural muscle damage (Clarkson & Tremblay, 1988; Nosaka et al., 2005; Teague & Schwane, 1995; Twist & Eston, 2005), it may be suggested that high level players have a higher capacity to cope with the match demands, and therefore be less susceptible to exercise-induce muscle damage and subsequent performance impairments. In this matter, study IV and others (Ascensao et al., 2008; Ascensao 2011; Fatouros et al., 2010) showed evidences that low-level soccer players have greater match-induced performance impairments and longer recovery periods after a match than high-level soccer players (S-V) (Andersson
et al., 2008; Krstrup et al., 2011; Rampinini et al., 2011). Accordingly, professional (S-V) but not semi-professional players (S-IV), recovered isokinetic strength as well as the majority of the different evaluated neuromuscular parameters after 24-h (only CMJ performance was impaired at 24-h). Additionally, semi-professional players (S-IV) showed greater performance impairments and longer recovery periods in jump and sprint performance as well as in knee extension and flexion isokinetic force production. It is plausible to assume that if muscle damage occurred, it was of minor magnitude in professional players. This was corroborated by a lower neuromuscular impairment, a shorter recovery period in addition with a lower efflux of intracellular proteins (S-V).

In study IV, we observed that sprint ability, in contrast with CMJ was more affected by the friendly-match than by the LIST. Probably, considering the distinct levels of motor coordination involved in the actions of jumping and sprint running, the repeated eccentric actions performed during the match might have caused disturbances in running movement control (Bottas et al., 2005). In fact, eccentric contractions may had affected more pronouncedly the running performance of these semi-professional players, which likely rely to a greater extent on muscular coordination than a single jump or leg curl and extension.

On the other hand, in study V, CMJ was the only neuromuscular parameter impaired at 24-h recovery. Accordingly, Andersson et al., (2008) and Ascensao et al., (2011) reported a longer impairment of CMJ, when compared to other neuromuscular parameters, after a friendly elite female soccer match. During recovery, the neuromuscular system can adjust to the contractile failure in a very flexible way (Nicol et al., 2006) that makes it difficult to establish a direct link to which mechanism can be related to the distinct recovery pattern observed. Moreover, the different neuromuscular parameters assessed (CMJ, sprint and COD) have been recently described as independent motor abilities (Salaj & Markovic, 2011). CMJ has been characterized as a concentric and slow SSC-related action (Salaj & Markovic, 2011) that in contrast with other activities (e.g., running) rely on slower transitions between stretch to shortening phases, as well as by a lower stretch velocity, which limits stretch reflex activation.
(Wilson & Flanagan, 2008). Certain specific components of the SSC might attenuate the detrimental performance effects associated with exercise-induced muscle damage (EIMD), e.g. increase of muscle pre-activation (Byrne et al., 2004). Nevertheless, studies investigating fatigue in SSC activities reported greater performance impairments in actions involving higher (e.g., sprint, drop jump) than lower (e.g., CMJ and Squat jump) ground impact forces (Nicol et al., 2006). However, others did not observe impairments in sprint performance (5-10-20-30-m) after EIMD (Semark et al., 1999) and after the match (Ascensão et al., 2011). Ascensão et al., (2011) assessed the effects of a single session of cold or thermoneutral water immersion after a match on muscular dysfunction and damage in soccer players. The previous authors observe that in contrast to sprint performance, high-level junior players had impaired CMJ performance during the 48-h of recovery period. Moreover, the maintenance of sprint ability and squat jump performance in one group of the examined players (cold water immersion group), occurred despite the increase in indirect markers of EIMD (CK and perceived muscle soreness increased and maximal isometric voluntary contraction decreased throughout 48-h recovery). In study V, despite the preservation of the majority of the examined neuromuscular parameters during the recovery period (e.g. muscle peak torque, COD and sprint ability recovery at 24-h and CMJ at 48-h) that may suggest indicate absence of muscle damage we examine increases in other indirect markers of EIMD (e.g., CK). It cannot be excluded, amongst other factors, a decrease in work capacity resulting of a lower force capability in all joint range of motion (e.g., force decrements or damage at specific muscle lengths). Also, EIMD may shift the length-tension relationship (Brown & Donnelly, 2011; McHugh & Tetro, 2003; Philippou et al., 2009; Prasartwuth et al., 2006). If these facts occurred, it might at least, in part, distinctly influence the recovery pattern of the different motor abilities due to their kinetics and kinematics characteristics. Therefore, the already mentioned dissimilarities between the recovery pattern of distinct parameters measured within our studies (S-IV and S-V) and the observations of others (Ascensao et al.,2008; Ascensao et al., 2011; Andersson et al 2008; Ispirlidis et al., 2008;
Krustrup et al. (2011; Rampinini et al. 2011) highlighted the complexity of the mechanisms of the neuromuscular SSC fatigue (Nicol et al., 2006). Andersson et al. (2008) suggested that the greater delay in CMJ recovery compared to other neuromuscular parameters was not solely dependent on the recovery of force production of the extensors and flexors muscles but also of additional mechanisms related with SSC fatigue. Nevertheless, it is important to highlight that post-match responses may be influenced by the used exercise model to analyse performance, and physiological and biochemical responses to the “match” (soccer-specific protocol vs. friendly-match vs. official match). In fact, as observed in study IV, although, the impact of distinct models (friendly match vs. LIST) did not differ regarding the observed muscle damage markers and some neuromuscular parameters, friendly-match required higher cardiac demand and induced higher changes on redox status, adenine nucleotide metabolism and on lymphocyte counts than LIST.

Testosterone (T) and cortisol (C) are two major endogenous indicators of tissue adaptation, and their ratio represents a biological marker of homeostasis reflecting the relationship between the anabolic and catabolic hormonal environment within the body (Filaire et al., 2003; Gorostiaga et al., 2004; Handziski et al., 2006; Hayes et al., 2010; Kraemer et al., 2004; Kraemer & Ratamess, 2005; Kraemer et al., 1998). According to our results (S-V), the official soccer match altered the catabolic/anabolic-related hormonal homeostasis towards a predominant catabolic response during the first 48 hours of the recovery period. This was expressed by the acute increase in cortisol response in the 48-h recovery period without alteration in total testosterone, which resulted in a significant decrement in the plasma total testosterone:cortisol ratio (T/C). Ispiridis et al., (2008) found significant increases in cortisol concentration immediately after a friendly soccer match. Increase in C concentrations and decreased T/C during the recovery period (24-h) has also been reported after other high-level football codes competitions (Cormack et al., 2008; McLellan et al., 2010). Also, in line with our findings, Ispiridis et al., (2008) did not observe alterations in plasma testosterone (free testosterone) concentration throughout the 144 hours of the recovery period. To
our best knowledge, no reports on T/C during the recovery period after an official soccer match have yet been described.

Study II showed that total plasma T concentration was stable during the soccer season. Filaire et al., (2003) found similar findings when salivary testosterone concentrations (biological active fraction) throughout the season and off-season were analysed. Also, Kraemer et al., (2004) observed that T did not change during the competition period while an increase in T was found just one week after the end of competitive period over the initial phase of competition period (Kraemer et al., 2004). Others (Handziski et al., 2006) showed that T in the week of taper that preceded the beginning of competitive season, was higher than in the start of pre-season training, but was lower at the end-of-season than in the former time-points. Concerning C concentrations, our results (S-II) showed a progressive decline in C throughout the competition period with significantly lower values at the end-of-season than in the start of both seasons and middle-of-season. Kraemer et al., (2004) did not examine changes in C concentrations during several time points of an 11-week competition period of academic soccer players. In contrast to our data, Handzisky et al., (2006) reported that cortisol decreased during preseason and was higher at the end-of-season than before and after pre-season period. Higher resting concentrations of salivary C at the end-of season than in start of season were also observed by others (Filaire et al., 2003). In study II, as a result of the T and C kinetics, we observed a progressive increase in T/C towards the end-of-season and a decrease in this ratio during off-season. Kraemer et al., (2004) observed that T/C increased in non-starters players after one week of recovery during off-season. However, no changes were examined in starters. In the study of Filaire et al., (2003), no differences were found in T/C in the different assessments carried out during the season (pre-and-post preseason, end-of-season and off-season). In contrast, others (Handziski et al., 2006) observed an increment in T/C after the preparatory period (week of taper preceding competition phase) and a higher decrement in the end-of-season than in the other time points (pre-and-post preseason). The different results observed between our (S-II) and previous studies could be explained, at least partially, by the differences in the
study designs. Handziski et al., (2006) and Kraemer et al., (2004) analysed the soccer season with different periodization’s structures (e.g., half-season and 11-wk competitive season, respectively). Perhaps the length of the preparation period and/or the different densities of match commitments during the weekly in-season cycles that characterize distinct periodization structures, may among other factors, explain the different hormonal kinetics.
In line with our observations (SHIV), studies in other football codes (Cormack et al., 2008) and team sports (Mazon et al., 2011) reported a decrease in C and increase in T/C during the competition period. Moreover, resting hormonal concentrations reflect the current state of muscle tissue and elevations or reductions may occur at various stages depending on substantial changes in the volume and intensity of training phases, i.e., number of weekly match commitments (Hakkinen et al., 1988). Research involving high-level basketball players has shown that both increases and decreases in catabolic and anabolic-related hormones may occur in distinct phases of the in-season (Martinez et al., 2010). The progressive decrease in C and increase in T/C ratio observed in our study may reflect an increase in the anabolic status of skeletal muscles in the phase that a higher accumulation of match-related physiological stresses occurs (e.g., middle and end-of-competitive phase). In fact, the decrease in cortisol parallels the increment in T/C, which reflects an enhanced anabolic environment throughout the competition phase (Kraemer et al., 1998). These results suggest that it is possible to correct or avoid situations of accumulated stress even in high competitive contexts (Martinez et al., 2010). Moreover, it is appealing to speculate from a hormonal perspective that players’ physical adaptations and/or specificities of their phenotype resulting from their training background led to a higher ability to cope with the stress induced by the intermittent nature of the activity in soccer. Another factor that cannot be excluded is a decreased influence of psychophysiological factors, since in our study (S-II) the end-of season blood samples were obtained as soon as the competitive season finished, and therefore, at least in part, may have contributed to the decrease in C concentration found in this period as team performance during season was very successful. The beginning of the season
can be associated with a high anticipatory level of psychophysiological stress that may influence C concentrations (Hoffman et al., 2005). In fact, it is recognized that preseason is a period devoted to players’ selection and squad definitions. On the other hand, Reinke et al., (2009) observed that the reduction of stress levels of professional soccer players during off-season was related to an increase in catabolic metabolism (e.g., increased in leukocytes counts and IL-8) and a decrease in soccer players lean body mass (Reinke et al., 2009). Therefore, since resting cortisol concentrations generally reflect long-term training stress (Kraemer & Ratamess, 2005), the higher C values found during off-season can be related, among other factors, to a process related to the metabolization of the damaged muscle cells during in-season (Reinke et al., 2009).

There are reports regarding academic starter players with higher C in the middle of competition phase than in other time points within the competitive period (Kraemer et al., 2004). Accordingly, in study II, we observed that individual match playing time (IMPT) at the middle of the season was positively associated with C values and negatively associated with T/C ratio. Nevertheless, in this time-point we also observed that players with higher match playing time (IMPT) were those with greater performance in power-based efforts, and no association between the hormonal data and performance was found. It is likely that these increased levels of catabolic hormonal-related evidences in players with higher IMPT (increased cortisol and decrease T/C ratio) could be related to muscle damage induced by the accumulation of training sessions and matches (S-IV and S-V). The fact that IMPT was associated with lower T/C and with higher performance raises the question on the extent to which anabolic:catabolic ratio can be related with performance, as well as which magnitude of variation of T/C could be associated with performance changes. Although a decrease in anabolic/catabolic ratio has been suggested to be associated with performance decreases (Adlercreutz et al., 1986), different investigations observed that soccer players performance increments can be related with both decrements (Gorostiaga et al., 2004) and increments in T/C (Kraemer et al., 2004). In study II, players with greater
individual increases in T/C from middle to end of season had the higher individual increases in KFD, KFND between the two periods. Furthermore, a positive association between T/C ratio and KED and KFND values and negative association between plasma C and isokinetic knee extensor and flexor force production were examined in the end-of-season. However, since we did not examine the hormones production controlled by the hypothalamo-pituitary-testicular and adrenal axis that mediate the regulation of T and C, as well as other important factors that influence the role of T and C in muscle (e.g., hormone bioavailability, binding proteins and membrane receptors), the present results should be interpreted with caution.

The mechanical stress induced by the eccentric components of the SSC activities that are common in soccer practice and competition induces skeletal muscle damage, results in morphological alterations within the muscle and led to loss of cellular integrity (Friden & Lieber, 2001; Peake et al., 2005; Teague & Schwane, 1995). The increase in plasma concentration and activity of certain intracellular proteins and serum enzymes (e.g., Mb, CK, LDH) has been widely used as stress-biomarker that reflects damage of the muscle tissue (Brancaccio et al., 2007; Ebbeling & Clarkson, 1989; Kanter, 1995; Powers & Jackson, 2008). Studies IV and V corroborate the post-match increase in muscle damage markers observed in similar investigations (Ascensao et al., 2008; Fatouros et al., 2010; Ispirlidis et al., 2008). Longer responses (72-h to 96-h post-match) were reported in semi-professional (study IV; Ascensao et al., 2008; Fatouros et al., 2010) compared with our and other studies with players of the same standard (study V) (Andersson et al., 2008; Krstrup et al., 2011; Rampinini et al., 2011) during the recovery period. Considering that baseline values in study V were obtained 96-h after the previous official game of the regular championship, probably as observed in study II, the higher pre-match CK values found in the professional players of the study V and others (Rampinini et al., 2011), might be associated with “residual” levels of muscle damage from previous match and regular training sessions (McLellan et al., 2010). On the other hand, the different magnitude of changes in CK in studies IV, V and others (Andersson et al., 2008; Ascensao et al., 2008; Fatouros et al., 2010).
2010; Ispirlidis et al., 2008; Krstrup et al., 2011; Rampinini et al., 2011), might also be related to the training status when the match was performed. In fact, training status has been shown to be a determinant factor in the magnitude of the post-exercise CK changes, since less trained subjects exhibited the higher post-exercise increases (Garry & McShane, 2000; Maxwell & Bloor, 1981). Nevertheless, given the large number of factors that are associated with plasma CK inter-individual variability (e.g., age, muscle mass, physical activity) both in rest and in response to exercise (low and high responders) (Brancaccio et al., 2007), it is difficult to compare the magnitude of the changes from different studies. Moreover, some studies evidenced that muscle enzyme release may not clearly reflect the size of the injury (e.g., evaluated by histological analysis), being the efflux of intracellular proteins a possible result of both damage and transient changes in membrane permeability (Malm, 2001). In studies II, IV, and V, both MDA content increases in post-match (24-h, 48-h) and during the competitive phase are consistent with alterations in membrane permeability that could, at least in part, contribute to an enhanced efflux of intracellular proteins to circulation despite the preservation of the functionality of the contractile machinery, and concomitantly of the performance observed in study II and V. Studies IV and V also corroborate the occurrence of an acute phase inflammatory response after the match. This was suggested by the increase of blood leukocyte counts (S-IV) (Andersson et al., 2010a; Ascensao et al., 2008; Ispirlidis et al., 2008), inflammatory mediators (Andersson et al., 2010a; Ispirlidis et al., 2008) and in cortisol levels (S-V) (Ispirlidis et al., 2008) that has been described after friendly-matches performed by professional and semi-professional soccer players. During inflammation, an increased in inflammatory mediators stimulate the synthesis of acute phase proteins such as C-reactive protein (CRP) (Ispirlidis et al., 2008). In accordance with our results (S-V), Ispirlidis et al., (2008) and Ascensão et al., (2011) observed that CRP content increased at the same time-point (24-h) of the recovery period after a friendly match, both in professional and semi-professional players. Concerning chronic variation in CRP content, we observed an increase from the start to the middle and end-of-season. Nevertheless, values observed in the middle and in the
end-of-season were not statistically higher than those at the beginning of the 2nd season. Other studies (Reinke et al., 2009) did not find differences in professional soccer players’ plasma CRP levels between the end-of-season, beginning and end of pre-season period.

Soccer presents an activity pattern highly demanding for neuromuscular system (e.g., acceleration and decelerations) and/or metabolic (e.g., elevated mitochondrial oxygen consumption) pathways. This is considered a prone condition to free radical-induced damage (Vina et al., 2000). In fact, the high absolute levels of mitochondrial oxygen consumption, the increased oxidation of circulating catecholamines, and the intermittent and repeated sprint actions-causing temporary ischemia-reperfusion events in skeletal muscle are plausible factors that may induce reactive oxygen and nitrogen species (RONS) production during and after a soccer match (Ascensao et al., 2008) as well as during training periods. An increase in RONS during and following acute exercise is believed to serve the necessary “signal” for the hormetic-associated up-regulation in antioxidant defence commonly observed with chronic exercise training (Fisher-Wellman & Bloomer, 2009). However, the chronic effects of high-level soccer practice in the antioxidant status and oxidative stress levels of professional players have not yet been investigated. In addition, studies examining acute changes in redox status during and after a match have been scarcely investigated (Ascensao et al., 2008; Fatouros et al., 2010). Although evidence suggests a higher increase in plasma activity of certain endogenous antioxidants such as uric acid (Cazzola et al., 2003), superoxide dismutase (Brites et al., 1999; Cazzola et al., 2003) and glutathione reductase (Banfi et al., 2006) in soccer players than in untrained individuals, there are contradictory results regarding a higher total antioxidant status (TAS) (Banfi et al., 2006; Brites et al., 1999). Moreover, although scarce and contradictory studies involving other team sports showed that training may induce improvements in both athletes total antioxidant enzymatic and non-enzymatic capacity [for reference see (Finaud et al., 2006)]. Nevertheless, data gathered in soccer players (Arent et al., 2010; Brites et al., 1999) revealed that the increasing free radical scavenging capacity by means of ergogenic supplementation or by the
endogenous up-regulation of antioxidant defences due to the training-related pro-oxidant insults, is not a guarantee of sufficient protection or a condition for a decrement in exercise-induced oxidative damage during periods of high-intensity training and competition. In study II, we did not observe chronic changes on TAS and UA levels throughout the soccer season. According to our best knowledge, only one study focused on the kinetics of these biochemical parameters during a soccer season, and analysed UA contents (Filaire et al., 2003). Filaire et al., (2003) observed higher plasma UA levels in professional soccer players in the beginning and at the end-of competitive period than before the beginning of the pre-season. We observed an increase in TAS during the post-match recovery period (S-IV and S-V). Previous studies with semi-professional soccer players reported that TAS increased immediately after a friendly-match and throughout the 48-h (Fatouros et al., 2010) and 72-h recovery period (Ascensao et al., 2010c). Consistent with these findings, Anderson et al., (2010c) observed increases in specific endogenous (non-enzymatic) compounds and in certain dietary antioxidants immediately and during the 72-h post-match recovery period, respectively. According to the authors (Anderson et al., 2008), this delayed increase in dietary antioxidants during recovery may serve as a putative mechanism to strengthen the endogenous antioxidant defence and restore the redox balance (Andersson et al., 2010c). Different studies seem to support the idea that a soccer match induces a pro-oxidant insult resulting in a compensatory response in plasma TAS immediately after or during the first 48-h of the post-match recovery period. Data examining post-match UA and IMP responses (Krustrup et al., 2006b) seem to suggest that a soccer match is associated with a significant degradation of purine nucleotides. Despite the absence of UA increases in study V (with professional players), in study IV and in others studies with semi-professional players an elevated plasma UA content was observed immediately after a friendly match (Ascensao et al., 2008) that was maintained until 48-h recovery period (Fatouros et al., 2010). In agreement with study V, match-related UA responses during the recovery period were lower in players of higher standard (Andersson et al., 2008). In fact, Andersson et al., (2010b) observed
UA levels of elite female soccer players increase immediately after a match but return to pre-match levels at 21-h of the recovery period. Altogether, data suggest that lower-level compared to high-level players seem to more strongly rely on the degradation of purine nucleotides as reflected by the higher increments in UA contents throughout recovery. In addition, the longer post-match recovery pattern reported by Ispirilidis et al., (Ispirilidis et al., 2008) e.g., physical parameters, CK and UA responses, in contrast to our results and others (Andersson et al., 2008; Krustrup et al., 2011; Rampinini et al., 2011) involving players of the same standard, could be related with seasonal variations in physical fitness.

As observed in study IV, the friendly-match induced higher increases in TAS and UA (30 min) contents than LIST. Moreover, MDA was higher and SH were lower after match than after LIST (30 min and 24 h). These results suggest that the match may induce higher changes on redox status and on adenine nucleotide metabolism than LIST. Moreover, it may also suggest that the match resulted in a higher pro-oxidant insult than the LIST.

In line with the above referred effects of a soccer match on antioxidant markers, specific enzyme activity tends to respond accordingly (Fisher-Wellman & Bloomer, 2009). Superoxide dismutase (SOD) is the major antioxidant defence upon superoxide radicals production and is the first defence line against oxidative stress (Finaud et al., 2006). Concerning the alterations in redox status throughout the post–match recovery period (S-V), we observed an increase in SOD activity during the 48-h of the post-match recovery period (S-V). Although no reports on SOD kinetic after a soccer match have been published, studies with untrained individuals and other athletic populations showed that exercise can result in increments or maintenance of SOD activity (Fisher-Wellman & Bloomer, 2009). Accordingly, a progressive increase in SOD activity during the middle and at the end-of-season period was found in study II. This up-regulation of SOD may result from the continuous pro-oxidant insult derived from the already described mechanism during the season, as corroborate by study IV and V. Since SOD is considered the first enzymatic defence line against oxidative stress (Finaud et al., 2006; Powers & Jackson, 2008) and that the
magnitude of exercise-mediated changes in skeletal muscle SOD activity increases in function of the intensity and duration of the exercise (Powers & Jackson, 2008), alterations may be expected due to the high physiological demands of a soccer match. Moreover, in conjunction with the protective role against free radical-induced muscle damage during intense exercise, antioxidants may have a positive effect on performance and on the prevention of fatigue (Vina et al., 2000). Although examined in animal models, superoxide radicals can attenuate the function and enhance the rate of fatigue from the exercised muscles (Barclay & Hansel, 1991). Arent et al., (2010) examined the effects of pre-season supplementation with a dietary containing an orally available form of SOD as part of an antioxidant and anticatabolic nutriceutical mixture. Although no differences between supplemented and control players were found in performance capacity, players submitted to the ergogenic intervention presented lower CK and 8-isoprostranes responses during the post-exercise recovery period (Arent et al., 2010). Curiously, although we did not observe any relationship, the lowest CK activity in-season was coincident with the higher SOD activity (middle season).

Glutathione peroxidase (GPX) reduces a wide range of hydroperoxides and therefore is an important intracellular antioxidant protecting against oxidative stress (Powers & Jackson, 2008). To exert its scavenging function, GPX requires a supply of reduced glutathione (GSH) to provide electrons (Powers & Jackson, 2008). The antioxidant enzyme glutathione reductase (GR) is involved in the reduction of oxidized glutathione (GSSG), and elevated activity is thought to be indicative of increased free radical generation (Kanter, 1995). With respect to the match-induced alterations in antioxidant glutathione cycle-related enzymes (S-V), although a decrease in GPX at 24-h of recovery period was observed, others (Fatouros et al., 2010) examining semi-professional players observed an increased activity during the 24 to 72-h post-match recovery period. On the other hand, an up-regulation of plasma GR activity at 24-h post-match was also found. Concerning the chronic alterations in resting antioxidant glutathione cycle-related enzyme activities, although not significant, lower values of GPX activity during competition period (middle and end-of season)
than in beginning of season were observed. To our best knowledge, studies II and V are the first that analysed the effect of a soccer season (S-II) and a soccer match (S-V) in plasma GR activity. The activity of GR decreased from middle to end-of-season. Taking into account the critical physiological role of the balance between the first (SOD) and second step (GPX and/or catalase) antioxidant enzymes, the significant increase in plasma SOD/GPX ratio observed after the game and throughout the competition period, mainly due to a decreased in post-match GPX activity and no changes throughout competition phase as referred to previously, seems to favor \( \text{H}_2\text{O}_2 \) accumulation. This fact could be indicative of a low plasma scavenging efficiency contributing to enhanced oxidative stress (Gaeta et al., 2002) that is consistent with the increased levels of MDA found in studies II, IV and V and others (Ascensao et al., 2008; Fatouros et al., 2010; Ispirlidis et al., 2008). These facts suggest that although an up-regulation of the first line of defence of the endogenous enzymatic antioxidant system (SOD) throughout the season may suggest a higher capability to cope with the soccer-related metabolic stresses (S-II), this was not evident in the secondary enzymatic line of defence, i.e., GPX did not change and GR decreased from middle to end-of-season (S-II). As previously mentioned, the increase in oxidative stress by-products (MDA) may suggest that an imbalance exists between oxidants and antioxidants due to the continuous pro-oxidant state associated with the soccer demands (e.g., muscle high metabolic rates). In fact, oxidative stress occurs both under conditions in which local antioxidant defences are depleted or when the rate of the radical production is greater than the capacity of the antioxidant defence mechanisms (Buettner, 1993). Taking into account the evidence of an increase in ROS production, i.e., up-regulation of certain antioxidant enzymes and the increase levels of lipid peroxidation by products (MDA) and other markers of oxidative stress (CK) during the soccer season (S-II) and post-match recovery periods (S-IV and S-V), it may be important to consider the beneficial effect of antioxidants supplementation in professional soccer players. Nevertheless, there are contradictions regarding their ability to enhance exercise performance as well as in the ability to help recovery of soccer players (Arent et al., 2010) and other
athletic populations (Finaud et al., 2006; Powers & Jackson, 2008). It is important to highlight that increased ROS could activate signal/transcription pathways and promote among other factors the hormentic-associated up regulation in antioxidant defence (Fisher-Wellman & Bloomer, 2009; Magalhaes et al., 2005). Even though if needed, an adequate antioxidant supplementation may help athletes to maintain an optimal health, which is a key conditioning to attaining the best performance (Finaud et al., 2006), there is a point at which increased ROS may represent an optimal redox state for greater performance, i.e., force production (Reid, 2001).

Literature regarding soccer nutritional strategies and metabolic demands of soccer training and match play focus on the role of the ingestion of proper amounts of carbohydrates before, during and after exercise (Bangsbo et al., 2006; Burke, 2010; Burke et al., 2006; Zehnder et al., 2001). Furthermore, it has been emphasized that optimal adaptations and enhanced recovery may occur when carbohydrate ingestion is complement with protein and/or amino acid administration in order to stimulate muscle protein synthesis, inhibit protein breakdown, and allow net muscle protein accretion (Beelen et al., 2010; Burke et al., 2006). Nevertheless, the increased levels of MDA in our studies (SI, S-IV and S-V) may also highlight the importance of lipid rich macronutrients, which could compensate for an eventual increase in lipid peroxidation (Magalhaes et al., 2005). In fact, since dietary fatty acids are exchanged with membrane fatty acids, dietary fat composition is reflected in membrane lipid composition and could influence cell function through effects on membrane properties (Magalhaes et al., 2005).

Concerning the influence of IMPT, it seems that not only influences some physical and hormonal parameters (S-I and S-II) but might also have some impact on the endogenous enzymatic and non-enzymatic antioxidant system (S-II). Study II showed that in the middle of season players with higher IMPT had increased levels of TAS and GPX activity and a lower ratio between the 1st and 2nd line of enzymatic defense. The increased levels of TAS and GPX activity in players with higher IMPT may act as a mechanism to strengthen the endogenous antioxidant defence and restore the redox balance (Andersson et
Another class of biomarkers of oxidative stress involves the evaluation of oxidative modified molecule (Powers & Jackson, 2008). Free radical reactions can cause damage to lipid membranes, proteins, DNA and other cellular constituents (Kanter, 1995) generating uniquely oxidized biomolecules that can be used as “finger-tips” to detect oxidative stress (Powers & Jackson, 2008). Regarding plasma sulfhydryl residues (-SH), we observed an increase during post-match recovery period (at 24-h; S-V) and an increase during the competitive period of professional players (S-II). These results showed decreased disulphide linkages (-S-S-) concentration from both proteins and reduced glutathione, and ultimately suggest a prevention of protein oxidation. In study IV and in Ascensao et al., (2008), decreased –SH contents were observed in semi-professional soccer players immediately after and throughout the first 48-h of the recovery period. Nevertheless, as previously stated, in study V involving professional players, a distinct response was observed, i.e., increased –SH contents at 24-post-match. A decrease in GSH and GSH/GSSG and increases in GSSG during the post-match recovery period have been observed in semi-professional male soccer players (Fatouros et al., 2010). Nevertheless, these findings were not confirmed in elite female players. Andersson et al., (2010c) observed that the immediately post-match increased in GSSG and decreased in GSH/GSSG returned to baseline at 21-h of the recovery period. Moreover, GSH content was unaltered during the recovery period (Andersson et al., 2010c). The point in the season when the match was performed might contribute to the different results between studies. In fact, although not observed in study II, some reports with other athletic populations suggest that the antioxidant status (e.g. TAS) might change within the season (Teixeira et al., 2009). In addition, as examined in study II, players match exposure from beginning to the middle of the season (higher IMPT) was associated to higher resting TAS at that time-point. These facts, among other factors, might influence the capacity of the antioxidant defence system and the ability to restore the redox balance. Players training background (Martinovic et
al., 2009) (e.g. years of practice) and standard (Nakagami et al., 2009) cannot be ruled out as factors that may influence the different results between studies, since they have been associated with higher concentrations of sulphydryl groups. Probably, vigorous training sessions and competitions resulting in oxidative stress may lead to an up regulation of antioxidant defences and associated shift in redox balance in favour of a more reducing environment, thereby protecting the athlete from excessive oxidative damage during subsequent training sessions (Fisher-Wellman & Bloomer, 2009).

One of the least understood and more scarcely studied phases of the soccer season training cycle is the off-season period (S-II). The off-season period appears to result in decrements of a variety of physiological, biochemical and functional parameters (R-I and S-II). There are reports that the off-season period seems to result in an increase in body fat of different standard of players (Caldwell & Peters, 2009; Reinke et al., 2009) and decreases in professional players lean body mass (Reinke et al., 2009). Despite the scarcity of reports, deconditioning during off-season resulting in VO$_2$ max decrements in players of different standards (Caldwell & Peters, 2009; Mohr et al., 2002b; Sotiropoulos et al., 2009) and slower VO$_2$ kinetics (75% MAS) (Christensen et al., 2011) have been described. In addition, a decrease in time to exhaustion during a maximal incremental lab test and increase in heart rate at sub-maximal intensity has been reported in professional players (Mohr et al., 2002). Regarding neuromuscular function, it seems that such decrements are more evident in muscle power ability than in muscle strength (Caldwell & Peters, 2009; Ostojic, 2003) (as indirectly confirmed by the data in review I and from study I). In addition, a decrease in the activity of certain muscle oxidative enzymes was found (Christensen et al., 2011). Accordingly, a decrease in the first line of defence of the antioxidant enzymatic system (SOD) and in plasma SOD/GPX ratio was analysed. In addition, the reduction in functional and metabolic demands produces a decrease in lipid peroxidation by products (MDA) and in –SH contents. Moreover, lower levels of plasma bio-markers of muscle damage (CK) were examined after off-season.
Conclusions
Conclusions

Based on the general conclusions of each of the different studies presented in this dissertation, it seems possible to highlight the following major conclusions:

a) Seasonal variations occur in different soccer specific and non-specific endurance and neuromuscular function parameters. Soccer specific endurance (game-physical performance) and soccer specific neuromuscular function (change of direction speed) increase during the competitive period.

b) Professional soccer players show an improved match activity and express low level of fatigue during the match towards the end-of-season.

c) Professional soccer players, although submitted to high levels of stress signalled by increased muscle damage and oxidative stress, seem to possess an efficient capability to sustain the soccer season stressful demands. In addition, the recovery period is related to a decrease in markers of muscle damage and oxidative stress.

d) Players’ individual match playing time influences physical, hormonal and oxidative-related parameters.

e) The interrelationship between lower limb muscle strength and soccer specific neuromuscular parameters (sprint and jump ability) highlight the importance of muscle strength and power training in soccer.

f) Professional players with high neuromuscular capabilities have lower performance decrements in game physical parameters. These results highlight the relevance of players’ neuromuscular function for physical performance in the match.

g) Friendly-match and LIST performed by semi-professional professional players did not differ regarding the observed muscle damage markers and some neuromuscular parameters, although friendly-matches require higher cardiac demand and induced higher changes on redox status, adenine nucleotide metabolism and on lymphocyte counts than LIST.
h) A high-level official match increases catabolic hormonal environment, muscle damage and inflammation, as well as oxidative stress markers at 24-h and 48-h of recovery period. Regardless these alterations, neuromuscular performance of professional players are affected to a small extent and only until 24-h of the recovery period.

In summary, this dissertation presents a scenario regarding the functional and biochemical changes induced by acute and chronic exposure of professional soccer players to high-level training and competition. Moreover, highlights the complexity of the established interactions between players’ game-physical performance (GPP), GPP throughout the season and players physical fitness (PPF). In fact, despite PPF shown to be related with GFP, a direct link between an in-season higher GPP and PPF could not be established. In fact, a higher GPP towards end-of-season may not necessarily be coincident with a higher PPF (e.g. expressed both by performance and biochemical measurements) in the same time point. Nevertheless, data suggest that neuromuscular capabilities play an importance role in high-level soccer performance and that individual playing time may influence the former as well as hormonal and oxidative parameters of professional soccer players.
References


Individual Match Playing Time During the Season Affects Fitness-Related Parameters of Male Professional Soccer Players

João R. Silva, José F. Magalhães, António A. Ascensão, Eduardo M. Oliveira, André F. Seabra, and António N. Rebelo

Abstract
Silva, JR, Magalhães, JF, Ascensão, AA, Oliveira, EM, Seabra, AF, and Rebelo, AN. Individual match playing time during the season affects fitness-related parameters of male professional soccer players. J Strength Cond Res 25(10): 2729–2739, 2011—The purpose of this study was to analyze the effects of an entire season on physical fitness parameters (PFPs) in male professional soccer players (N = 18). Performance in 5- and 30-m sprint (T5 and T30), countermovement jump (CMJ), agility (T-test), knee extensor (KE) and knee flexor (KF) isokinetic strength, hamstrings/quadriceps strength ratio (H/Q) and bilateral differences (BDs), and Yo-Yo intermittent endurance test 2 (YYIE2) was evaluated in 4 moments (E1–E4) throughout the season. Individual match playing time was quantified. Significant improvements in CMJ and YYIE2 from E1 to E2 were observed (p < 0.05–0.01). The T30 improved from E2 to E3 (p < 0.01). The CMJ decreased from E2 to E3 and E4, and YYIE2 from E2 to E4 (p < 0.05). There were increments in the H/Q ratio and Agility from E1 and E2 to E3 and E4 (p < 0.05–0.01). Significant correlations were found in all evaluation points between different PFPs and between changes in strength parameters and agility, T5 and T30, CMJ, and YYIE2 (p < 0.05–0.001). Influence of individual match playing time was correlated to changes in T5 (E1 to E3; r = −0.705), KE nondominant leg (KEND; E2 to E3; r = 0.786), and KF (E3 to E4; r = 0.575–0.590). The interrelationship between muscle strength (e.g., KE), sprint (e.g., T5), and jump abilities (CMJ) suggests the importance of muscle strength and power training for soccer. This study suggests that the systematic participation of the players in soccer matches favors the increase and maintenance of soccer players KE and KF muscle strength and sprint ability (T5). Thus, given the unique demands of actual match play, coaches should try to incorporate a competitive friendly match in the weekly training cycle of nonstarter players.

Keywords: soccer season, training schedule, training status, strength, muscle power, intermittent endurance

Introduction
The regular evaluation of soccer players’ physical fitness using different tests is of special importance because players should be able to successfully compete during 10–11 months. Data from different studies suggest that elite soccer players cover 8–13 km during matches (7) at a mean intensity close to the anaerobic threshold (AT) (43). Moreover, energy expenditure during match averages 70–75% of maximal oxygen consumption (VO2max) (7,38), which suggests that performance at the elite level may, in part, be determined by aerobic fitness (38). However, despite low- to medium-intensity running being the predominant activity pattern of soccer players, muscle power-based efforts, such as sprints, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power of the neuromuscular system (15), are essential factors to successfully perform in soccer. Therefore, it is consensual among the literature that soccer players’ performance is intimately related to the efficiency of different energy-related systems (43). During season, players are usually under prolonged physiological stress, as has been described in different studies. Analysis of soccer player during the season has revealed signs of oxidative (9) and functional stress (7,25), and immune system (36) and hormonal stress-related imbalances (25). In such conditions, the maintenance or improvement of player performance is not only determined by appropriate conditioning but also by the ability of the body systems to recover and regenerate after multiple
stress stimuli (25). Therefore, to better understand the recovery pattern of the player under such demanding competitive stress conditions, a longitudinal coverage of physical performance in several time points throughout the season may be useful. However, there is a lack of studies concerning the effects of an entire season on physical fitness of professional players engaged in sport activities, including soccer. Most of the studies rely on particular functional or physiological data such as anthropometrics (11,13,25,31), \( \dot{V}O_2\max \) and AT measurements (11,13,31,32), specific intermittent endurance test (6,36), exercise performance in incremental treadmill tests until exhaustion (11), muscle power through jump and sprint tests (4,11,30), and lower limb strength evaluated by isokinetic devices (30,31). Inconsistency has been revealed in the literature regarding the impact of training and competition in the variation of certain fitness parameters (4,10,11,13,25,30–32). Moreover, these observations resulting from longitudinal scanning of players fitness, and from the analysis of the short-term impact of diverse forms of strength (29,49) and endurance training (42,49), did not consider the influence of the cumulative amount of competition playing time. A study performed by Impellizzeri et al. (23), wherein rating of perceived exertion (RPE) was used to quantify the internal training load (RPE-Tld), showed that in weeks comprising 2 official matches, the RPE-Tld can represent about 50% of total weekly training load decreasing to 25% in weeks with only 1 match performed. Hence, a possible determinant factor of physical fitness over the season could be the cumulative amount of match playing time, a subject scarcely explored. Therefore, the purposes of this study were to examine the impact of the season in a group of fitness-related parameters and the association between their changes, considering the influence of individual match playing time (IMPT).

**METHODS**

**Experimental Approach to the Problem**

As presented in Figure 1, the different physical tests were performed in the same order and in the same period of the day on 4 time points of a soccer season (10–11 months) as follows: first (E1)—before the soccer season; early July); second (E2)—end of the preseason period (early August); third (E3)—middle of the season (January); fourth (E4)—end of the competitive season (May).

In each moment, players were evaluated for basic anthropometry, countermovement vertical jump (CMJ), sprint and agility abilities, leg extensor and flexor maximal strength, and Yo-Yo intermittent endurance test 2 (YYIE2). However, no YYIE2 data were collected at the midseason (E3) because of Club heavy match commitments at that time. Additionally, the players’ individual time spent in the matches was recorded. The physical fitness measurements throughout different time points of the season allowed the analysis of seasonal fitness alterations, the relationship between the different fitness parameters, and the IMPT in physical fitness. The selected variables provide valid and reliable data for the evaluation of physical fitness parameters (PFPs), known to be directly (agility, sprint, jump, intermittent endurance) and indirectly (isokinetic strength parameters) related to soccer performance.

**Subjects**

A group of 23 male professional players competing in the Portuguese elite championship (rank 6 of the Union of European Football Associations) was initially involved in the protocol. As a result of injuries (\( n = 2 \)) and transfers to other clubs (\( n = 3 \)), 5 players were not considered in the analysis (\( n = 18 \); Table 1). Only players free from injury involved in the full training schedules were tested. In accordance with the Club policy, all soccer players underwent usual physical examination in the beginning of the season (e.g., blood sample analyses, rest electrocardiogram, lung x-ray).

The experimental protocol followed the Declaration of Helsinki of the World Medical Association for research with humans and was approved by the local Ethics Committee. All participants were fully informed about the aims, experimental protocol, and procedures and provided written informed consent.

![Figure 1](image.png)

**Figure 1.** Season time line. E1 = evaluation moment 1 (before preseason); E2 = evaluation moment 2 (end of preseason); E3 = evaluation moment 3 (midseason); E4 = evaluation moment 4 (end of season); YYIE2 = Yo-Yo intermittent endurance test 2; IS = isokinetic strength; CMJ = countermovement jump; TS = 5-m sprint time; T30 = 30-m sprint time; W = week; 8FM = 8 friendly matches; 1OM = 1 official match per week; 2OM = 2 official matches per week; 1FM = 1 friendly match per week; 2FM = 2 friendly matches per week; 3FM = 3 friendly matches per week.
Evaluation Procedures

The CMJ, sprint ability, and agility tests were conducted in indoor facilities to exclude possible ground surface variations in the soccer pitch throughout the season. Before the tests, all the players performed a 10–15 minutes of warm-up consisting of light jogging, specific mobility exercises and stretching routine, and 10-m sprints. Players completed 2 rounds of each test in the same sequence, and the best result was considered for analysis. Each subject was allowed a minimum of 5-minute rest between tests to ensure adequate recovery. All the evaluations took place at the same time of the day, after regular overnight sleep. Players were instructed to maintain normal routines for daily food and water intake and followed the same dietary recommendations defined by the medical staff. In addition, during the days of physical tests, players were instructed to refrain from drinking beverages containing caffeine and alcohol and from consuming food during the 3 hours before testing. In the 2 days preceding evaluations, the players had a day off (first day) and a training session (second day) including low-intensity exercises aiming to improve postmatch recovery.

Physical Performance Tests

Countermovement Jump. The CMJ was performed using a platform, Ergojump (Digitime 1000, Digitest. Jyvaskyla, Finland) according to Bosco et al. (8), whereby the highest vertical jump (centimeters) and the longest flying time (seconds) were registered. The best trial of the 2 jumps was considered. The apparatus consisted of a digital timer connected by a cable to a jump platform, Ergojump (Digitime 1000, Digest). The timer is triggered at take-off and then by participants’ feet at the moment of touchdown. The subject started from an upright standing position on the platform, and immediately after an eccentric phase (corresponding to a semi squatting position), the participants jumped vertically without using arms (arms remained at both sides, hands on the hip throughout the tests).

Sprint Time. Sprint measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-TI75, Draper, UT, USA) mounted on tripods positioned approximately 0.75 m above the floor and situated 3 m apart facing each other on either side of the starting line (0 m), at 5 and 30 m. The players stood 0.3 m behind the starting line, started at their own discretion, being time activated when players cross the first pair of photocells, and they ran as fast as they could to complete 30-m distance. The fastest trial was considered.

Agility. Agility was evaluated through the T-test following the protocol of Semenick (41) with modifications (Figure 2). The subjects began with both feet 0.3 m behind the starting point A. At their own discretion, each subject sprinted forward 9.14 m (10 yd) to point B and touched the base of the cone with the right hand. They then sprinted to the left 4.57 m (5 yd) and touched the base of a cone (C) with the left hand. The subjects then sprinted to the right 9.14 m (10 yd) and touched the base of a cone (D) with the right hand. They then sprinted to the left 4.57 m back to point B and touched the base of a cone with the left hand. They turned 270° and then ran to point A, passing the finishing line. Two test trials were performed, and times

<table>
<thead>
<tr>
<th>Variable</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.7 ± 4.6</td>
<td>25.4 ± 4.7</td>
<td>26.9 ± 4.3</td>
<td>27.1 ± 4.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.5 ± 9.2</td>
<td>76.2 ± 9.3</td>
<td>76.3 ± 8.4</td>
<td>77.7 ± 9.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.1 ± 5.7</td>
<td>177.8 ± 5.7</td>
<td>178.7 ± 6.6</td>
<td>179.1 ± 6.6</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>9.8 ± 3.7</td>
<td>9.5 ± 2.7</td>
<td>9.3 ± 3.0</td>
<td>9.1 ± 3.1</td>
</tr>
</tbody>
</table>

*E1 = evaluation 1 (before preseason); E2 = evaluation 2 (end of preseason); E3 = evaluation 3 (midseason); E4 = evaluation 4 (end of season).
†Values are mean ± SD.

Figure 2. Layout of the T-test. Modified from Semenick (41). Brief explanation.
were recorded to the nearest 1/100th of a second. As described for sprint ability, measurements were carried out using telemetric photoelectric cells (Brower Timing System, IRD-T175). The players stood 0.5 m behind the starting line, being time activated when they passed the electronic sensors, and the clock stopped the instant players again crossed Point A. The fastest trial was considered.

**Isokinetic Strength.** To evaluate the players’ lower limb muscle function, maximal gravity corrected concentric peak torque of quadriceps and hamstring, bilateral leg strength differences (BD), and ratio between concentric hamstring (H) and quadriceps (Q) peak torque values (H/Q) were measured during isokinetic knee joint movement (Biodex System 2, New York, NY, USA) of the dominant and nondominant (ND) leg at the angular velocity of 110° s⁻¹ (1.57 rad s⁻¹), according to Magalhães et al. (27). After individual self-report, the dominant leg was determined by a routine visual inspection in a simple target-kicking test requiring accuracy. Before muscle function measurements, subjects performed a standardized warm-up consisting of 5-minute period on a cycle ergometer (Monark E-824) with a fixed load corresponding to 2% of body weight. Players were then seated on the dynamometer chair at an 85° inclination (external angle from the horizontal) with stabilization straps at the trunk, abdomen, and thigh to prevent inaccurate joint movements. The contralateral leg was not secured to avoid influencing the strength developed by the knee being tested. The knee to be tested was positioned at 90° of flexion (0° = fully extended knee), and the axis of the dynamometer lever arm was aligned with the distal point of the lateral femoral condyle. Before the anatomical alignments and procedures, all the subjects were instructed to kick and also to bend the tested leg as hard and as fast as they could through a complete range of motion (from 90° to 0°). The subjects were also instructed to hold their arms comfortably across their chest to further isolate knee joint flexion and extension movements. All subjects also performed a specific submaximal warm-up protocol on the Biodex device to familiarize with the isokinetic device and test procedure. Three maximal repetitions at angular velocity 90° s⁻¹ (1.57 rad s⁻¹) were therefore carried out. The highest peak torque found during all the repetitions was chosen for the calculation of the BD. This parameter was calculated as follows: [(dominant concentric leg strength − ND concentric leg strength)]/dominant concentric leg strength × 100 and is expressed from absolute values as percentage, that is, independently of the difference direction (from dominant [D] to ND or ND to D). The ratio between concentric hamstring (H) and quadriceps (Q) peak torque values (H/Q) was also determined and expressed as percentage.

**Yo-Yo Intermittent Endurance Test Level 2.** The Yo-Yo tests were designed to measure the ability to perform bouts of repeated intense intermittent exercise. After a 10-minute warm-up, the players performed the test, which consists of repeated 2 × 20-m runs back and forth between the start and finish lines at a progressively increased speed controlled by audio beeps from a CD-ROM (5). The initial speed was 11.5 km h⁻¹ (12.5 seconds for 2 × 20 m), and between running bouts the participants had a 5-second rest period. The test was considered ended when the subjects failed twice to reach the starting line (objective evaluation) or the participant felt unable to complete another shuttle at the dictated speed (subjective evaluation) (12). The total distance covered during the YYIE2 (including the last incomplete shuttle) was considered as the testing score. Heart rate was measured during the YYIE2 and recorded every 5 seconds using an HR monitor (Polar Team System™, Polar Electro, Kempele, Finland). Field testing sessions were performed on the football pitch where players undertake their daily training sessions on several marked 2-m-wide and 20-m-long running lanes.

Intraclass correlation coefficients of all physical fitness tests were estimated using a test–retest procedure, with a random subsample of 9 subjects in each evaluation moment. The intraclass correlation coefficients (R) of all variables were high as follows: Height, weight, and fat mass: 0.93 ≤ R ≤ 0.99; countermovement jump: 0.80 ≤ R ≤ 0.88; sprint time: 0.71 ≤ R ≤ 0.87; agility: 0.70 ≤ R ≤ 0.85; Yo-Yo intermittent: 0.80 ≤ R ≤ 0.97; and isokinetic strength: 0.78 ≤ R ≤ 0.98.

**Playing Schedule and Training Program**

The generic training and competition plan completed by the soccer players involved in this study was supplied by the technical staff of the team. As can be seen by the season time line (Figure 1), 32 training sessions (6.4 per week) each lasting for 90–120 minutes per session, and 8 “friendly” matches for a total duration of 3,400 minutes were comprised between E1 and E2 (5 weeks). Training contents during this period consisted of 2 sessions per week of aerobic and strength training from week 1 to weeks 3 and 2 “friendly” matches. From weeks 4 to 5, 1 session per week of aerobic and strength training and 6 “friendly” matches were performed, 3 matches in each week. Aerobic training sessions consisted of general (interval running) and specific exercises (small-sided games and soccer specific circuits). Strength training sessions followed the specificity of strength training based on complex and contrast training (14).

Before training sessions, players performed a warm-up lasting approximately 20–30 minutes (5–10-minute jogging and low-intensity running, flexibility and mobility exercises, technical drills, short and brief explosive actions). After the training sessions, a warm-down that lasted approximately 10–15 minutes (jogging and stretching) was fulfilled.

Between E2 and E3 (25 weeks), first half of the in-season, the team performed 150 training sessions (6 per week) with a total duration of 13,300 minutes and played 26 official matches (1.04
matches per week). In this period, players performed in each week 1 session of aerobic high-intensity training (high-intensity interval training and small-sided games), 1 session of functional strength training (plyometric training, resistive sprints, agility drills) and 1 session of others sprint ability exercises (with and without changes of directions).

Between E3 and E4 (17 weeks), second half of the in-season, players were engaged in 90 training sessions (5.3 per week) with a total duration of 7,200 minutes and played 26 official matches (1.5 per week). In this period, the number of matches increased, and the number of fitness training sessions decreased.

**Statistical Analyses**

All data are reported as mean and SD. Normality was tested with the Shapiro–Wilks test. Intraclass correlation coefficient was calculated to estimate the reliability of the physical fitness tests. Analysis of variance for repeated measures was used to compare the differences between evaluations. Pearson correlation coefficients (r) were used to determine association between tests, tests changes, and their relationship with individual match playing time. The SPSS statistical package (version 14.0; Inc., Chicago, USA) was used. Statistical significance was set at p ≤ 0.05.

**Table 2. Variation of different performance measurements in the 4 evaluation moments throughout the soccer season.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>GS</th>
</tr>
</thead>
<tbody>
<tr>
<td>T5m (s)</td>
<td>1.032± 0.11</td>
<td>1.056± 0.05</td>
<td>1.017± 0.06</td>
<td>1.033± 0.07</td>
<td>1.033</td>
</tr>
<tr>
<td>T30m (s)</td>
<td>4.164± 0.18</td>
<td>4.217± 0.15</td>
<td>4.137± 0.14‡</td>
<td>4.16± 0.17</td>
<td>4.169</td>
</tr>
<tr>
<td>CMJ (cm)</td>
<td>42.44± 4.04</td>
<td>44.64± 4.5§</td>
<td>42.84± 4.4</td>
<td>42.23± 4.38</td>
<td>43.08</td>
</tr>
<tr>
<td>T-test (s)</td>
<td>8.714± 0.33</td>
<td>8.745± 0.36</td>
<td>8.408± 0.27∥</td>
<td>8.498± 0.27∥</td>
<td>8.591</td>
</tr>
</tbody>
</table>

*E1 = evaluation moment 1 (before preseason); E2 = evaluation moment 2 (end of preseason); E3 = evaluation moment 3 (midseason); E4 = evaluation moment 4 (end-of-season); GS = global sample; T5m = 5-m sprint time; T30m = 30-m sprint time; CMJ = countermovement jump.

†Values are mean ± SD.

‡p < 0.05 E2 vs. E1, E3, and E4.

§p < 0.05 E2 vs. E1, E3, and E4.

∥p < 0.01 E3 and E4 vs. E1 and E2.

**Table 3. Variation of isokinetic strength measurements in the 4 evaluation moments throughout the soccer season.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>GS</th>
</tr>
</thead>
<tbody>
<tr>
<td>KED 90°·s⁻¹ (N·m)</td>
<td>238.95± 39.8</td>
<td>240.73± 51.4</td>
<td>239.13± 45</td>
<td>243.96± 52.8</td>
<td>240.64</td>
</tr>
<tr>
<td>KFD 90°·s⁻¹ (N·m)</td>
<td>131.22± 22.1</td>
<td>131.58± 21.9</td>
<td>135.36± 25.1</td>
<td>138.03± 24.2</td>
<td>134.05</td>
</tr>
<tr>
<td>H/Q D (%)</td>
<td>54.91± 6</td>
<td>54.65± 5</td>
<td>56.6± 8.5‡</td>
<td>56.4± 7.3‡</td>
<td>55.64</td>
</tr>
<tr>
<td>KEND 90°·s⁻¹ (N·m)</td>
<td>241.748± 39</td>
<td>241.338± 47</td>
<td>241.85± 40</td>
<td>243.82± 53.3</td>
<td>241.417</td>
</tr>
<tr>
<td>KFND 90°·s⁻¹ (N·m)</td>
<td>128.992± 19.6</td>
<td>128.561± 23.4</td>
<td>133.01± 20.1</td>
<td>133.86± 23.6</td>
<td>130.74</td>
</tr>
<tr>
<td>H/QND (%)</td>
<td>53.35± 4.9</td>
<td>53.2± 5.2</td>
<td>55.02± 5.4‡</td>
<td>54.9± 6.1‡</td>
<td>54.1</td>
</tr>
<tr>
<td>BDKE (%)</td>
<td>5.76± 5.36</td>
<td>5.57± 5.42</td>
<td>5.70± 4.95</td>
<td>8.54± 4.91</td>
<td>6.39</td>
</tr>
<tr>
<td>BDKF (%)</td>
<td>6.64± 6.3</td>
<td>5.59± 4.7</td>
<td>7.66± 5.4</td>
<td>7.6± 5.8</td>
<td>6.87</td>
</tr>
</tbody>
</table>

*E1 = evaluation moment 1 (before preseason); E2 = evaluation moment 2 (end of preseason); E3 = evaluation moment 3 (midseason); E4 = evaluation moment 4 (end-of-season); GS = global sample; KED = peak torque in knee extension dominant legs; KFD = peak torque in knee flexion dominant legs; H/Q D = concentric hamstrings/quadiceps strength ratio dominant leg; KEND = peak torque in knee extension nondominant legs; KFND = peak torque in knee flexion nondominant legs; H/QND = concentric hamstrings quadiceps strength ratio nondominant leg; BDKE = bilateral strength differences in extensors muscles; BDKF = bilateral strength differences in flexors muscles.

†Values are mean ± SD.

‡p < 0.05 E3 and E4 vs. E1 and E2.
### Results

#### Physical Performance Tests

The results of the physical tests performed in the 4 time points throughout the season are shown in Tables 2–4. The best 5-m (T5) and 30-m (T30) results were obtained at E4, but significant differences were only observed between E2 and E3 in the T30 (Table 2).

The results of CMJ were significantly higher in the E2 compared with the other 3 time periods (Table 2). No significant changes were found between E3 and E4 nor between E1 and E2. The total distance covered during the YYIE2 significantly increased in E2 and E4 compared with E1. However, significant differences were found between E2 and E3 and E4.

No significant changes were found in knee extension (KE) and knee flexion (KF) strength in both dominant (D) and ND legs (peak torque in knee extension dominant legs [KED], peak torque in knee extension nondominant legs [KEND], peak torque in knee flexion dominant legs [KFD], peak torque in knee flexion nondominant legs [KFND]), and in BD in leg extension and flexion in the 4 time points (Table 3). However, a significant increase in the H/Q ratio was found from E1 and E2 to E3 and E4.

As mentioned, YYIE2 was only evaluated in E1, E2, and E3. The best 5-m (T5) and 30-m (T30) results were obtained at E4, but significant differences were only observed between E2 and E3 in the T30 (Table 2). No significant changes were found between E3 and E4 nor between E1 and E2. The total distance covered during the YYIE2 significantly increased in E2 and E4 compared with E1. However, a significant decrease was also observed from E2 to E4 (Table 4).

#### Association between Tests

In E1, T5 was correlated with T30 ($r = 0.662; p < 0.001$) and T-test time ($r = 0.577; p < 0.01$). The T30 was also correlated with CMJ ($r = −0.456; p < 0.05$) and T-test ($r = 0.441; p < 0.05$). Moreover, significant correlations were observed between CMJ and KE values ($r = 0.524$ and 0.534 for KED [p < 0.05] and KEND [p < 0.01], respectively). In E2, significant correlations were found between CMJ and T30 ($r = −0.723; p < 0.01$), T5 ($r = −0.526; p < 0.01$), and T-test time ($r = −0.556; p < 0.01$). The T30 was also correlated with T5 ($r = 0.667; p < 0.01$) and T-test time ($r = 0.725; p < 0.01$). In E3, correlations were found between CMJ and T5 ($r = −0.508; p < 0.05$) and T30 ($r = −0.686; p < 0.01$), and also between T5 and T30 ($r = 0.801; p < 0.01$). Additionally, significant correlations between H/QND and the T-test were found ($r = 0.607; p < 0.01$). In E4, correlations between T5 and CMJ ($r = −0.730; p < 0.01$; Figure 3) were observed. The KED was correlated with the T5 ($r = −0.547; p < 0.05$), and H/QND was correlated with ke CMJ ($r = 0.623; p < 0.01$). In all the evaluations, body mass was correlated with the peak torques from both KE and KF (r ranging from 0.6 to 0.8 and $p < 0.01$ to 0.001).

#### Association between Changes in the Tests

From E1 to E2, significant correlations were observed between individual changes in T30 and changes in CMJ ($r = −0.546; p < 0.05$) and KED ($r = −0.623; p < 0.01$). Changes in T-test time were correlated with changes in KFD.

### Table 4. Variation of YYIE2 measurements in the 4 evaluation moments throughout the soccer season.*†

<table>
<thead>
<tr>
<th>Variables</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>GS</th>
</tr>
</thead>
<tbody>
<tr>
<td>YYIE2 (m)</td>
<td>1.120 ± 187.6</td>
<td>2.250 ± 296.4‡</td>
<td>1.640 ± 196§</td>
<td>1.670</td>
<td></td>
</tr>
<tr>
<td>HRmax (b-min⁻¹)</td>
<td>197.9 ± 8.3</td>
<td>196 ± 7.1</td>
<td>196.1 ± 9.0</td>
<td>196.9</td>
<td></td>
</tr>
<tr>
<td>HRmean (b-min⁻¹)</td>
<td>181.6 ± 9.6</td>
<td>180.9 ± 7</td>
<td>176 ± 9.8</td>
<td>179.5</td>
<td></td>
</tr>
</tbody>
</table>

*E1 = evaluation moment 1 (before preseason); E2 = evaluation moment 2 (end of preseason); E3 = evaluation moment 3 (midseason); E4 = evaluation moment 4 (end-of-season); GS = global sample; YYIE2 = Yo-Yo intermittent endurance test level 2; HRmax = maximal heart rate; HRmean = mean heart rate.

†Values are mean ± SD.
‡p < 0.01 E2 vs. E1.
§p < 0.05 E4 vs. E1 and E2.
(r = 0.522; p < 0.05) and H/QD (r = 0.768; p < 0.001). Also, a significant correlation between individual changes in KED and YYIE2 was observed (r = −0.568; p < 0.05). From E2 to E3, significant correlations were observed between individual changes in T-test time and H/QD (r = 0.726; p < 0.05). Also, changes in KEND were correlated with T5 (r = −0.638; p < 0.05; Figure 4), and changes in T-test time were correlated with H/QND (r = 0.622; p < 0.05) from E3 and E4.

Association between Match Playing Time and Changes in the Tests

The individual match playing time from E1 to E3 was correlated with the individual changes in T5 (r = −0.705; p < 0.01; Figure 5). From E2 to E3, the IMPT was significantly correlated with KEND (r = 0.786; p < 0.05) and H/QND (r = −0.738; p < 0.05). From E3 to E4, the IMPT was correlated with KFD (r = 0.590; p < 0.05), KFND (r = 0.575; p < 0.05) and H/QND (r = 0.794; p < 0.05, Figure 6).

**DISCUSSION**

The purposes of this study were to examine the impact of the season in a group of fitness-related parameters and the association between their changes, considering the IMPT.

Our data showed that very short-sprint performance measured by the T5 is a stable physical ability throughout the soccer season. However, significant differences were observed in the T30, where the time to perform the 30-m distance decreased from E2 to E3 (p < 0.01) evidencing better sprint performance in the middle of the competitive period. In contrast with our data, Aziz et al. (4) reported a gradual improvement in sprint performance over the season in both 5- and 20-m sprint tests.

The soccer players showed a significantly higher CMJ performance in E2 than in E1, E3, and E4 (Table 2). Accordingly, Clark et al. (13) also found variations in CMJ throughout the soccer season. However, discrepancies regarding seasonal variation in CMJ performance have been reported in the literature. In fact, in contrast with our results, others did not report variations in CMJ during the season (11, 30, 31). The increase in CMJ performance in E2 could possibly be explained by the higher number of sessions dedicated to improve strength and muscle power during preseason. During this period, high training volumes are usual aiming to improve the general fitness levels (45) including muscle power. Moreover, around December before E3, and from E3 to E4, we observed an increase in the number of games (see Figure 1) requiring more time given to recovery interventions, which implies a reduction in the volume of strength and power training and potentially explain CMJ decrement from E2 to E3 and E4. The predominant use of functional strength or solely functional type of muscle training during in season may result in a decrement of the jump ability gains attained during preseason training (10, 39). Nevertheless, in a study by Caldwell and Peters (10), semiprofessional players only
performing functional strength training during in season were able to further increase and maintain the improvements obtained during preseason.

Some researchers have suggested the importance of the agility as an independent physical variable in the assessment of male football players’ performance (44,48). Our data showed improvements in agility performance from E1 and E2 to E3 and E4, with the highest scores being observed in E3. A better coordination between agonist and antagonist muscles allows an improved ability to stop, start, and turn rapidly while maintaining balance without loss of speed (41), which may explain, at least in part, our results. These actions that are systematically performed during training and games could likely contribute to the agility increase observed in E3 and E4.

All together, the results of the agility and sprint tests suggest that activities related with the stretch-shortening cycle (SSC [26]) were improved in the midseason (E3). However, CMJ was not improved in this period. One possible reason is that CMJ might not fulfill the criteria of SSC as would running-based exercises, which lie in a very fast transition from stretch to shortening phases and in a greater stretch velocity, both critical for stretch reflex activation (47).

Generally, data from our study showed that players maintain the same levels of strength in both knee extension and flexor muscles and bilateral strength differences throughout the soccer season. In fact, only significant increases in the H/Q ratio from E1 and E2 to E3 and E4 were observed. A stability of isokinetic strength parameters (e.g., KE) of professional soccer players over the season was also observed by other investigators (30). In contrast, amateur players showed a significant decrease of KE during an 11-week competitive soccer season (25). This fact may highlight the possible influence of training time in exercises specifically targeting muscle strength. Curiously, the KE peak torques observed in this study during the season were higher than those reported in professional soccer players even at lower angular velocities (60°·s⁻¹) (30,31). Possible differences in time devoted to strength training may contribute to explain, at least partially, the referred differences between studies. Nevertheless, it is important to refer that despite the popularity of isokinetic tests in research, they are less predictive of performance and longitudinal changes than isoinertial measures, because a high sensibility is expected when the mode of training matched the mode of testing (22).

Despite the multifactorial etiology of muscle injury, some evidence suggests that imbalances in H/Q, and BD (2,16), previous injury (2,17) and lower resistance to fatigue (e.g., hamstrings) (40), are major risk factors for soft tissues injuries of soccer players. Throughout the time course of our study, the H/Q ratios observed in the different time points were identical to those reported previously by our group (27) and lower than the results reported by others (31). Furthermore, Mercer et al. (31) did not report any significant changes in H/Q from the beginning to the end of preseason (E1 to E2). According to the improvements in CMJ during preseason and in agility during in season, it seems reasonable to suggest that a detraining effect may occur during offseason. In fact, there is scientific evidence that detraining effects are more marked in muscle power than in strength, the former being more dependent on muscle coordination (24,34).

The Yo-Yo Test is widely used to evaluate the ability of soccer players to intermittently perform exercise and has been considered a sensitive tool to detect seasonal changes in the fitness of soccer players (6). Our data showed an increase in the YYIE2 performance after the preseason period followed by a decrease at the end of season (E4). These results are in agreement with previous data reporting soccer players’ lower intermittent endurance performance at the beginning of the season in field and laboratory tests. In fact, data obtained through different tests, such as the incremental treadmill test to exhaustion (32), exercise tolerance at VO₂ max, Intermittent Field Test (36), and the Yo-Yo Intermittent Recovery Test (6) expectedly revealed lower performance at the beginning of the season than during the competitive period. Unfortunately, because of practical constraints related to the competitive calendar of the team engaged in this study, no data have been collected during the midseason (E3), which enables one to assess the impact of the first half of the season in the ability to perform intermittent endurance exercise.

Despite some controversies regarding the eventual contribution of the players’ aerobic power, the increments in YYIE2 performance in E2 and E4 compared to E1 may also be related to improvements in other endurance-related physiological features. Rampinini et al. (35) recently observed that time constant of VO₂ Kinetics and the ability to maintain acid–base balance are also important physiological factors to the performance in Yo-Yo tests. As so, improvements in the former factors throughout the season may have increased the capacity to repeatedly perform intermittent endurance exercise. On the other hand, the lower levels of intermittent endurance observed in E1 may also explain the significant increment observed during preseason (E2), because in such conditions, higher adaptations should be expected.

The decline in YYIE2 performance observed in our study at E4 compared to that at E2 might be related to the specificity of the competition schedule. In fact, Mohr et al. (33) reported decreases in the ability to perform high-intensity running during games in elite soccer players involved in National and European competitions (2 matches per week) toward the middle of the season, during which there is limited time to fitness training and more time to recovery is needed. Accordingly, Kraemer et al. (25) observed that starter players who usually accumulate more match playing time experience greater performance decrements in some fitness parameters. Curiously, no relationship was observed in this study between changes in YYIE2 and IMPT.

An intriguing finding was that players with lower increments or higher decrements in KFD (E1 to E2), H/QD (E1 to E2 and E2 to E3), or H/QND ratio (E3 to E4) showed...
higher improvements in agility performance. These results suggest that improvements in flexors strength and H/Q ratios seem to be in the opposite direction of agility development. A possible explanation could be that the decrease in the H/Q strength ratio allowed the increment of acceleration capacity resulting from the increase of KE activation and from the reduction of the antagonist KF coactivation during the knee extension task (50).

The present results showed that improvements in KED from E1 to E2, KEND from E3 to E4 (Figure 4), and CMJ from E1 to E2 were significantly correlated with the performance improvement in short sprints. The KE also showed significant correlations with the CMJ (E1) and T5 (E4; KED). Moreover, high correlations between CMJ and T30 (E1, E2, and E3) and T5 (E3 and E4; Figure 3) were observed. Previous studies (19,48) showed that players with improvements in CMJ and in strength experienced improvements in short-sprint performance. As proposed by other researchers (19), this may suggest a possible transfer from leg power strength gains into enhanced short sprint performance. In fact, there were significant associations between the different functional tests that rely on power activities (CMJ, T5, T30, and Agility; Figure 3) in the different evaluation time points. Moreover, from E1 to E2, we observed that the players with higher improvements in KED showed lower improvements in YYIE2 (r = −0.568; p < 0.05). However, during the same period, a higher improvement in YYIE2 was parallel to an improvement in a muscle power test (CMJ). It has been reported that concurrent muscle training and high-intensity interval training performed during pre-season could lead to improvements both in muscle power (10- and 30-m sprint, vertical jump) and in intermittent endurance performance (YYIR1)(49). However, after the observation of an increment in both CMJ and YYIE2 performance during pre-season in this study, no relationships between the 2PFPs and/or their changes were found.

Top-level soccer players are usually involved in the weekly matches of the national leagues and very often in international commitments. These competitive demands may impose strains to various physiological systems including musculoskeletal, nervous, immune and metabolic (7,9,25,36), to a point where the managing of player’s physical fitness trough training and recovery strategies became influential to maximize match performance. Thus, the knowledge on the relationship between IMPT and PFP throughout the season would be helpful in the management of training programs including the time scheduling of official and “friendly” matches and specific training sessions.

During the 90 minutes of the match, soccer players can perform 10–20 sprints, a high-intensity running every 70 seconds, about 15 tackles, 10 headings, 50 involvements with the ball, and 30 passes, and changing pace and sustaining forceful contractions to maintain balance and control of the ball against defensive pressure (43). Therefore, high stress levels are imposed on the neuromuscular system to cope with this essential muscle power-based efforts. As already observed in other intermittent team sports such as handball (18,20), individual match playing time (IMPT) seems to influence players’ fitness during season. In fact, according to our results, players with more accumulated IMPT from E1 to E3 showed higher improvements in T5 (r = −0.705; p < 0.01; Figure 5). This suggests that the short bursts of acceleration required during games could have a positive effect in developing players’ ability to accelerate (15,48).

It was observed that players with higher IMPT showed both higher increments and lower decrements in KEND (r = 0.786; p < 0.05; E2 to E3), KFD, and KFND (r = 0.575 and 0.590, respectively; p < 0.05; E3 to E4). Kraemer et al. (25) observed that during and after a 11-week competitive soccer season, collegiate soccer players’ KF strength did not significantly decrease as much as KE did. Moreover, the authors observed that starter players showed higher values of KF in the different evaluation points. Additionally, we observed that the correlation coefficients between IMPT and individual changes in the H/Q ratio were r = −0.738 (p < 0.05) from E2 to E3 and changed to a positive value of r = 0.794 (p < 0.05) from E3 to E4 (Figure 5), reflecting the different impact of IMPT in KE and KF in the different evaluations throughout the competitive period. The observed relationship between IMPT and these PFP suggests that male professional soccer players with higher IMPT have higher capabilities to increase or maintain muscle strength (KE and KF) and sprint (T5) throughout the season, that is, competition time may possibly contribute to influence certain physical characteristics of professional soccer players. In fact, it was evidenced that after the game, KF is affected by fatigue and muscle damage and the KF reduction follows the increase in some indirect markers of muscle damage (1,3,28).

Moreover, KF was also referred as a muscle group less resist to fatigue than KE (40) that shows after a match higher force decrements (1,21) and longer period of muscle soreness (46) than extensor muscles. This may explain at least partially the higher adaptations found in this muscle group in players with more IMPT between E3 and E4. In fact, muscle adapts to training regimens that engage them in SSCs and are impaired less with repeated exposure (37).

**Practical Applications**

This study showed that changes in different parameters of soccer players’ fitness are observed throughout a soccer season. In this way, a proper control of training and competition workloads and their impact should be monitored. The preseason improvements in players’ physical fitness from start (E1) to the end of the season (E4) makes it logical to conclude that during offseason players experienced pronounced effects of detraining in jump ability and agility, and intermittent endurance capacity. Thus, male professional soccer players should perform a specific training program or participate in active leisure activities to attenuate reductions in training status that result from offseason period and to better cope with preseason training loads. Coaches should be aware...
that the interrelationship between KE strength, 5- and 30-m sprint time, and CMJ performance suggests that muscle strength and power training should be an important component of soccer training. Moreover, given that during the in-season, the isolated completion of functional strength exercises may be insufficient to maintain the jump ability of professional players, the incorporation of some form of weight training (e.g., combined weight and plyometric training) might be beneficial. This study suggests that the systematic participation in soccer matches favors the increase and maintenance of male professional soccer players’ muscle strength and sprint ability. Thus, given the unique demands of actual match play, coaches should try to incorporate a competitive friendly match in the weekly training cycle of nonstarter players.

ACKNOWLEDGMENTS

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REFERENCES


TRAINING STATUS AND MATCH ACTIVITY OF PROFESSIONAL SOCCER PLAYERS THROUGHOUT A SEASON

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ABSTRACT

Silva, JR, Magalhaes, JP, Ascensoa, AA, Seabra, AF, and Rebelo, AN. Training status and match activity of professional soccer players throughout a season. J Strength Cond Res XX(X): 000–000, 2012—The purpose of this study was to examine match activity (MA) and fatigue development (FD) during official soccer games in different moments of a season and the influence of training status (TS) on MA and FD. Match activity of 13 professional players was examined by time-motion analysis at 4 time points of a competitive season. In addition, a time point within the 3-week period between the 2 games video-taped, players performed the following physical tests: countermovement jump, 5- and 30-m sprints, change of direction, trunk extensor and flexor isokinetic strength, and Yo-Yo intermittent endurance test level 2. The players covered a greater high-intensity distance running (Hi; p < 0.05) in the last quarter of the season (E4) than in the second (E2) and the third (E3) quarters. Within each assessment period, a greater distance was covered in Hi during the peak 5-minute period of the match (P5-min) than in the 5-minute period after P5-min (Next5 min) and the remaining 6-minute periods (AV6 min); p < 0.05) of the match. Also, P5-min was higher in E4 than in the beginning of the season (E1, E2, and E3; p < 0.05). The physical fitness variables, composite scores of power-related and isokinetic strength tests were correlated with changes in MA and FD. The players with a greater muscle strength and power expressed lower performance decrements in the GPPs. In conclusion, the results highlight the relevance of players’ neuromuscular function on game physical performance.

KEY WORDS: performance analysis, competitive period, strength, muscle power, fatigue

INTRODUCTION

Match analysis is a widely used instrument in professional soccer to study tactical and physical performance of players and referees (2). Research with some of the most up-to-date technologies such as the multicamera method (2,4,44), global position systems (44), and video-based time-motion analysis (30,44) revealed detailed information about players’ movement patterns (30,44). Moreover, these methods seem to be able to detect performance decrements during soccer games and thereby enable the study of game-induced fatigue (44). Data revealed that several signs of fatigue can be manifested temporally during a game (6,30,31), toward the end of the game (6,30,31), and persist afterward (6,30,31) with a time dependency for the fitness parameter evaluated. Also, it has been observed through these methods that performance during the match is dependent on multiple factors (7,25,26,30,42).

Recent investigations observed that physical performance during the game changes throughout the season (30,42) and is related to players’ training status (25,26,41). In fact, some studies showed that soccer players’ performance in the Yo-Yo intermittent recovery test in both men (25) and women (26), in incremental field tests (shorter version of the University Montreal Track Test (41), and in the repeated shuttle sprint ability test (41) are indicators of game-related physical performance.

However, data on locomotive activity and on unorthodox movements (e.g., sluffling, diving, soccer-specific movements (e.g., heading, blocking), and accelerations and decelerations are generally omitted or not taken into account.
Training Status and Match-Related Physical Performance

In fact, a massive metabolic load is imposed on players, not only during the more intense parts of the game but also every time acceleration occurs, even when the speed is low (36). The regular evaluation of soccer physical fitness using different tests is of special importance, because soccer players should be able to successfully perform over competitive seasons of around 10–11 months. Although seasonal variations in game-physical performance have been analysed (39,42), these investigations usually involve 3 time points of the season (beginning, middle, and end-of-season). Therefore, research assessing a higher range of time points throughout the season will be more fruitful when analyzing seasonal variations in game-related physical parameters.

Moreover, scrutinized research of seasonal alterations in fatigue patterns (e.g., temporary fatigue) of soccer players during games has never been reported. Thus, we aimed to analyze game-related physical performance and fatigue development (FD) during official soccer games during 4 time points of the season.

Rapid force production is considered essential for a wide range of athletes (1,28,34). In fact, soccer players’ muscle strength (3,16) and performance in sport-specific muscle power efforts (15,33) were reported to be related to the players’ and team’s competitive levels. Moreover, improvements in coordination specificities (17) and a greater agility (19) have been positively associated with performance and the ability to delay fatigue. In accordance with recent reports, enhancement of neuromuscular function can be a determinant in improving short- and long-term endurance capacities (1).

Despite these indications and high stresses being imposed on the neuromuscular system during soccer games, until now, no study addressed whether muscle strength and power can be indicators of game-related physical performance. Therefore, we also aim to investigate whether physical conditioning evaluated in field and laboratory tests is related to game-physical performance and FD. We hypothesized that during the season, soccer players may experience performance alterations in certain game physical parameters (GPPs) analyzed by time motion. Moreover, given the high-stress levels imposed on the neuromuscular system, soccer players’ training status might be associated with some game-related physical parameters.

**Methods**

**Experimental Approach to the Problem**

Match activity (MA) and fatigue during games have been a topic of increased research in recent years (4,5,28,29,40). Moreover, players’ physical and physiological characteristics have already been extensively described (6,16,20). However, the seasonal alterations in MA and the influence of players’ training status in game physical performance had been scarcely investigated. Therefore, to analyze seasonal alterations in GPPs and in fatigue patterns (e.g., temporary fatigue), a sequence of time-motion analysis of 13 players was performed over 8 videotaped matches during 4 time points of a competitive season (Figure 1). Temperature and relative humidity during matches at different time points were as follows: E1: first and third official games, temperature ranging from 24 to 26°C and relative humidity from 40 to 44%; E2: eighth and tenth official games, temperature ranging from 19 to 21°C and relative humidity from 53 to 60%; E3: 15th and 16th official games, temperature ranging from 13 to 15°C.
and relative humidity from 65 to 65%; E4: 24th and 26th official game, temperature ranging from 14 to 15°C and relative humidity from 50 to 75%. In addition, to analyze the influence of player training status in game physical performance, the players underwent a group of laboratory and field physical tests within 2 weeks of each of the 2 time points of competitive games video-taped (E1 and E3, Figure 1). The players performed the countermovement jump (CMJ), 5-m (T5) and 30-m (T30) sprints, change-of-direction (COD) ability (t-test), knee extensor (KE) and knee flexor (KF) maximal isokinetic strength, and the Yo-Yo intermittent endurance test level 2 (YYIE2). These tests provide valid and reliable data allowing an evaluation of the physical fitness parameters directly (agility, sprint, jump, and intermittent endurance) and indirectly (isokinetic strength) related to soccer physical performance. In addition, composite scores were determined to provide a more complex operational indicator of physical fitness. With this option, we aimed to investigate the association between GPFs and certain soccer players’ specific physical fitness parameters to provide a more global indicator of the soccer players’ training status (e.g., the sum of the scores in the soccer-specific muscle power-related tests).

**Subjects**

A group of 13 professional male soccer players (1 defender, 5 midfielders, and 4 attackers; mean ± SD: 25.7 ± 4.6 years, body mass 76.3 ± 9.2 kg, height 176.1 ± 5.7 cm, and 9 ± 3.7% fat percentage) from a professional team competing in the Portuguese championship (Professional Soccer League) was involved. All the subjects had a minimum of 3 and a maximum of 10 years of senior soccer professional activity. Only injury-free players participating in full training schedules were tested. In accordance with the professional club policy and medical requirements, all the soccer players underwent physical examinations both at the beginning and throughout the season (e.g., blood sample analysis, resting electrocardiogram, lung X-Ray). All the subjects were informed of the purpose of the study, and written informed consent was obtained.

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### Table 1. Rank of opposing team, frequency of activities, and distance covered in different locomotion categories throughout the different evaluation moments of the season (mean ± SD).*

<table>
<thead>
<tr>
<th>Frequency (n)</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1,028 ± 251</td>
<td>1,077 ± 230</td>
<td>1,183 ± 184</td>
<td>1,155 ± 143</td>
</tr>
<tr>
<td>FLI</td>
<td>930 ± 88</td>
<td>711 ± 93</td>
<td>1,018 ± 124</td>
<td>850 ± 80</td>
</tr>
<tr>
<td>FFH</td>
<td>190 ± 27</td>
<td>130 ± 24</td>
<td>130 ± 261</td>
<td>169 ± 41</td>
</tr>
<tr>
<td>Distance covered (km)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>9.15 ± 0.34</td>
<td>9.3 ± 0.41</td>
<td>9.35 ± 0.47</td>
<td>9.6 ± 0.31</td>
</tr>
<tr>
<td>TDft run</td>
<td>4.5 ± 0.33</td>
<td>4.5 ± 0.24</td>
<td>4.7 ± 0.27</td>
<td>4.8 ± 0.23</td>
</tr>
<tr>
<td>TDft walk</td>
<td>4.05 ± 0.39</td>
<td>4.5 ± 0.22</td>
<td>4.6 ± 0.19</td>
<td>4.9 ± 0.16</td>
</tr>
<tr>
<td>TDft trot</td>
<td>4.2 ± 0.33</td>
<td>4.4 ± 0.44</td>
<td>4.7 ± 0.41</td>
<td>4.3 ± 0.39</td>
</tr>
<tr>
<td>TDft walk</td>
<td>2.1 ± 0.31</td>
<td>2.2 ± 0.24</td>
<td>2.4 ± 0.28</td>
<td>2.2 ± 0.36</td>
</tr>
<tr>
<td>TDft trot</td>
<td>2.1 ± 0.27</td>
<td>2.2 ± 0.21</td>
<td>2.3 ± 0.23</td>
<td>2.1 ± 0.26</td>
</tr>
<tr>
<td>TDft walk</td>
<td>0.64 ± 0.20</td>
<td>0.5 ± 0.06</td>
<td>0.62 ± 0.07</td>
<td>1.0 ± 0.31</td>
</tr>
<tr>
<td>TDft trot</td>
<td>0.72 ± 0.26</td>
<td>0.65 ± 0.05</td>
<td>0.84 ± 0.08</td>
<td>0.9 ± 0.25</td>
</tr>
</tbody>
</table>

*E1 = evaluation 1; E2 = evaluation 2; E3 = evaluation 3; E4 = evaluation 4; FLI = frequency of low-intensity activities; FFH = frequency of high-intensity activities; LI = low-intensity running; MI = medium-intensity running; TD = total distance; V = victory; T = draw.  
*Significantly different from E4 (p < 0.05).  
†Significantly different from E3 and E4 (p < 0.05).  
‡Significantly different from E1.

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Table 2. Distance covered in different categories of HI throughout the season (mean ± SD).*

<table>
<thead>
<tr>
<th>Category</th>
<th>HI (m)</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td>Moderate-speed running</td>
<td>399±16</td>
<td>399±16</td>
<td>399±16</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td></td>
<td>First half</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td></td>
<td>Second half</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td>High-speed running</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td></td>
<td>First half</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td></td>
<td>Second half</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td>Sprint</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td></td>
<td>First half</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
<tr>
<td></td>
<td>Second half</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
<td>500±25</td>
</tr>
</tbody>
</table>

*E1 = evaluation 1; E2 = evaluation 2; E3 = evaluation 3; E4 = evaluation 4; HI = high-intensity running.
†Significantly different from E4 (p < 0.05).

Evaluation Procedures

Match Analysis. To avoid variations in pitch dimensions, all videotaped records were restricted to home-played matches (as 'Home team') and were filmed by the same group of researchers. Game score and rank of the opposing team data are presented in Table 1.

Time-motion was performed according to the procedures described by Kristrup et al. (26). Each player was filmed close up during the entire match by a VHS movie camera (DVC-HC3AE, Sony, Japan) positioned at the side of the field, at a height of about 15 m, and at a distance of 30–40 m from the touchline. The videotapes were later replayed on a monitor for computerized coding of activity patterns. The following locomotor categories were used: standing (0 km·h⁻¹), walking (6 km·h⁻¹), jogging (8 km·h⁻¹), moderate-speed running (12 km·h⁻¹), moderate-speed running (15 km·h⁻¹), high-speed running (18 km·h⁻¹), sprinting (30 km·h⁻¹), and backward running (10 km·h⁻¹). The locomotor categories were chosen in accordance with the results of Bangsbo et al. (7), whereas the mean speed for each category was determined after detailed studies of the videotapes. Thus, the time for the players to pass landmarks in the grass, center circle, and other known distances was used to calculate the speed for each locomotor activity. The above activities were later divided into 4 locomotor categories: (a) standing; (b) walking; (c) moderate-speed running, encompassing jogging, low-speed running, and backward running; and (d) high-intensity running (HI), consisting of moderate-speed running, high-speed running, and sprinting.

The frequency and duration of each activity were recorded at 5-, 15-, 45-, and 60-minute periods throughout the game. The distance covered by each locomotor activity was determined in 5-minute intervals as the product of the total time and mean speed for that activity. The total distance (TD) covered during a match was calculated as the sum of the distances covered during each type of activity. The peak distance covered in HI in a 5-minute period is also presented. This period

Figure 3. Results of post-distance covered in high-intensity in a 5-minute period (P5–min). In the rest 5-minute period (RS5–min). In the average distance covered in the remaining 5-minute periods (AV5–min) and variation from peak period to rest or final period (DIFF–5–min). *Significantly lower than P5–min (p < 0.05); †Significantly higher than RS5–min in E1, E2, and E3 (p < 0.05); ‡Significantly lower than AV5–min in E4 (p < 0.05).
represents that particular 5 minutes that comprises the most HI in a game and is specific to each of the monitored players. All the match recordings were analyzed by an experienced observer. Kriznap and Bungo (21) observed that the coefficients of variation for test-retest analysis were 1.2, 2, 3.3, and 3% respectively, for the TD covered, walking, low-intensity running, HI, and backward running. In this study, the coefficient of variation (CV) for test-retest analysis in different locomotor categories was <9%. Each player’s locomotive style was previously extensively analyzed and several validation tests were performed according to the predetermined locomotor categories (26). Both halves were analyzed in a random order.

**Physical Fitness Testing**

All the evaluations took place at the same time of the day, after the players had a regular overnight sleep. The players were instructed to maintain routine exercise for daily activity and water intake, and they followed the same dietary recommendations defined by their medical staff. In addition, during the days of physical tests, the players were instructed to refrain from drinking beverages containing caffeine and alcohol and from consuming food during the 3 hours before testing. In the 2 days preceding evaluations, the players had a day off (first day) and a training session (second day) involving low-intensity exercises aimed to improve post-match recovery.

The CMJ, sprint, and COD tests were conducted indoors to exclude the influence of ground surface variations of the soccer pitch on the results. The YVIE2 was performed on a field of natural grass where the team normally conducted their training sessions. The order of the tests was as follows: (a) CMJ, (b) sprint, (c) COD ability, (d) KE and KF isokinetic strength, and (e) YVIE2. Before the tests, all the players performed a 10- to 15-minute warm-up consisting of light jogging, specific mobility exercises and stretching routines.
Table 4. Correlations between time-motion variables and sprint, agility, CMJ, and CSPRT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>T2G (s)</th>
<th>T3G (s)</th>
<th>COD (s)</th>
<th>CMJ</th>
<th>CSPRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Next 5 min</td>
<td>-0.579 (p = 0.059)†</td>
<td>-0.631 (p = 0.021)†</td>
<td>-0.545 (p = 0.054)</td>
<td>0.409 (p = 0.175)</td>
<td>0.577 (p = 0.011)†</td>
</tr>
<tr>
<td>%20-40 m HR 1st</td>
<td>0.569 (p = 0.042)†</td>
<td>0.622 (p = 0.032)†</td>
<td>0.501 (p = 0.060)†</td>
<td>-0.516 (p = 0.071)</td>
<td>-0.726 (p = 0.003)†</td>
</tr>
<tr>
<td>HR Set</td>
<td>-0.674 (p = 0.012)†</td>
<td>-0.473 (p = 0.103)</td>
<td>-0.561 (p = 0.040)†</td>
<td>0.195 (p = 0.522)</td>
<td>0.396 (p = 0.063)†</td>
</tr>
<tr>
<td>HR Set</td>
<td>-0.693 (p = 0.023)†</td>
<td>-0.581 (p = 0.067)†</td>
<td>-0.473 (p = 1.003)</td>
<td>0.220 (p = 0.443)</td>
<td>0.534 (p = 0.066)†</td>
</tr>
<tr>
<td>HR Set</td>
<td>-0.642 (p = 0.017)†</td>
<td>-0.549 (p = 0.244)</td>
<td>-0.103 (p = 0.906)†</td>
<td>0.559 (p = 0.042)</td>
<td>0.413 (p = 0.101)†</td>
</tr>
<tr>
<td>SP 2nd row</td>
<td>-0.559 (p = 0.034)†</td>
<td>-0.568 (p = 0.115)</td>
<td>-0.147 (p = 0.835)</td>
<td>0.178 (p = 0.748)</td>
<td>0.344 (p = 0.250)†</td>
</tr>
<tr>
<td>SP Set</td>
<td>-0.661 (p = 0.046)†</td>
<td>-0.728 (p = 0.005)†</td>
<td>-0.541 (p = 0.254)</td>
<td>0.547 (p = 0.245)</td>
<td>0.522 (p = 0.023)†</td>
</tr>
<tr>
<td>SP Set</td>
<td>0.621 (p = 0.023)†</td>
<td>-0.650 (p = 0.016)†</td>
<td>-0.299 (p = 0.332)</td>
<td>0.609 (p = 0.772)</td>
<td>0.521 (p = 0.098)†</td>
</tr>
<tr>
<td>SP 2nd row</td>
<td>-0.562 (p = 0.034)†</td>
<td>-0.482 (p = 0.068)</td>
<td>-0.104 (p = 0.525)</td>
<td>0.101 (p = 0.747)</td>
<td>0.430 (p = 0.143)†</td>
</tr>
</tbody>
</table>

*CSPRT = composite score of power-related tests; T2G = 5-m sprint time; T3G = 30-m sprint time; COD = change of direction test; CMJ = countermovement jump. Hash tag † = p < 0.05. Hash tag † = p < 0.01.

Training Status and Match-Related Physical Performance

Inc., Chicago, IL, USA) was used. Statistical significance was set at p ≤ 0.05.

RESULTS

Seasonal Variations

Results regarding seasonal variations in the frequency of activity changes, the number of runs in low intensity and in high intensity (FHI), in the TD covered during the game, and in each type of movement during the 2 halves are presented in Tables 1 and 2. The TD in the first 15-minute period was 10, 7, and 17% lower (p < 0.05) in E2 than in E1, E3, and E5, respectively, and 11% higher (p < 0.05) in E4 than in E3. In the last 15 minutes of the game, the TD was 14 and 8% higher in E4 than in E2 and E3, respectively (p < 0.01). The distance covered in FHI in the first 15 minutes of the first half of the match in E4 was 49, 37, and 33% higher (p < 0.05) than in E1, E2, and E3, respectively. Also, the HI in the last 15-minute period of the game in E4 was 43% higher than in E3 (p < 0.05). The sum of the distance covered in the HI in the 2 last 15-minute periods of each half was 36 and 38% higher in E4 than in E2 and E3, respectively (p < 0.05). The peak 15-minute period of HI was 30, 37, and 34% higher in E4 than in E2, E3, and E5, respectively (p < 0.05), whereas the lower 15-minute period of HI during the game was 58, 44, and 41% higher in E4 than E1, E2, and E3, respectively (p < 0.05).

Results from the peak distance covered in HI in a 5-minute period (P5-min), in the next 5-minute period (Next5-min), in the remaining 5-minute periods (Av5-min), and the variation of distance covered from P5-min to Next5-min (%Dep/Next) are presented in Figure 3. The players covered a higher distance in HI in the P5-min (p < 0.05) from E2 than in the remaining P5-min periods of the other time points. Furthermore, in all assessments points of the season, the distance covered in HI was higher in F5-min (p < 0.05) than in Next5-min and Av5-min within each assessment period. Moreover, higher (p < 0.05) Av5-min values were observed in E4 than in E2 and E3. No significant differences were observed between the distance covered in HI in the Next5-min and Av5-min within the different assessment periods.

Physical Fitness Results

Results of the different physical tests performed within the 2-week period between video-taped matches are presented in Table 3.

Training Status in Relation to Match Analysis

Time-motion variables showed that the TD and the distance covered in HI for the first and second halves were not significantly different (4,585 ± 265 vs. 4,570 ± 301 m and 532 ± 75 vs. 680 ± 150 m, respectively). The distance covered in HI during the first 15-minute period of each half (first and fourth 15-minute periods of the game) was higher (p < 0.05) than in the last 15-minute period of each half (third and sixth periods of the game, respectively; Figure 4). Tables 4 and 5 show the relationship between time-motion variables and the different muscle power-related tests, isokinetic parameters, and tests composite scores, respectively. Performance in sprint tests (T5 and T30) and in the CSEP1 was the physical related parameter that showed the highest correlations with time-motion variables. The T30 and CSEP1 showed significant correlations (r = 0.422-0.725) with the distance covered in HI in the 5-minute period of the game after P5-min (Next5-min), with the decrement (%) in HI from P5-min to Next5-min (%Dep/Next) and with the distance in sprinting in the second half of the game (SP2nd half; Table 4).

Knee extension peak torque of the nondominant leg showed significant correlations (r ranging from 0.55 to 0.73) with the following time-motion parameters: decrement (percent) in HI between the highest (fourth) and the lowest (sixth) intense 15-minute periods of the second half (HI4th-HI6th), average decrement (percent) in HI from the highest to the lowest intense 15-minute periods (first to third and fourth to sixth) of both halves (HI1st-3rd-HI7th-9th), decrement (percent) in HI from the first to the last 15 minute periods (HI1st-15th) of the game (Table 5). The decrement (%) in HI from the highest to the lowest intense 15-minute periods (first to third and fourth to sixth) of both halves (HI4th-HI6th) was also correlated (r = 0.59-0.73) with knee flexion peak torque of the nondominant leg, and with the composite scores of knee extension (CSEK), knee flexion (CSF), and knee extension and flexion (CSEF). The distance covered in the T10E2 was not correlated with any of the time-motion parameters analyzed.

DISCUSSION

The main findings of this study were that alterations in GPPs of professional soccer players occur during the season and that their training status is related to a greater ability to maintain HI-related performance variables during the match. Time-motion analysis of the matches performed at different time points of the season showed that the players covered greater total and HI distances in the last quarter of the season (E4; Table 1). Also, it was observed that players were more frequently engaged in HI activities (FHI) during the last quarter of the season (E4; Table 1) than in the remaining season periods (E2, E3, and E5). Although differences in HI match running were only significant from E2 and E3 to E4, the analysis of each match half showed that the players performed more HI in the first half in E4 than in the first half of the remaining periods (E1, E2, and E3; Table 1). A greater distance in HI was covered in the peak and in the lowest 15-minute periods of the match in E4 than in the corresponding 15-minute periods of the other time points of the season. Moreover, the amount of HI performed in the last 15-minute period of each half, which is indicative of the ability to maintain performance during the game (26), was again higher in E4. In agreement with our findings, others observed that players covered a greater distance in HI at the end than in the middle of the competitive season.
Although not controlled in this study, an improved physical capacity in the last part of the season could explain, at least in part, our results. In fact, increases in match-related physical performance could be attributed to an improved physical capacity (42). Several studies observed an increase in different physical parameters toward the end of the season (13,23,25,33). Studies involving longitudinal analysis of soccer players' physical capacity throughout the season observed increases in soccer-specific endurance fitness (25,35), repeated-sprint ability (21), speed (13), agility (15), and jump performance (35) toward the end of season. Nevertheless, it is important to note that these findings have not been corroborated by others (14,29).

The performance in a group of physical fitness measures and in a CSPET (T5, T30, COD, CMJ), and CSPF (1) was related to certain time-motion GTs (Table 3). In fact, players with better T5, T30, COD abilities, and CSPF showed an increased performance in Next5-min, a lower decrement from T5-min to the Next5-min (1000mEPOSS), and achieved higher sprint distances during the second half of the game. These findings suggest that soccer players with improved capacity to perform sport-specific maximal dynamic activities have an increased ability to maintain performance during short periods of high-intensity intermittent exercise and a greater fatigue resistance in the second half of the game.

Also, some isokinetic strength parameters (Table 4) and CSKE, CSKF, and CSKF were correlated with game-related physical performance (Table 5). Relationships were observed between KE and XP muscle strength of the lower limbs (2,23,31). CSKE and CSKF and the following fatigue parameters, namely, decrement in HI from the first and to the final 15-minute periods of the game and decrement in HI from the highest to lowest intense 15-minute periods of both values. These results suggest that greater levels of lower-limb strength are related to a higher ability to maintain performance during games (Table 5). This seems to suggest that players with greater ability to rapidly produce force, allowing fast accelerations and decelerations and to quickly and efficiently perform complex and coordinated movements, are able to perform at high level in certain game-related physical parameters.

Accordingly, some studies showed that strength (16) and sport-specific muscle power evaluated by sprint ability (19) and agility (33) are influenced by the competitive level of the players. Also, high performance levels in jump ability, leg extension strength (1), and in intermittent exercise protocols (43) were observed in players from more successful teams compared with their less successful counterparts.

Rampini et al. (41) did not observe any relationships between both the best time during an RSSA test (RSSA,cm) and jump ability (squat jump), with different match time-motion variables (TD, HI, very high-intensity running, and sprinting). However, despite the fact that low- to medium-intensity running is the predominant activity during the match, power-based efforts such as sprints, COD and change-of-speed, jumps, duels, and kicking, which are mainly dependent on maximal strength and anaerobic power, are widely accepted as essential factors for success in soccer performance (16). In fact, a massive metabolic load is imposed on players not only during the maximal intensities phases of the game but also every time acceleration occurs, even when speeds are low (36). These speed and direction of movement changes performed during games impose high levels of stress to the involved musculature, thereby affecting energy usage and resulting in a higher physiologic impact than habitual forward movements (18). Indeed, higher VCO2 (11), blood lactate (11,15), HR (18), and rate of perceived exertion (18) values were observed during high-intensity intermittent exercise during shuttle mode than during in-line format. Some possible reasons could be the involvement of additional muscles, such as upper body muscles (22) and different neuromuscular activation patterns during COD activities (11). At high-speed displacements and during intermittent shuttle running, running technique becomes more important, and anaerobic power is essential because players might accelerate after timing to reach the desired speed (18). The physiological demands and functional characteristics that are typical of soccer-specific activity patterns may explain the observed correlations found in this study between power-related tests and game-physical parameters.

Fatigue is a complex phenomenon that cannot be simply explained by a single factor (19). The ability to resist fatigue has been related to different functional and physiological features (6,12,19,31). Improvements in coordination specificities (12) and a greater ability to effect COD (19) are positively associated with performance and with an attenuated fatigue response. Indeed, an optimized performance during the game and particularly during the most intense periods would be expected in players with neuromuscular features tuned for the movements performed during the game (15).

Soccer players with greater CSPET values may have a higher ability to accelerate, decelerate, and cut a COD than the players with lower values, leading to an improved physical capacity. This greater physical capacity may likely affect some of the mechanical and neuromuscular effects of repeated stretch-shortening cycle fatigue (15). For instance, improved training status seems to be related to the higher ability to regulate joint stiffness more efficiently during exposure to maximal intensity repeated sprints (15).

As games progress, some examples of game-induced fatigue are the reductions in concentric (42,44) and eccentric muscle strength (46), and in electromyography activity (39) of the major lower-limb muscles. Game-induced performance decrements are also evident in players' decreased ability to perform certain strength-dependent actions such as sprint (42,44) and jump (42). In accordance, players' fatigue reflected in reduced electrical activity of muscles and in compromised strength toward the final periods of the
Training Stages and Match-Related Physical Performance

match may cause a lower work-rate toward the end of a soccer match (59,60). Moreover, because each specific game action requires breaking and propulsive forces, the importance of the strength and endurance capacities of leg muscles likely increases as the game progresses (10,11). Thus, strength decrements during the game could affect the performance of explosive actions such as jumping, sprinting and CODs, which require high quadriceps strength at the initiation of movement when the joint extension velocity is low (60). It is plausible to assume that soccer players with higher levels of strength have greater ability to maintain strength toward the final stages of each half of the game than players with less strength and thus show a lower decrement in work rate.

Data suggest that the athletes' ability to exercise during longer periods of time is usually related to their endurance capability (e.g., anaerobic threshold, VO2max). Nevertheless, improved endurance is also influenced by factors related to muscle recruitment and force production (37,38). It was recently observed that distance running performance and running economy of well-trained distance runners are related to the neuromuscular capacity to produce force (54). Also, improvements in soccer players' maximal strength resulting from training and neural adaptations lead to improved running economy by 6.9%, at both the lactate threshold and a fixed velocity in a treadmill test (20). Rapid force production is considered essential for a wide range of athletes (20,34), with recent reports highlighting that an enhancement in neuromuscular function can be a determinant in improving short- and long-term endurance capacities (1). In fact, there is a consensus that training-related improvements in motor unit recruitment and synchronization result in force potentiation, improving efficiency and coordination, which may delay the onset of fatigue (17,37,38).

In summary, this study gives empirical support to the neuromuscular parameters measured by sprint, COD, jump ability, strength parameters, and their composite scores as indicators of physical performance of soccer players during games. However, it is important to refer that moderate correlations do not affirm a direct cause and effect (11). Indeed, it should be considered that although data from laboratory and field tests are useful in providing information on the players' general physical profile and soccer-specific fitness, test results should not be used to predict the overall performance during match play because of the complex nature of the demands of the game (17).

Practical Applications

Our results highlight the importance of strength and power for soccer players. In fact, this study reports an association between muscle strength and power and performance decrements in game-related physical parameters. Thus, the soccer players' training should incorporate specific exercise programs to improve the athletes' strength and power during the performance of soccer-specific activities. Also, the regular evaluation of the capacity of the players' neuromuscular system to produce force and to perform powerful specific sport activities (e.g., sprint, COD) is advised. In fact, athletes need to successfully perform over competitive seasons of around 10–11 months and, according to our results, these functional qualities of players' physical fitness seem to be associated with their physical performance during games. Interventional studies designed to analyse the relationship between improvement in these physical parameters (e.g., strength, power, COD), and enhanced game-related physical parameters are warranted.

Acknowledgments

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References


Impact of Loughborough Intermittent Shuttle Test versus soccer match on physiological, biochemical and neuromuscular parameters

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Abstract The aim of the present study was to analyze the impact of Loughborough Intermittent Shuttle Test (LIST) versus soccer match on heart rate (HR), muscle damage, redox status, blood leukocytes and neuromuscular function throughout 72 h recovery. Sixteen male soccer players (21.3 ± 1.1 years; 175.0 ± 6.0 cm; 70.7 ± 6.3 kg) completed LIST and performed a soccer match separated by 2 weeks and data were collected before, 30 min, 24, 48 and 72 h after LIST and match. HR, plasma creatine kinase (CK) activity, myoglobin (Mb), uric acid (UA), protein sulfhydryls (–SH), malondialdehyde (MDA) contents, total antioxidant status (TAS), blood leukocyte counts, delayed onset muscle soreness, 20 m sprint and jump performances, and maximal isokinetic knee extension and flexion were analyzed. HR after LIST was significantly lower than after the match. Post-match TAS was lower and UA was higher than after LIST. Thirty minutes and 24 h after soccer MDA was higher and –SH was lower than after LIST (P < 0.05). LIST and soccer match induced elevation in total leukocytes and a reduction in lymphocytes at 30 min. This reduction in blood lymphocytes 30 min after match was lower than after LIST. In conclusion, the impact of both exercises did not differ regarding the observed muscle damage markers and some neuromuscular parameters, although soccer requires higher cardiac demand and induced higher changes on redox status, adenine nucleotide metabolism and on lymphocyte counts than LIST, which should be taken into account when using LIST to simulate a match to study these type of physiological and biochemical-related endpoints.

Keywords · Intermittent exercise · Muscle damage · Oxidative damage · Antioxidants · Fatigue and recovery

Introduction

The physiological, biochemical and neuromuscular impact of intermittent multi-sprint sports, including soccer, has been studied through different methodologies. Time-motion analysis of soccer match provides information regarding total distance covered and type of movement, number of physical contacts, tackles, headers and kicks performed by players. Moreover, specific field approaches during soccer matches (Krustrup et al. 2006), tentative replications of match demands in laboratory (Drust et al. 2000) or a combination of field and lab tests (Greig et al. 2006) have been designed to monitor heart rate (HR), blood and muscle...
metabolite alterations, estimated energy expenditure and oxygen uptake of soccer players. These laboratory and field tests are used to uncover the lack of control of the activity pattern and exercise intensity in a game, resulting from the randomized order of players’ actions, thus allowing a standardized analysis of metabolic, biochemical and functional features of intermittent exercise. Among several intermittent field tests, the Loughborough Intermittent Shuttle Test (LIST) has been used to study the effects of the ingestion of carbohydrate-electrolyte solutions or meals (Foskett et al. 2008; Nicholas et al. 1995, 1999), fluid ingestion and gastric emptying (Leiper et al. 2005), muscle metabolism and temperature (Morris et al. 2005), muscle soreness and damage (Thompson et al. 1999), heat acclimatization protocols (Sunderland et al. 2008), cryotherapy treatment against muscle damage (Bailey et al. 2007), as well as the influence of antioxidants (Kingsley et al. 2001a; Thompson et al. 2001a) on intermittent exercise performance and recovery with special reference to soccer. In fact, some authors have suggested that LIST is a field test that simulates the activity pattern and the workload imposed by soccer, i.e., was designed to mimic the activities performed and the distance covered in a typical soccer match (Bishop et al. 1999; Nicholas et al. 2000). This was based on data from time-motion analysis and estimated indirect calorimetric variables (Nicholas et al. 2000). However, the analysis of the distance covered by players during the match tends to underestimate the energy expended as many unorthodox modes of motion, such as running backwards and sideways, jumping, decelerating and changing direction, as well as the dribbling and contesting possession accentuate the mechanical and metabolic loading (Reilly 1997). Moreover, even considering the pre-defined turns implying acceleration and decelerations during LIST, the great quantity and variety of soccer actions associated with eccentric contractions during a match are expected to cause additional muscle disturbances in soccer when compared to LIST.

Recent studies from our lab and others have analyzed the response of performance indices, muscle damage, inflammation as well as oxidative stress and damage markers of male and female soccer players during the recovery from a match (Andersson et al. 2008; Ascensao et al. 2008; Ispiridis et al. 2008), as the recovery is thought to be influenced by changes in these functional and biochemical parameters. Some of these endpoints have also been measured during the recovery period after the LIST (Bailey et al. 2007; Kingsley et al. 2005; Thompson et al. 1999, 2001a, 2003). In addition to the comparison of time-motion analysis and estimated indirect calorimetric variables between LIST versus soccer match (Nicholas et al. 2000), no data had been published comparing the impact of LIST versus soccer on biochemical and neuromuscular parameters. Therefore, it would be of interest to examine whether, and to what extent, muscle damage, plasma oxidative stress and damage and blood inflammatory markers, as well as lower limb neuromuscular variables such as jump, 20 m sprint ability and strength performance, are altered in response to LIST versus soccer. Thus, the present study aimed to comparatively analyze the effect of LIST versus soccer match on muscle damage, plasma antioxidant capacity, oxidative damage, blood leukocyte counts and neuromuscular variables throughout 72-h post-exercise recovery period. Our hypothesis is that soccer induces higher levels of muscle and oxidative damage than LIST.

Methods

Subjects

Sixteen male soccer players from 2nd and 3rd Portuguese divisions participated in this study after being informed about the aims, experimental protocol, procedures and after delivering writing consents. At the time of the experiments, the players were in the competitive period of the season, performing 4–5 training sessions per week. The experimental protocol was approved by the Ethical Committee of Faculty of Sport, University of Porto, Portugal, and followed the Declaration of Helsinki of the World Medical Association for research with humans.

Experimental design and procedures

The players performed the LIST and one soccer match separated by 2 week (Fig. 1). For 2 weeks prior to data collection and during the protocol period, soccer players were instructed not to change their normal eating habits and to refrain from additional vitamin, antioxidant dietary supplementation or any recovery treatment such as cryotherapy. Subjects were also instructed to abstain from exhaustive exercise during the 72-h pre- and post-LIST and match, with exception of the functional evaluation tests. The overall set-up was performed during a 3-week interruption of the competitive period. During the remaining days, with the exception of the 72 h pre- and post-protocol periods, the players were engaged in normal training routines.

The temperature at the days of field exercise (LIST and soccer match) was around 16°C.

Blood samples and functional data (jump and sprint performance, quadriceps and hamstrings muscle strength) were assessed pre-LIST/match and at 30 min, 24, 48 and 72 h of the recovery period. On the day of the LIST/match, players arrived at the laboratory after an overnight fast between 10.00 and 12.00 h. A resting blood sample was taken after subjects had been standing for at least 15 min, after which subjects consumed a light standardizing meal.
and drink and rested for 2 h. According to Thompson et al. (2003), the meal consisted of 1.7 g/kg white bread and 0.3 g/kg of low-fat spread. Rest muscle strength, jump and sprint performance were assessed during the 2-h period between the consumption of pre-exercise meal and the start of the LIST/match.

For 3 days after the LIST/match, subjects returned to the laboratory. A blood sample was taken from the forearm in the same conditions described above. Subsequently, the players performed the sprint and strength tests as outlined below.

Preliminary measurements

The players performed an incremental (0.1 m/s increase each 1 min step) treadmill (Quasar-Med, Nussdorf, Germany) test until voluntary exhaustion to determine maximal oxygen uptake ($V_O^{2max}$) and maximal HR ($HR_{max}$, Vantage NV, Polar Electro, Finland). Expired respiratory gas fractions were measured using an open circuit breath-by-breath automated gas-analysis system (Cortex, Metalyzer, 3B, Germany). From the $V_O^{2max}$, running speeds corresponding to 55 and 95% $V_O^{2max}$ were calculated. Thereafter, the subjects performed the LIST for 30 min to familiarize with the test. Jump and 20 m sprint abilities and strength performance were also evaluated at baseline as described below.

The LIST

The 90-min shuttle run test was conducted according to Thompson et al. (1999) in a natural green soccer pitch. Briefly, the participants were required to run between two lines, 20 m apart, at various speeds dictated by an audio signal and based on the velocities corresponding to their individual $V_O^{2max}$. The exercise periods were designed as follows:

- 3 × 20 m walking
- 1 × 20 m maximal running sprint
- 4 s recovery
- 3 × 20 m at a running speed corresponding to 55% $V_O^{2max}$
- 3 × 20 m at a running speed corresponding to 95% $V_O^{2max}$

This pattern was repeated for each 15-min exercise block followed by the corresponding 3-min recovery period for five times.

HR during the LIST was measured and recorded every 5 s.

Match time-motion analysis

For time-motion analysis, each player was video-filmed close up during the entire match. The videotapes were later replayed for computerized time-motion analyses according to the procedures described by Mohr et al. (2003) The used motor pattern categories included standing (0 km h$^{-1}$), walking (6 km h$^{-1}$), jogging (8 km h$^{-1}$), low-speed running (12 km h$^{-1}$), moderate-speed running (15 km h$^{-1}$), high-speed running (18 km h$^{-1}$), sprinting (30 km h$^{-1}$), sideways, and backwards (10 km h$^{-1}$) running. The match activities were later analyzed considering standing, walking, jogging, cruising, sprinting, backwards running and sideways running.

HR was also measured during the match as previously described.

Delayed onset muscle soreness (DOMS)

After LIST/match and prior to blood sampling, each subject was asked to complete a leg muscle soreness questionnaire, in which they rated their perceived muscle soreness on a scale from 0 (normal absence of soreness) to 10 (very intense sore).

Blood sampling and preparations

All venous blood samples were taken by conventional clinical procedures as described previously (Magalhaes et al. 2007). An aliquot of the whole blood was used to perform leukocyte counts as indirect markers of muscle damage. The remaining freshly withdrawn blood was immediately centrifuged at 3,000 rpm during 10 min to obtain plasma. Plasma was separated into aliquots and rapidly frozen at...
–80°C for later biochemical analysis of the muscle damage markers myoglobin (Mb) and creatine kinase (CK), as well as the redox state using total antioxidant status (TAS), malondialdehyde (MDA), protein sulfhydryl groups (SH) and uric acid (UA).

Biochemical assays

Muscle damage

Plasma CK activity was determined spectrophotometrically using a commercial kit (ABX A11A01632, Mompelier, FR). Plasma Mb concentration was assessed using a commercial kit (myoglobin bioMerieux 30446, Carnaxide, PT).

Leukocyte count was assessed by an automatic cell counter (Horiba 60; ABX Diagnostics, France). Whole blood smears on glass slides (VBS 655/A Microscope, Biosigma) were used for white blood cell differential analysis. Smears were stained using Wright coloring (Merck) and air-dried. Cell differentials were performed using an Olympus microscope equipped with 1,000× oil immersion lens.

Redox state

Total antioxidant status was measured spectrophotometrically using a commercial kit (Randox NX2332 Crumlin, UK). Uric acid was determined by an enzymatic method using a commercial kit (Horiba ABX A11A01670, Montpellier, France).

Plasma MDA was assayed according to Rohn et al. (1993) with some modifications and measured by the formation of thiobarbituric acid reactive substances at 535 nm. Plasma SH was spectrophotometrically evaluated at 414 nm according to Hu (1990). Protein content was spectrophotometrically assayed using bovine serum albumin as standard according to Lowry et al. (1951). Samples were analyzed in duplicate and the mean of the two values was used for statistical analysis.

Jumping performance

Vertical jumping was evaluated on a Bosco’s mat (Ergojump, Globus, Italy). In accordance with Hertogh et al. (2005), free counter-movement jumps with extension of both upper limbs were chosen to simulate spontaneous jumping movements. The depth of the counter-movement was self-selected and represented each players’ optimal depth for maximal jump. Each athlete performed three jumps and the best result expressed as jump height was recorded.

20 m sprint ability

Sprint ability measurements were carried out using telemetric photoelectric cells placed at 0 and 20 m (Brower Timing System, IRD-T175, USA). The players stood 1 m behind the starting line, started on a verbal signal being time activated when players cross the first pair of photocells, and then ran as fast as they could to complete the 20-m distance. Players completed two runs interspersed by 1-min recovery period and the best time was registered.

Strength assessment

In order to evaluate muscle function, subjects were familiarized with the muscle function test on at least two occasions during preliminary visits to the laboratory. Maximal gravity corrected concentric peak torque of quadriceps and hamstrings was measured during isokinetic knee joint movement of dominant leg at an angular velocity of 90 s⁻¹ (1.57 rad s⁻¹) using a isokinetic dynamometer (Biodex System 2, USA) as described previously by our group (Magalhaes et al. 2004).

Fluid loss and intake

In order to determine sweat loss, the players were weighed wearing dry shorts immediately before and after the LIST and match using a digital weight (Tanita Scale BC533). The subjects were allowed to drink water ad libitum during both the LIST and the match, and their water intake was recorded.

Statistics

Mean, standard deviation and standard error mean were calculated for all variables. A Kolmogorov–Smirnov test was used to test whether physiological, biochemical and neuromuscular-related variables were normally distributed. Two-way analysis of variance (ANOVA) for repeated measures followed by the Bonferroni post hoc test was used to compare variables between LIST and soccer at the analyzed time points (before vs. 30 min vs. 24 vs. 48 vs. 72 h). When there were only single comparisons, a paired sample t test was used to determine whether any differences between LIST and soccer existed. All data analysis was performed using SPSS 17.0 package. The significance level was set at 5%.

Results

Physiological and anthropometric characteristics of the soccer players are presented in Table 1.

Time-motion analysis showed that players were around 80 min of match time involved in low-intensity activities including standing, walking, jogging and cruising, and 8 min in high-intensity activities including sprinting,
Symptoms of muscle damage decreased significantly during the recovery period (Fig. 6c) and reached baseline values at 72 h recovery. No significant differences were observed between LIST and match in the analyzed neuromuscular parameters with the exception of sprint performance, which was less affected after LIST than after soccer at 30 min and 24 h recovery (P < 0.05).

The fluid loss during LIST versus match was 0.88 ± 0.17 L versus 0.90 ± 0.2 L, or 1.2 ± 0.3% versus 1.2 ± 0.5% of the body mass, respectively. The fluid intake was 0.74 ± 0.1 L versus 0.65 ± 0.1 L. Thus, the total fluid loss was similar between the two conditions, respectively 1.62 ± 0.4 L versus 1.55 ± 0.3 L, corresponding to 2.3 ± 0.3% versus 2.2 ± 0.4% of the body mass.

Discussion

Intensity of the soccer match

The match examined in the present study was a friendly game played by secondary division players, and it should be thus considered how far the intensity is from games played at an elite level. Nevertheless, the mean and absolute HR values were similar to those reported for Danish soccer players from similar level (Krustrup et al. 2006). Additionally, time-motion analysis showed that the frequency and the percentage of time both at low- and high-intensity activities were also similar to that described for players of the same level, although below to those observed in elite players (Mohr et al. 2003). These observations may suggest that the analyzed match intensity was somewhat similar to other non-elite games, and probably lower than the intensity performed by elite soccer players.

Muscle damage

The tendency and magnitude of changes induced by both LIST and match in muscle damage-related parameters were

Table 1 Anthropometric and physiological characteristics of the subjects before LIST and match

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before LIST</th>
<th>Before match</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>21.3 ± 1.1</td>
<td>21.3 ± 1.1</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>70.7 ± 6.3</td>
<td>69.8 ± 5.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.0 ± 6.0</td>
<td>175.0 ± 6.0</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>8.3 ± 1.9</td>
<td>7.9 ± 2.9</td>
</tr>
<tr>
<td>VO2max (mL kg⁻¹·min⁻¹)</td>
<td>55.1 ± 5.1</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are mean ± SD; VO2max maximal oxygen uptake

backwards and sideways running, corresponding to approximately 92 and 8% of the total match time, respectively (Table 2).

The mean HR during the match was 173.0 ± 8.8 bpm and the peak HR was 195.6 ± 6.0 bpm, which corresponds to 87.1 ± 3.2% and 99.7 ± 7.0%, respectively, of the maximal HR previously determined. Mean HR during the match (including 1st and 2nd halves) was significantly higher than during the LIST (Fig. 2).

Plasma Mb content increased 30 min after both LIST and match (P < 0.05), returning to baseline at 24, 48 and 72 h recovery. No significant differences were found between LIST and soccer in any analyzed time point (Fig. 3a). Plasma CK activity and DOMS increased at 30 min, 24, 48 and 72 h after LIST and match when compared to pre-exercise values (P < 0.05), but no significant differences were found between protocols at any time point (Fig. 3b, c).

Plasma TAS (Fig. 4a) increased significantly at 30 min, 24, 48 and 72 h recovery time points after both LIST and match. Plasma UA only increased at 30 min after both LIST and match (Fig. 4b). The increases in TAS and UA at 30 min were higher after the match than after the LIST (P < 0.05). Plasma MDA and SH levels (Fig. 4c, d), respectively, increased and decreased at 30 min, 24, 48 and 72 h both after LIST and soccer (P < 0.05). The increase in MDA and the decrease in SH contents 30 min and 24 h after the match were significantly higher than after LIST.

LIST and match increased blood leukocyte counts (Fig. 5a) at 30 min (P < 0.05) which returned to baseline values at 24, 48 and 72 h recovery. No differences were observed between LIST and match for any time point. After both LIST and match, lymphocyte counts at 30 min were significantly lower than pre-exercise values, returning to baseline values at 24 h. However, match induced a significantly higher lymphopenia than LIST at 30 min.

LIST and match induced significant reductions in jump (Fig. 6a) and sprint (Fig. 6b) abilities as well as in isokinetic peak torques for knee extension (Fig. 6c) and flexion (Fig. 6d) until 72 h recovery. No significant differences were found between LIST and match in the analyzed neuromuscular parameters with the exception of sprint performance, which was less affected after LIST than after soccer at 30 min and 24 h recovery (P < 0.05).

The fluid loss during LIST versus match was 0.88 ± 0.17 L versus 0.90 ± 0.2 L, or 1.2 ± 0.3% versus 1.2 ± 0.5% of the body mass, respectively. The fluid intake was 0.74 ± 0.1 L versus 0.65 ± 0.1 L. Thus, the total fluid loss was similar between the two conditions, respectively 1.62 ± 0.4 L versus 1.55 ± 0.3 L, corresponding to 2.3 ± 0.3% versus 2.2 ± 0.4% of the body mass.

Discussion

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Muscle damage

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Table 2 Frequency, mean duration and percent of match time spent on the considered motor categories

<table>
<thead>
<tr>
<th></th>
<th>Standing</th>
<th>Walking</th>
<th>Jogging</th>
<th>Cruising</th>
<th>Sprinting</th>
<th>Backwards running</th>
<th>Sideways running</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (n)</td>
<td>114 ± 44.8</td>
<td>408.6 ± 93.8</td>
<td>441.9 ± 96.2</td>
<td>69.6 ± 10.4</td>
<td>41.7 ± 18.0</td>
<td>122.1 ± 26.6</td>
<td>64.9 ± 4.8</td>
</tr>
<tr>
<td>Mean duration (min)</td>
<td>7.0 ± 2.5</td>
<td>39.5 ± 3.6</td>
<td>31.8 ± 7.4</td>
<td>2.9 ± 1.5</td>
<td>2.2 ± 1.5</td>
<td>4.3 ± 1.3</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Total time (%)</td>
<td>7.8 ± 3.4</td>
<td>43.8 ± 7.9</td>
<td>35.3 ± 5.6</td>
<td>5.8 ± 2.3</td>
<td>2.5 ± 1.3</td>
<td>4.8 ± 1.9</td>
<td>1.6 ± 0.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD
somewhat expected and in the range of similar exercise protocols (Andersson et al. 2008; Bailey et al. 2007; Kingsley et al. 2005; Thompson et al. 1999, 2001b, 2004), being the significant alterations observed after the match in plasma CK, Mb, DOMS and leg strength close to some reported after LIST protocol (Kingsley et al. 2005; Thompson et al. 1999, 2001a, 2003).

Although the activity pattern of LIST is representative of the typical activities of soccer, there are activities such as jumping, running backwards and time in possession of ball that are not included. Some of these unorthodox activities, together with tackles and sudden direction changes rely greatly on eccentric contractions, probably increasing the neuromuscular demands imposed by match when compared with LIST. The considerable amount of this type of lengthening-based contractions characteristic of soccer were initially expected to induce additional signs of muscle injury in match when compared to LIST. However, no differences in plasma CK and Mb, as well as in DOMS levels and lower limb strength were observed between the match and the LIST during recovery. One hypothetical reason to explain this absence of differences might be the number of turns, including accelerations and decelerations during LIST.

As reported after other types of exercise (Ascensao et al. 2007; Magalhaes et al. 2007) and also after match (Ascensao et al. 2008; Ispirlidis et al. 2008), data showed that LIST and match induced a leukocytosis. This can be ascribed to the mobilization of blood cells from marginal pools by hemodynamic redistribution and augmentation that resulted from exercise-related metabolic conditions, such as enhanced catecholamine secretion (Bangsbo 1994). Our results also reported a marked lymphocytopenia during the subsequent period after both exercise protocols. Nevertheless, lymphocyte counts differed significantly between LIST and match at 30 min recovery being lower in match

![Fig. 2 Mean heart rate (bpm) values observed during LIST and soccer (1st and 2nd halves and overall). Values are mean and SD. Asterisk versus LIST](image)

![Fig. 3 Plasma myoglobin content (a), creatine kinase activity (b) and perceived delayed onset muscle soreness (c) during the 72-h recovery following LIST (triangles) and soccer (squares). Values are mean and SEM. Asterisk versus pre-exercise for both exercises (LIST and soccer); no significant differences were found between LIST and soccer](image)

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than in LIST. In comparison to concentric exercise, eccentric activity has been shown to result in a greater release of immune system modulators such as proinflammatory cytokines, acute phase proteins, and the recruitment of phagocytic cells with the potential to release ROS (Malm et al. 2000). These signals may favor additional oxidative stress and damage, as well as apoptosis in several tissues and cells, including lymphocytes. However, the hypothesis that a higher apoptosis-induced lymphocytopenia observed after match than after LIST can be attributed to the referred unorthodox eccentric activities during match is unlikely, as Simpson et al. (2007) reported that the levels of blood lymphocytopenia apoptosis observed after downhill running were not different compared with intensive running. The precise reasons for the lower lymphocyte counts after match should be further investigated.

Redox status

Match induced higher increases in UA (30 min) and MDA (30 min and 24 h) contents than LIST. Moreover, TAS (30 min) and SH (30 min and 24 h) were lower after match than after LIST. These results suggest that match may induce higher changes on redox status and on adenine nucleotide metabolism than LIST. Given that around 1–5% of the total oxygen uptake results in the generation of
superoxide radical and given the elevated oxygen consumption accompanying both LIST and match, it is not surprising its impact on these biomarkers of oxidative stress and damage. Furthermore, other sources of free radicals can influence cellular and blood antioxidant status. For example, stress hormones undergoing autoxidation (Cooper et al. 2002) and circulating neutrophils-induced oxidative burst (Quindry et al. 2003) can contribute to blood oxidative stress and damage. The influence of eccentric exercise-mediating muscular damage-like events on the formation of free radicals has also been reported (Lee and Clark son 2003). Considering the specific physiological demands imposed by the intermittent exercise models used, none of these potential-free radical sources should be ruled out, although we cannot conclusively demonstrate a causal link between any of those potential sources and the increased plasma oxidative stress and damage.

This study also shows that plasma TAS and UA increased at 30 min after both LIST and match. The observation that plasma UA levels increased in response to LIST and match is consistent with the findings from other studies (Ascensao et al. 2007; Magalhaes et al. 2007). However, match induced a higher increase in UA levels than LIST (at 30 min), which should probably due to an enhanced contribution of purine metabolism during match than during LIST. Recent data from Krustrup et al. (2006) showed a significant decrease in muscle ATP levels after an intense exercise period in the second half and after the entire soccer match, as well as significant increase in muscle inosine monophosphate content after an intense exercise period in the second half. Moreover, increased blood ammonia, plasma UA and hypoxanthine contents were earlier reported (Bangsbo 1994). Therefore, it is likely that the observed increased oxidative stress and damage during the intense exercise periods comprised during the match might have a higher contribution from xanthine oxidase-free radical generating system than during LIST.

The enhanced oxidative damage induced by the match compared to LIST can also be observed by the accumulation of lipid peroxidation by-products, measured as plasma MDA. Accordingly, the match also induced a significant decrease in plasma SH, suggesting increased disulphide linkages (–S–S–) from both proteins and reduced glutathione (GSH).

Since both LIST and match did not seem to represent sufficient severe muscular stimuli to cause leukocyte infiltration, as shown by the maintenance of blood leukocyte counts from 24 to 72 h recovery period compared to baseline, the possible effects of neutrophils-related oxidative burst on muscle damage induced by match should probably be ruled out. However, other immune cell-mediated free radical production during the post-exercise periods might be considered, such as monocyte and macrophage oxidative burst (MacIntyre et al. 1995). It is possible that a delayed

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**Fig. 6** Jump performance (a), sprint performance (b), isokinetic peak torque for knee extension (c) and flexion (d) during the 72-h recovery following LIST (triangles) and soccer (squares). Values are mean and SD. Asterisk versus pre-exercise for both exercises (LIST and soccer); #LIST versus soccer.
and continuous monocyte mobilization from bone marrow, thus compensating infiltration of these cells into muscle after damaging exercise (MacIntyre et al. 1995) had occurred masking leukocyte count changes in blood.

Neuromuscular function

In accordance with previous reports (Andersson et al. 2008; Bailey et al. 2007; Kingsley et al. 2005; Krstrup et al. 2006; Nicholas et al. 2000; Thompson et al. 1999, 2001b, 2003, 2004), both LIST and match impaired neuromuscular parameters assessed through measurements of sprint, jump and strength performance. Interestingly, only sprint ability was more affected by match than by LIST. Accordingly, Mohr et al. (2005) observed that soccer players decreased their ability to sprint after intense periods of the game, as well as after the end of the first and second halves. This temporary fatigue-related impairment should also be expected in jump and strength performance. In fact, considering that, at least in part, similar involvements of metabolic pathways should occur in energy turnover during these maximal power-elicited running and jumping tests, these distinct results were unexpected. An hypothetical explanation based on the presumable changes in the force-velocity relationship as a result of selective damage to type II muscle fibers induced by the great amount of eccentric exercise during match is truly appealing (Twist and Eston 2005). In fact, a greater reliance on intra-muscular high-energy phosphates in the countermovement jump instead of glycogen was likely to occur. The relatively longer duration of the 2- to 3-s sprint test would rely more heavily on glycolgen metabolism, which may be affected by the presumable eccentric exercise-induced muscle-damage and could, at least partially, explain differences between the two tests (Twist and Eston 2005). In fact, both LIST and match induce significant glycogen depletion in skeletal muscle fibers (Krstrup et al. 2006; Nicholas et al. 1999), although the effects of glycogen depletion on high intensity/short duration performance should only occur below critical levels. Alternatively, considering the distinct levels of motor coordination involved in the actions of jumping and sprint running, the repeated eccentric actions performed during the match might cause disturbances in movement control (Bottas et al. 2005) affecting more pronouncedly the running performance, which likely rely to a greater extent on muscular coordination than a single jump or leg curl and extension.

In summary, the impact of LIST and match did not differ regarding the observed muscle damage markers and some neuromuscular parameters, although match requires higher cardiac demand and induced higher changes on redox status, adenine nucleotide and on lymphocyte metabolism than LIST, which should be taken into account when using LIST to simulate a soccer match to study these type of physiological and biochemical-related endpoints.

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