

**HOW DOES A SIMULATED SOCCER MATCH AFFECT
REGIONAL DIFFERENCES IN BICEPS FEMORIS MUSCLE
ARCHITECTURE?**

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ABSTRACT

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Soccer is played by thousands of athletes across the globe and its participation increases the overall risk of injury, in particular, hamstring strain injuries (HSI). Biceps femoris (BF) has been shown to be involved in 5 out of 6 HSI cases and risk factors including fatigue and short BF fascicle length (FL) have been identified. Furthermore, previous studies suggest that different muscle regions may undergo different strains during dynamic tasks, which could contribute to injury risk. The primary aim of this study was to evaluate the effects of a soccer match on regional differences in the BF muscle architecture. A secondary aim was to assess the reliability of the extended field of view (EFOV) 2D ultrasound imaging to measure muscle architecture parameters.

Muscle architecture was assessed, using ultrasound, in 9 amateur soccer players and 5 physically active men, before and after a 45 minutes soccer specific fatigue protocol (SAFT) or 20 minutes of rest, respectively.

Significant muscle architecture changes were found after SAFT, however, these were smaller than the minimal detectable change associated with the scanning method. No correlations were found between force reductions and muscle architecture changes. Good reliability was found for FL measurements but poor reliability was found for pennation angle and muscle thickness.

Muscle architecture changes after 45 minutes of a football match may not be a mechanism to explain the increased HSI rates. Furthermore, when using EFOV ultrasound, care must be taken when interpreting statistically significant results, since these can be below the minimal detectable change or not reliable for all the parameters.

Key words: Biceps femoris; EFOV; fatigue; hamstring strain injury; muscle architecture; soccer.

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LIST OF ABBREVIATIONS

BF = biceps femoris muscle

CNS = central nervous system

EMG = electromyography

EFOV = extended field of view

FL = fascicle length

GPS = global positioning system

HSI = hamstring strain injury

ICC = intra-class correlation

LG = lateral gastrocnemius muscle

LIST = Loughborough intermittent shuttle test

MG = medial gastrocnemius muscle

MT = muscle thickness

MVIC = maximum voluntary isometric contraction

PA = pennation angle

SAFT = soccer-specific aerobic field test

SM = semimembranosus muscle

ST = semitendinosus muscle

SSC = stretch-shortening cycle

SOL = Soleus muscle

TE = typical error

VL = Vastus lateralis muscle

VO₂max = maximal oxygen uptake

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1 INTRODUCTION

Soccer is one of the most popular sports all over the world with around 200 million participants (Turner et al. 2014) and was estimated to have over 1.3 billion fans all over the world by the Fédération Internationale de Football Association (FIFA). The term soccer is going to be used during this thesis to the detriment of football to avoid misunderstandings for the reader, since there are many other sports so called football other than Association Football (e.g. American football, Gaelic football, rugby football, Australian football, etc.).

The hamstring group consists of these 3 muscles, biceps femoris (BF), divided in a long and short head, semitendinosus (ST) and semimembranosus (SM) and its main functions are knee flexion and hip extension (Turner et al. 2014). Hamstrings strain injuries (HSI) were shown to represent between 11 and 21.5% of the total injuries in soccer and up to 84% of these involved the biceps femoris, particularly its long head, while semimembranosus and semitendinosus were affected in 12% and 4% of the cases, respectively (Ekstrand et al. 2016a,b; Turner et al. 2014; Woods et al. 2004). Running has shown to be responsible for around 57% of the HSI, especially in a fatigue state, as in late stages of a match (Woods et al. 2004). Also, a soccer match play has been shown to result in decreased performance that may impair the ability of the muscle and the central nervous system to respond to the demands of the game and, result in an increased risk of injury (Rampinini et al. 2011; de Hoyo et al. 2016; Marshall et al. 2014)

Some protocols have been designed to induce soccer specific fatigue, for instance, SAFT90 (Marshall et al. 2014) and Loughborough Intermittent Shuttle Test (LIST) (Cohen et al. 2014; Coratella et al. 2015; Delextrat et al. 2010). For the LIST, maximal oxygen uptake (VO₂max) and maximum aerobic speed are required to determine the different intensities of running, while in the case of SAFT90, five standardized speeds are used. None of the protocols used include kicking, passing or other soccer specific tasks with ball, however their validity to replicate the demands of a soccer match play has been verified in previous research (Small et al. 2008; Nicholas et al. 2000).

Recently, researchers have focused their work on relating the injury incidence to numerous intrinsic and extrinsic factors such as age, previous injury, fatigue, maximum strength or muscle architecture (see 2.3.3).

From all this factors, a special attention has been given to muscle architecture of the BF (Timmins et al. 2014,2015; Alonso-Fernandez et al. 2017). Some researchers suggest that athletes with shorter BF fascicles are at higher risk of injury (Timmins et al. 2015; Bourne et al. 2016). However, existing research did not focus on muscle architecture alterations immediately after a soccer match. Furthermore, BF architecture has mainly been analysed by placing the ultrasound probe on a single site where the whole length of the fascicles cannot be seen and thus, fascicle length (FL) must be estimated (Timmins et al. 2014, 2015; Potier et al. 2009; Alonso-Fernandez et al. 2017). Since HSI seem to occur unevenly in different regions of the muscle (Ekstrand et al. 2016a), comparing muscle architecture changes between regions is totally pertinent. Consequently, not only it is impossible to see the total length of the fascicles by placing the probe at one single site but regional differences in muscle architecture are also not taken into account. Although different techniques for comparing regional muscle architecture have been validated in the literature (see 2.2), none of them have been used to evaluate architectural changes in the BF after a fatigue protocol. Hence, it is relevant to understand how fatigue-induced reductions in muscle function affect BF muscle architecture parameters in different regions. With this knowledge, a better comprehension of mechanisms behind hamstring strains will help researchers and practitioners improve intervention programs and, with that, reduce the incidence of hamstring strain injuries in different sports, specifically, in soccer.

2 LITERATURE REVIEW

2.1 Muscle-tendon function during exercise

Muscles transform chemical energy into mechanical work to produce force and consequently produce movement. As initially introduced by Hill (1938), human and animal muscles can be described as a “two-component system” involving a contractile and elastic element. Different Hill-type muscle models have later been developed aiming to describe and predict muscle behaviour (Siebert et al. 2015). From a mechanical point of view, these models refer to the contractile element as a component to actively produce force while elastic element is responsible for passive forces. Furthermore, the elastic element differentiates in the muscle in series and parallel elastic element (Siebert et al. 2015). The parallel elastic element comprises connective tissue and titin and behaves analogous to the muscle, i.e., stretches when the muscle stretches and shortens when muscle shortens (Siebert et al. 2015). On the other hand, the series elastic component (tendon and aponeurosis) is stretched when the contractile element shortens (Hill 1938; Siebert et al. 2015; Zatsiorsky & Prilutsky 2002, p.206). This series elastic component is able to store energy when stretched and thus, can contribute to increased shortening velocity and force during human movement (Hill 1938; Komi 1984).

Unlike during isometric contractions, human locomotion, and a large number of sports activities, involve muscle contractions that are characterized by a combination of lengthening (eccentric) and shortening (concentric) muscle actions in cyclic patterns (Komi 1984; Nicol et al. 1991; Strojnik & Komi 1998). These actions are defined in the literature as stretch-shortening cycle (SSC) and take advantage of the series elastic element of the muscle to enhance the concentric and propulsive phases of the movements (Komi 1984; Nicol et al. 1991). A soccer match or training performance are highly dependent on the ability of performing them as fast and powerful as possible once they require high intensity running, changes of direction and jumping (Bangsbo 2014; Di Salvo et al. 2009; Mallo et al. 2015). Muscles behaviour has shown to be different depending on the action they are involved in

(concentric, eccentric, isometric or SSC) (Komi 1984; Reeves & Narici, 2003). For instance, muscle activity patterns (Søgaard et al. 1998; Tax et al. 1990) and mechanical properties, such as fascicle behaviour (Finni 2006; Ishikawa & Komi 2004; Reeves & Narici 2003), have found to vary between isometric, dynamic and SSC type of exercise. During isometric and concentric contractions, fascicles are shorter and pennation angle (PA) is greater compared to resting state (Reeves & Narici 2003). However, this shortening of the fascicles is greater during isometric compared to concentric actions due to the compliance of the series elastic component of the muscle. Alternatively, when muscles act eccentrically, fascicles seem to behave quasi-isometrically (Reeves & Narici 2003; Finni 2006; Ishikawa & Komi 2004; Cronin et al. 2013; Péter et al. 2017) (see 2.2.1)

During SSC activities, when changes between muscle actions occur fast enough, research has shown a potentiation of muscle force compared to isometric or concentric only (Finni et al. 2000; Fukutani et al. 2015; Komi 1984). Walking and jumping also depend on stretch reflexes in addition to pre-activation (Ishikawa & Komi 2004; Komi & Gollhofer 1997) and tendon stored elastic energy (Finni et al. 2000; Fukutani et al. 2015; Ishikawa & Komi 2004) as mechanism contributing to these force enhancements.

Along with global muscle function (activity and mechanics), previous literature has also focused on region-specific adaptations to exercise. For instance, if a particular region of the muscle is more affected during an injury (Ekstrand et al. 2016a), it is of extreme importance to identify what are the mechanisms causing such regional impairment and later investigate solutions to induce regional adaptations (see 2.2.1 and 2.3.2). This regional dependent changes in muscle activity (Cronin et al. 2015; Watanabe et al. 2014), muscle architecture (Bennett et al. 2014; Blazevich et al. 2006; Kellis et al. 2010; Tosovic et al. 2016) and muscle fatigue (Watanabe et al. 2013) have been demonstrated. However, ongoing research from our laboratory (Hegyí et al. 2017, unpublished) suggests that, during running, BF activity is similar along all the muscle regions. Consequently, knowing how different muscles work in various types of exercise is important not only to understand the mechanisms behind certain adaptations but also to develop high quality training and rehabilitation programs for athletes.

The focus of this research relates to muscle architectural adaptations resulting from a fatiguing soccer match, more specifically on the BF muscle. For this reason, this review will focus mainly on SSC activities, even though examples from other exercise modalities might be evoked when pertinent.

2.2 Implications of muscle architecture to exercise performance

Muscle architecture has been studied across different leg muscles at rest and during contraction (Bennett et al. 2014; Blazevich et al. 2006a, b; Brancaccio et al. 2008; Cronin et al. 2013; Finni et al. 2000; Péter et al. 2017; Reeves & Narici 2003; Timmins et al. 2015; Timmins et al. 2014; Tosovic et al. 2016). These architecture adaptations are often assessed using 2D ultrasonography although other methods can be used such as magnetic resonance imaging (MRI) (Budzik et al. 2007; Oudeman et al. 2016) or 3D ultrasound (Rana & Wakeling 2011). However, MRI and 3D ultrasound are expensive methods and require long scanning times (2 to 15 minutes) (Rana & Wakeling 2011). One problem with measuring muscle architecture using 2D ultrasound is the probe positioning (Finni 2006). Due to a limited two-dimension perspective, the image analysis cannot account for the muscle fibres rotation that occurs during contractions in other planes. For this reason, calculations of reliability of the measurements are often reported (Bennett et al. 2014; Timmins et al. 2014). Although 2D ultrasound has shown to be reliable and to be a valid method to measure muscle architecture of the biceps femoris muscle, this method is highly user-dependent. Thus, a “skilled operator” is recommended to perform the measurements (Timmins et al. 2014) and test reliability of his/her own measurements.

Muscle architecture parameters such as PA, FL and muscle thickness (MT) are involved in the mechanical properties of the muscle that increase stiffness and efficiency of MTU complex and contribute to enhancement of performance during fast SSC movements (Finni et al. 2000; Ishikawa & Komi 2004). Furthermore, muscle architecture seems to be related to muscle function (Blazevich et al. 2006a; Cronin et al. 2013; Reeves & Narici 2003) and to be highly adaptable (Blazevich 2006b; Fukutani et al. 2015; Kanehisa et al. 2003). That said,

to comprehend muscle function, it is necessary to understand some of the short- and long-term exercise induced muscle architecture adaptations.

2.2.1 Short-term muscle architecture adaptations during isometric contractions

One generally accepted feature of muscle architecture is the shortening of the fascicles and increase in PA under pure isometric contractions (Bennett et al. 2014; Finni 2006; Timmins et al. 2014). Finni (2006) concluded that, under isometric contractions, biarticular muscles shorten more (30-40%) when compared one-joint muscles (25%). However, the BF was not included in her review and, until this date, no comparisons with other muscles have been made in this matter. Others studies have showed that biceps femoris FL decreases and PA increases during isometric contractions, and reported highly reliable results (Bennett et al. 2014; Timmins et al. 2014, 2015). Although, the majority of previous research has used a unique probe site, analysing, this way, a limited region of the muscle (Kellis et al. 2016; Timmins et al. 2015, 2014) and extrapolating the fascicle length using a mathematical formula.

To my best knowledge, only three studies examined regional differences in the hamstring muscles (Bennett et al. 2014; Kellis et al. 2010; Tosovic et al. 2016) and none of these analysed the effects of fatigue.

In two studies, cadavers were used to measure BF muscle architecture. Kellis et al. (2010) measured directly the FL and PA while Tosovic et al. (2016) assessed the reliability of the measurements comparing 2D ultrasound with direct measurements in the cadavers. Timmins et al. (2014) assessed the reliability of 2D ultrasound measurements just for one region of the muscle (mid-belly). A comparison between studies can be found in the TABLE 1. Studies where reliability of PA and MT in different regions of the muscle was verified (Kellis et al. 2010; Tosovic et al. 2016), found poor reliability while Timmins et al. (2014) showed highly reliable PA and MT measures with the probe placed over the middle region of the muscle.

As one can see in the TABLE 1., all the previous studies found shorter fascicles in the distal regions compared to middle and proximal. However, studies that measured FL directly showed lower absolute FL (Kellis et al. 2010; Tosovic et al. 2016) when compared to studies where FL was estimated (Timmins et al. 2014). The exception was the study from Bennett et al. (2014) who used EFOV ultrasound. Nonetheless, the real FL data is just a speculation from the figures in the paper since they did not present actual values.

Bennett et al. (2014) analysed proximal and distal regions of the muscle at rest and at different sub-maximal isometric contractions. The results suggested that, although fascicles were shorter in distal region, during contraction the fascicle strains were not significantly different between the two regions. In other words, fascicles in the proximal and distal regions, have shown to have similar relative fascicle length change under contraction.

TABLE 1- Comparison between FL measurements in different studies. Some measurements during contraction. 0=rest; 1 =25%MVC; 2=50% MVC; 3=75%MVC; + for this study the authors did not present FL numbers. These are approximations based on the graphic values. Regions of the muscle were divided in Prox = proximal, Mid1 = middle/proximal, Mid2= middle/distal and Dist = distal. The reliability was assessed in different studies either by using intra-class correlations (ICC) or percentage of typical error (%TE). ICC was interpreted as high (>0.9), moderate (0.80-0.89) and poor (<0.80). %TE was considered a good reliability indicator when below 10%.

	Prox	Mid 1	Mid 2	Dist	ICC / %TE*
Tosovic et al. (2016) (male)⁰	80.5±12.0	82.7±12.7	77.6±13.3	70.6±15.1	-
Tosovic et al. (2016) (cadaver-US)⁰	82.8 ±13.7	80.5 ±13.5	73.3 ±17.1	69.7 ±9.3	0.82
Tosovic et al. (2016) (cadaver-direct)⁰	88.3 ± 19.9	83.5 ± 20	79.8 ± 20.6	74.3 ± 20.2	0.82
Kellis et al. (2010)⁰	71.2 ± 4.8	62.2 ± 4.8	63.0 ± 8.4	63.5 ± 8.9	9.84*
Timmins et al. (2014)⁰		107.1 ± 14.5			0.97
Timmins et al. (2014)¹		102.1 ± 13.6			0.97
Timmins et al. (2014)²		94.6 ± 10.8			0.97
Timmins et al. (2014)³		88.8 ± 9.7			0.95
Bennett et al. (2014)⁰⁺	130			90	0.98

2.2.2 Short-term muscle architecture adaptations during dynamic movement

Given that soccer involves series of dynamic actions and locomotive tasks, it is also important to understand muscle architecture acute changes during activities consist of these actions. Although no studies have directly investigated BF muscle architecture behaviour in vivo during dynamic or locomotive activities, some pilot investigations from our laboratories have shown this to be challenging due to its complex architecture. Thus, to better picture architectural changes in the muscle during dynamic tasks, research from other muscle groups involving tasks similar to the ones included in a soccer match play may be valuable.

Many studies have investigated muscle architectural changes during walking (Cronin et al. 2013; Péter et al. 2017; Lai et al. 2015), running (Chumanov et al. 2011; Thelen et al. 2005, Hooren et al. 2016) and jumping tasks (Finni et al. 2000). During the stance phase of walking, studies have shown a rather isometric behaviour of the plantar flexors muscle fibres (Cronin et al. 2013; Péter et al. 2017) or a small shortening when compared to the total MTU shortening (Lai et al. 2015). This shows the importance of the elastic component of the MTU in contributing to force production enhancement during SSC activities. Similar to walking, during jumping muscle fibres behave quasi-isometrically while MTU is lengthening, indicating that the elastic component of the muscle elongates during the eccentric phase and recoils during the concentric phase of the movement, therefore, enhancing performance (Finni et al. 2000). In contrasts, during toe-off muscle fibres were shown to shorten and to elongate during the swing phase allowing a new gait cycle to begin (Cronin et al. 2013; Péter et al. 2017).

For the BF muscle, available data is less clear and hard to interpret. Most of the knowledge on the MTU behaviour is originated from models (Chumanov et al. 2011; Thelen et al. 2005) or animal experiments (Brooks et al. 1995; Gillis et al. 2005). Kinematic data has shown MTU shortening during stance and early swing phases of running and lengthening during mid and late swing (Chumanov et al. 2011). The same authors computed muscle forces during running and found that for the BF, peak forces occur when the muscle is at its

maximum elongation (late swing phase). The authors suggested that the BF undergoes active lengthening contractions and greater mechanical loading only during the swing phase of running (specially the late swing phase). These high eccentric forces, together with muscle lengthening, are indicated as a possible mechanism for hamstring muscle injuries (including BF) during high speed running (Chumanov et al. 2011; Thelen et al. 2005). Despite no changes in the hamstrings MTU peak length with increased speeds, the net musculotendon work increases, thus, placing the hamstrings to higher loads (Chumanov et al. 2011; Thelen et al. 2005). The higher risk of injury during the swing phase of running is also supported by experiments using animal muscle preparations (Brooks et al. 1995). The authors showed that, during active lengthening, the stretch required to cause muscle fibre strain and reduction in muscle function is 20% lower when compared to a passive condition (Brooks et al. 1995). This way the negative work done by the hamstring muscles through high speed running (swing phase), increases the risk of muscle fibres strain.

Although this results are based on biomechanical models and interpretations of indirect findings, other researchers might have other opinion regarding the role of the BF during running (Hooren et al. 2016). Based on studies using animal muscle observations (e.g. Gillis et al. 2005), Hooren et al. (2016) suggest that BF muscle fibres lengthen passively during the initial swing phase and behave isometrically during the late swing phase. In their review, the same researchers also proposed an eccentric action or active lengthening as a mechanism for hamstring muscle injury. However, this mechanism is suggested as a malfunction rather than a normal behaviour of the MTU during high speed running. Gillis et al. (2005) also suggest that experiments using animal samples have shown large variability in their results, therefore, interpretation of these results and assumptions to human muscle behaviour should be taken carefully. Thus, the true mechanism of hamstring injury is still unclear and further research is needed on the muscle activity and mechanical changes, *in vivo*, during high speed running.

2.2.3 Long-term adaptations of muscle architecture to exercise

Considering muscle chronic adaptations to long term training or to training interventions are two approaches to understand how muscle architecture is altered in function of exercise.

These adaptations are of interest for coaches and medical staff who pretend to create programs to prevent or rehabilitate muscle injuries (Opar et al. 2012). By identifying training programs or exercise methods that are more to induce the desired adaptations, risk factors may be counterbalanced and injury incidence may decrease (Opar et al. 2012) (see 2.3.3).

Various studies have compared athletes who have systematically participate in certain sporting activities for many years to identify how these activities affected their muscle architecture. For instance, when comparing the Medial Gastrocnemius (MG) and Vastus Lateralis (VL) of resistance trained and untrained men, researchers have found that trained men have increased PA and MT but similar FL (Fukutani et al. 2015). Similarly, Abe et al. (2000) compared VL, MG and Lateral Gastrocnemius (LG) of sprinters and long distance runners and found longer FL and lower PA in sprinters for all the three muscle groups. The same research group also found that sprinters who perform better at the 100m race have longer fascicles and lower PA for the same leg muscles (Kumagai et al. 2000), indicating that muscle architecture plays a determinant role in sprinting performance. Kanehisa et al. (2003), similarly, compared elite swimmers and soccer players (male and female) and found significantly larger VL muscle thickness in swimmers and similar MG muscle thickness for both groups. Interestingly, the authors discussed that male football players have VL and MG fascicles with lengths (77mm and 56 mm, respectively) in between values found for sprinters (88mm and 66mm, respectively) and long distance runners (63mm and 54mm, respectively). This data suggests that soccer specific training induces increases PA and decreases FL (Kanehisa et al. 2003) and the muscle architecture profile of soccer player . When comparing cyclists to Australian Rules Football (ARF) players, Brughelli et al. (2010) found that football players have longer fascicle in the VL and reduced PA when compared to similar competitive level cyclists. ARF is a sport similar to soccer regarding physical demands and incidence of HSI rates (25.7 vs 27.5 injures per 1000 match hours, respectively) (Ekstrand et al. 2011b; Orchard & Seward 2002) which allows some level of transfer from research done in either of the sports. With the limitation of retrospective studies in mind, the previously presented studies show that soccer players may have greater FL and lower PA when compared cyclists and long distance runners but shorter fascicles and greater PA than sprinters and swimmers.

Other methodology to study chronic adaptations to exercise is to conduct an exercise intervention and assess differences between interventional groups regarding parameters of interest. In this case, one can test the effectiveness of a specific protocol to alter the desired parameters. Several studies have investigated the effects of eccentric training on hamstring fascicle length (Alonso-Fernandez et al. 2017; Fukutani et al. 2015; Potier et al. 2009; Seymore et al. 2017) due to the idea that longer fascicles reduce the risk of sustaining a hamstring strain injury (Timmins et al. 2015). Regarding the effects of eccentric training on muscle architecture, some studies showed a significant effect of training for increasing FL (Alonso-Fernandez et al. 2017; Fukutani et al. 2015; Potier et al. 2009) while others showed no significant changes (Seymore et al. 2017). Also, Blazevich et al. (2007) found no difference in the VL fascicle length after a 5 week isokinetic training programme despite significant increases in eccentric (42.8%) and concentric (8.7%) force production. The authors suggested that short-term strength adaptations are not associated with muscle architecture adaptations. However, previous research including two hamstring focused exercises (Nordic Hamstring Curl and Hip Extension) found that, after 5 and 10 weeks, FL increased significantly with no difference between both exercises (11mm and 16mm, respectively, for hip extension exercise; 15mm and 24mm, respectively for Nordic Hamstring Curl).

Although the literature on this topic remains a bit controversial, a recent short review concluded that muscle architecture adaptations to strength training, are highly dependent on the contraction type (Franchi et al. 2016). In brief, and according to this review, concentric type of contractions favour PA adaptations while eccentric actions lead to more pronounced adaptations towards longer fascicle lengths.

2.3 Specifications of soccer

After analysing the general adaptations of the muscles to SSC activities, it is the aim of this section to introduce the demands of soccer as collective sport. These demands were studied extensively in the literature and allow researchers, coaches, trainers and medical staff to

recognize the physical, physiological, technical and local requirements of this sport in order to plan adequate programs for improving performance and minimizing injury risk.

2.3.1 Physical and physiological demands of soccer

Soccer is a collective, high-intensity, intermittent, multi-sprint sport where a player covers, on average, between 10km and 13km per game (Bangsbo 2014; Bangsbo et al. 2007; Dellal et al. 2011; Mallo et al. 2015; Nicholas et al. 2000). During soccer matches, players complete around 726 ± 203 turns and 111 ± 77 ball touches (Bloomfield et al. 2007) including dozens of passes and kicks (Russell et al. 2011). It's physical and physiological demands have been studied for several decades and, according to a recent review (Bangsbo 2014), the initial attempts to study the physical demands of soccer players date from the 1960s in Sweden. Since then, various research has focused on defining average Heart Rate and VO₂max during a soccer match. The results indicate that players perform a match at an average of 80-85% heart rate maximum and 70-75% VO₂max, respectively (Bangsbo et al. 2007; Bangsbo et al. 2006). Despite spending most of the time, 70% - 96%, in low intensity aerobic activities as walking (Bangsbo 2014; Bangsbo et al. 2006; Dellal et al. 2011; Mallo et al. 2015), soccer players' performance is highly dependent on anaerobic energy systems (Bangsbo 2014; Di Salvo et al. 2009; Ingebrigtsen et al. 2012). This dependence is corroborated by the levels of Blood Lactate (up to 10 mmol/L) and Blood Glucose concentrations (3.8- 6.5 mmol/L) after a match (Bangsbo 1994; Bangsbo et al. 2007). After comparing elite and sub-elite players, Mohr et al. (2003) concluded that elite players performed 28-58% more high intensity running activities than the lower level players. Additionally, this high intensity running seems to be related not only to physical fitness of the player but, also, to the tactical situation of the matches (Di Salvo et al. 2009; Dupont et al. 2010a) and team performance (Di Salvo et al. 2009), with higher placed teams performing significantly less high intensity activities compared to lower placed counterparts.

Different methods have been used to analyse physical demands in soccer such as global positioning system (GPS) technologies (Mallo et al. 2007), semi-automatic (Di Salvo et al. 2009) or automatic video analysis (Dellal et al. 2011). All these analyses have shown

significant differences in the total distance covered and total high intensity running between players from different positions. Centre midfielders are the ones who cover more distance and the central defenders the players who run less (Mallo et al. 2015). However, wide midfielders cover most distance at high intensities and forwards, full backs and wide midfielders are the players who sprint the most (Dellal et al. 2011; Mallo et al. 2015). It seems, however, to exist a big variance between data resulting from different collection methods (Di Salvo et al. 2009; Mallo et al. 2015) which explains some of the differences found in the literature regarding the total distance covered and total high intensity running per player position.

Owing to the high volume of running (especially at high intensities), neuromuscular fatigue has been shown to be present after soccer match play (de Hoyo et al. 2015; Magalhaes et al. 2010; Rampinini et al. 2011) and soccer match simulations (Cohen et al. 2014; Coratella et al. 2015; Delextrat et al. 2010; Marshall et al. 2014; Rahnama et al. 2003). Some differences have been showed in cardiac and redox demands in soccer matches when compared to simulations but not in muscle damage and neuromuscular parameters (Magalhaes et al. 2010). Since the main purpose of this study is to investigate the effects of a soccer match on muscle architecture, a match simulation seems to be more effective due to its standardized protocol (similar for every player).

2.3.2 Hamstring strain injuries in soccer

Epidemiologic studies on injuries in soccer have been conducted for decades in Europe attempting to describe the most common injuries in this sport for further relevant investigation (Eirale & Ekstrand 2013). In soccer, muscle injuries account for around 31% of the number of total injuries. Hamstring strains alone account for around 12% of the total amount of injuries being the most common in subtype of injury followed by adductor/ groin pain (9%) and ankle sprains (5%) (Arnason 2004; Aus Der Fünten et al. 2014; Ekstrand et al. 2011a, b). Muscle injuries, such as HSI, are described as “a traumatic distraction or overuse injury to the muscle, leading to a player being unable to fully participate in training or match play” (Ekstrand et al. 2011). Previous literature suggests that these injuries occur

mainly during sprinting or high speed running (Askling et al. 2007; Woods et al. 2004) during the late swing phase (before the heel contact the ground) where the muscle-tendon strain is expected to be higher (Chumanov et al. 2012; Thelen et al. 2005). While this idea of a late swing phase injury is yet to prove, the kinematic analysis of one event of HSI injury during a treadmill running trail (Heiderscheit et al. 2005) seems to corroborate the idea that running is the main mechanism for non-contact HSI (Woods et al. 2004).

Despite numerous interventions and attempts aiming to understand risk factors and effective training protocols to reduce these injuries (Ekstrand et al. 2016a; Marshall et al. 2015; Opar et al. 2013), HSI incidence has been increasing for the last 13 years (Ekstrand et al. 2016b). Around ninety six percent (96%) of HSI have been shown to occur in non-contact situations with matches having 5 to 8 times higher injury rates compared to practices (Arnason 2004; Ekstrand et al. 2011a; Woods et al. 2004). These results indicate that contact with other players or objects is not a major risk factor for the occurrence of these injuries. Therefore, when designing fatigue protocols to simulate a soccer match, it is not crucial to include contact activities or opponent interactions to study hamstring strains injury mechanisms.

Although there are some opposing findings (De Smet & Best 2000; Schache et al. 2009), HSI in soccer seem to occur mainly at distal and middle thirds of the muscle (Ekstrand et al. 2016a). These contradictory findings seem to have less validity since the sample size was smaller when compared to the study which found higher incidence in the proximal portion of the muscle (10 vs 255). Furthermore, in the first cases, the sample included athletes from a large variety of sports, not only soccer. Thus, there is no consensus in the current literature regarding the most common location of HSI mainly owing to differences in the injury definition and the structural incidences, i.e. if the injury involves muscle, myotendinous junction or tendon. However, data from MRI and clinical examinations on injured sprinters (Askling et al. 2007) have shown that injuries involving the proximal free tendon have a longer recovery period than the ones involving myotendinous junction. For this reason, it is relevant to analyse the muscle in different regions and test if these differences might influence the injury risk.

2.3.3 Risk factors for HSI

Although BF is, without any doubt, the more commonly affected among the hamstring muscles regarding strain injuries (Ekstrand et al. 2016a), several investigations have been conducted attempting to determine the factors that are associated with the incidence of injuries in the knee flexor muscles. Intrinsic or non-modifiable and extrinsic or modifiable risk factors have been described in the literature, being the modifiable factors more interesting since those are the ones that can be manipulated by coaches and clinicians (Turner et al. 2014). Modifiable and non-modifiable risk factors and factors with unclear results and with no relation with HSI are presented in the TABLE 2.

TABLE 2. Risk factors for sustaining a hamstring strain injury (HSI) during soccer participation 1 (Woods et al. 2004); 2 (Arnason 2004); 3 (Ekstrand et al. 2011b); 4 (Yeung, Suen, & Yeung 2009); 5 (Malone et al. 2016); 6 (Ristolainen et al. 2009); 7 (Evangelidis et al. 2015); 8 (Fiorentino & Blemker 2014) 9 (Schuermans 2016); 10 (Timmins et al. 2015); 11 (van Dyk et al., 2016); 12 (Askling et al., 2007);13 (Opar et al., 2013); 14 (Opar et al., 2014); 15 (Croisier et al. 2008); 16 (Malone et al., 2016);17 (Bengtsson et al. 2013);18 (Dellal et al. 2013).

Non-modifiable	Modifiable		
	Possible association	Unclear	No association
Age ^{1,9,13}	Fatigue ^{1,2,3}	Hamstring strength ^{11, 12,13,14,}	Muscle composition ²
Previous injuries ^{1,2,3,9,10,13}	Playing surface ³	Strength ratios ^{11,14,15}	Jumping ability ²
Season time ^{1,4,5}	Aponeurosis area ^{7,8}	Workload ^{16,17,18}	VO2max ²
Gender ⁶	Endurance strength ⁹	Neuromuscular control ¹²	Limb dominance ¹
Ethnicity ¹	Fascicle length ¹⁰		

Some physical parameters, for instance, VO₂max, body composition, jumping ability (Arnason 2004), muscle fibre composition (Evangelidis et al. 2016) or limb-dominance (Woods et al. 2004) seem to have no influence in the injury incidence, while others have, however, not yet been clearly associated with HSI due to contradictor findings among the literature. Hamstrings flexibility, core stability, hamstrings strength, strength ratios between hamstrings and quadriceps and angle of peak torque are some of the most controversial factors found in the previous research (Askling et al. 2007; Schuermans et al. 2017; Timmins et al. 2015; van Dyk et al. 2016)

High levels of physical conditioning seem to reduce the risk of HSI, however the absolute strength during knee flexion has not clearly been shown as a risk factor for HSI (Arnason 2004; Askling et al. 2003; Duhig et al. 2016).

Match and training load, fatigue, age, previous injuries, aponeurosis size, gender, playing surface, fascicle length, activity pattern and endurance strength of the hamstrings (Arnason 2004; Bengtsson et al. 2013; Carling et al. 2015; Dupont et al. 2010; Ekstrand et al. 2011a, b; Ekstrand et al. 2016a; Schuermans et al. 2016; Timmins et al. 2015; Woods et al. 2004) have been suggested as potential risk factors for HSI. It is quite clear from the literature that muscle injuries tend to occur less in artificial turf when compared to natural grass (Ekstrand et al. 2011a) and less in female subjects when compared to male (Ristolainen et al. 2009). On the other hand, the presence of match congestion periods seems to have some contradictory results in the literature. Some studies including small sample sizes, have shown evidence that fixtures congestion and injury incidence may not be associated (Carling et al. 2015; Dellal et al. 2013). However, a study involving an 11 years follow-up supports the theory of increased injury incidences during match congestion periods (Bengtsson et al. 2013). These differences were observed when comparing 4 vs 6 days of rest between matches but no differences in the injury incidence were found if players have less than 3 or 4 days between matches. The notion that muscle injuries, specifically hamstring strains, are related with higher work load is supported by other authors (Duhig et al. 2016; Dupont et al. 2010; Malone et al. 2016). Testing players from different professional soccer leagues, Malone et

al. 2016) investigated the association between workload (training and match) together with aerobic fitness and overall injury risk. The authors suggested that players with lower aerobic fitness, (assessed using Yo-Yo IR1 performance test) are at 3 to 5 times higher risk of injury, compared to the aerobically fitter counterparts.

Schuermans et al. (2014,2016) elaborated an interesting research protocol to assess hamstring muscle activity differences between injured and non-injured soccer players. They performed two functional MRI scans, with a bout of knee flexions until failure in between, with the aim of measuring metabolic activity of the different muscles as an indication of activity patterns between the hamstring complex. Later, the researchers followed the players for 1,5 seasons and recorded the injury incidence. Their results have shown that not only previous injured players but player who sustained later injuries have a lower activation of the ST muscle, compensated by a larger activation of the BF and SM. As stated by the authors, the different activation patterns used by injured athletes may contribute to a less efficient force production and an earlier onset of fatigue. This inefficiency in generating force over time together with increased workload (either from more congestion fixtures or training sessions) may place the players in an augmented risk of injury.

Regarding strength levels, various studies indicate that player who get injured have significant lower force levels (Opar et al. 2013; Schuermans et al. 2016; Timmins et al. 2015; van Dyk et al. 2016). However, van Dyk et al. (2016) showed that conclusions of differences between injured and uninjured groups should be taken carefully. Their data suggests that, despite a discrepancy on the average strength values between groups, it is challenging to clearly define a cut-off value that predicts a hamstring injury, contrary to what other authors suggest (Croisier et al. 2008; Opar et al. 2014). Nonetheless, in line with Malone et al. (2016) results about fitter players having less injuries, Croisier et al. (2008), found that players with strength imbalances that performed a training program and normalized their test results, were at reduced injured risk compared to the ones who did not participate in the intervention program. Similarly, Askling et al. (2003) showed a lower incidence of hamstring injuries for a group of players who performed pre-season training compared to a group who did not train. The authors found no correlation between strength levels and injury rate.

The above cited risk factors (such as strength, muscle architecture, flexibility, age...) are often measured at baseline with the idea of, prospectively, association with injuries (Schuermans et al. 2016; Timmins et al. 2015; van Dyk et al. 2016; Yeung et al. 2009). However, as previously shown by Malone et al. (2016), there is an accumulated effect of workload which may increase the risk of injury. This shows how dynamic the human body is and how these baseline parameters might change easily with external factors involved in the training and competition processes. Cook (2016) addresses this problem in the present injury predicting model and suggests a more dynamic assessing model, following the athletes and not predicting injuries based on non-dynamic measurements.

Since fatigue, acute (resultant from one match) and accumulated (following several matches and training sessions), has shown to increase the risk of injury, it is important to understand its mechanisms to understand the underlying effects in the muscle-tendon unit.

2.4 Mechanisms of muscle fatigue

Enoka and Duchateau (2008) in their review about muscle fatigue distinguished muscle fatigue from failure to perform a task, explaining this last definition would include a large complex of phenomena which could lead to “reduced likelihood that the cause of fatigue can be identified”. For this reason muscle fatigue is going to be approached during this thesis as an exercise-induced decrease in the capacity of the muscle to produce force, which is reversible by rest (Enoka & Duchateau 2008; Gandevia 2001). Thus, muscle fatigue should not be mistaken with “task failure” or “exhaustion”, terms used to describe the point where the exercise can no longer be maintained (Gandevia 2001).

To my best knowledge the first experiment attempting to study the effects of fatigue on human performance was performed in 1892 (Lombard 1892) using one subject to study the effects of various factors in maximal voluntary contraction. Since then many experiments have been conducted attempting to describe the mechanisms of fatigue in different activities. The mechanisms contributing to muscle fatigue have been studied either in single fibre

preparations (Allen et al. 2008; Edman & Lou 1990) or *in vivo* (Bigland-Ritchie et al. 1982; Goodall et al. 2015), intending to explain different mechanisms contributing to this phenomenon.

Experiments conducted *in vitro* (using, for instance, frogs muscle fibres preparations) supported the idea that the lack of calcium and increases in carbon dioxide in the cell are two of mechanisms causing reduced muscle fibre capacity of force production after fatigue stimulation (Allen et al. 2008; Allen et al. 1989; Edman & Lou 1990). However, these experiments were done in single fibre preparations and might not represent the behaviour of the whole muscle-tendon unit *in vivo*.

Denny-Brown (1928) firstly introduced a method later developed by Merton (1954) as interpolated twitch technique (ITT). He showed that if an electrical stimulation, when applied to the motor nerve during maximal voluntary contraction, does not produce an increased twitch, the muscle fibres are fully activated. By that time, the method was not well developed (Gandevia 2001) which led the investigators to the wrong conclusion of similar muscular responses with and without motor nerve electrical stimulation. (Bigland & Lippold 1954; Merton 1954). This technique has been validated for plantar flexors, dorsiflexors (Belanger & McComas 1981; Cooper et al. 2013) and for knee extensors (Oskouei et al. 2003), however it remains to validate for the hamstring muscles.

According to Bigland-Ritchie (1984), fatigue can be measured by the degree of force loss, during or immediately after an exercise. Muscle fatigue can result from failure to activate the muscle (central fatigue) and failure at the contractile material (peripheral fatigue). Voluntary Activation (VA) is commonly used to describe the capacity of the CNS to activate the muscle and can be used as an indicator of the site of fatigue (Gandevia 2001). One's VA can be estimated using ITT, by the formula:

$$VA = \frac{1 - T_{intrapolated}}{T_{control}} \times 100$$

where $T_{intrapolated}$ and $T_{control}$ represent the twitch during maximal voluntary isometric contraction (MVIC) and rest, respectively (Gandevia 2001). In short, if the central nervous

system (CNS) is not able to recruit all the motor units at optimal firing frequency on the target muscle, a supramaximal electrical stimulus will result in additional force (Gruet et al. 2013). Exercise-induced reductions in VA indicate failure of corticospinal mechanisms while a depression in the resting twitch indicates failure at the neuromuscular junction or in the contractile elements of the muscle (Gandevia 2001; Gruet et al. 2013; Rampinini et al. 2011). Although ITT allow us to distinguish between peripheral and central sources of fatigue, it does not separate fatigue driven caused by reductions in the cortical drive or spinal excitability. For this, other technique so called transcranial magnetic stimulation (TMS) can be applied (Gruet et al. 2013).

To quantify central and peripheral fatigue induced by activities involving locomotion, previous researchers have been mainly tested marathons, ultra-marathons, sprinting, cycling and even soccer matches (Goodall et al. 2017; 2015; Marshall et al. 2014; Millet et al. 2011; Petersen et al. 2007; Rampinini et al. 2011; Temesi et al. 2014; Thomas et al. 2015). It has been suggested that fatigue is highly task dependent and it is very difficult to say, for instance, if men are more fatigable than women or old people are more fatigable than young, due to interindividual variability (Enoka & Duchateau 2008). Nonetheless, some consensus does exist regarding the influence of central and peripheral factors to muscle fatigue, depending on the intensity, duration and mode of the exercise.

2.4.1 Central versus peripheral fatigue

Central fatigue is defined as the reduction in force production resulting from reduced muscle voluntary activation by the CNS (Bigland-Ritchie & Woods 1984; Gandevia 2001). In cycling a higher influence of central fatigue in long lasting exercises when compared to shorter races (40km vs 4km) has been shown (Thomas et al. 2015). The same has been shown in marathon and ultramarathon runners (Millet et al. 2011; Petersen et al. 2007; Temesi et al. 2014). Thus, in more prolonged activities, failure of the neural input to the muscle (central fatigue) seems to account for most of the exercise-induced loss of force. It is not in the scope of this review to present in detail corticospinal mechanisms of fatigue (for more details please read a review from Gandevia 2001 and Gruet et al. 2013). Millet & Lepers (2004) concluded

that, in long lasting activities, the contribution of central fatigue to strength losses was greater for running when compared to cycling and cross-country skiing. The authors suggested that not only the stretch-shortening cycle is a factor contributing to this differences in the source of fatigue, but the impact is too. The less impact of skiing compared to running, is thought to decrease the muscle damage associated the activity (Millet & Lepers, 2004). This is corroborated when comparing decreases in VA form studies including running (16% to 26%) (Goodall et al. 2015; Temesi et al. 2014) and studies including cycling (8% to 11%) (Lepers et al. 2002; Thomas et al. 2015).

Alternatively, the peripheral component of fatigue refers to a decline in the capacity of the muscle to produce force owing to mechanisms at or distal to the neuromuscular junction. This can be seen by the reduction of the resting twitch when used ITT (Bigland-Ritchie & Woods 1984; Gandevia 2001). When using ITT, the electrical stimulation applied to the peripheral nerve induces electromyographic responses in the muscle with different timings (Palmieri et al. 2004). First, at low stimulation intensities, the Ia afferent nerve fibres are stimulated transmitting the signal (synapse) at the spinal cord to the alpha motoneuron (efferent) that innervates the muscle and produces there a twitch response in the electromyograph (EMG) (Palmieri et al. 2004). This response is called H-reflex and has been largely used in the literature to assess spinal excitability (Palmieri et al. 2004; Walton et al. 2002). The H-reflex is normally defined as an electrically evoked analogue of a stretch reflex, a muscle response to a fast and unexpected increase in length (Enoka 2008, 261). Additionally, when the stimulation intensity increases and is sufficient to create direct action potentials in the efferent nerve fibre, a direct EMG muscle response occurs at a shorter latency. The higher intensity required to stimulate the efferent fibres when compared to the Ia afferent relates to the diameter of the fibres. Larger fibres such as the motoneurons are larger in comparison to smaller sensory neurons (Palmieri et al. 2004). Since the direct response did not pass through the spinal cord it is not called a reflex but M-wave (Palmieri et al. 2004). It has been used to measure the propagation of the action potentials at the neuromuscular junction (i.e. the excitation-contraction coupling) (Bigland-Ritchie & Woods 1984).

Peripheral fatigue has been shown to occur in shorter, however more intense muscle activities, either during isometric (Bigland-Ritchie et al. 1982; Schillings, Hoefsloot, Stegeman, & Zwarts 2003) or locomotive tasks (Millet et al. 2011; Petersen et al. 2007; Temesi et al. 2014).

Fatigue has also been studied in resistance or isometric type of exercise. In their review, Enoka & Duchateau (2008) highlighted that fatigue resultant from long sustained submaximal isometric contractions (>40 min), when compared to maximum isometric contractions sustained for shorter periods (3 min), is more related to reductions in VA (central origin). Babault et al. (2006) showed that, for long lasting submaximal contractions, fatigue subsequent to an isometric task induces greater reductions in VA (36%) when compared to a concentric task (27%). These findings are in accordance with the ones from locomotive tasks (e.g. Goodall et al. 2015), where reductions in VA (23%) in more prolonged activities indicate greater influence of central fatigue.

Only two studies investigated reflex behaviour on the hamstring muscles using sciatic (Floy 2012) and sural nerve stimulation (Baken et al. 2005). No investigation has been conducted regarding changes in the hamstring muscles corticospinal excitability. Nonetheless, findings from other muscles can be used to estimate possible adaptations. Nicol et al. (1996) studied the effect of an exhaustive SSC type of exercise on the stretch reflex responses of soleus (SOL) and LG. Interestingly, they found a decrease in the stretch reflex amplitude for the LG but not for the SOL which contradicts other researchers (Hortobágyi et al. 1991). These differences might be due to protocolary dissimilarities since Hortobágyi et al. (1991) included only 50 drop jumps and the subjects did not exercise until exhaustion. No relation between the lactate accumulation and the reductions in the reflex responses of the LG have been found (Nicol et al. 1996). The H-reflex has also been used as a measure spinal excitability after fatigue (Walton et al. 2002). Walter et al. (2002) studied the effects of a sustained submaximal (30%) isometric fatiguing protocol on endurance and sedentary people. Despite similar levels of maximal strength between groups, endurance runners showed longer times (31.3min vs 17.6min) until fatigue. Fatigue, in this case, was defined as a decrease in the MVIC to 70% or less. This may indicate that maximal strength has little influence in the reduction of force production during submaximal activities. Additionally, they showed a

greater suppression of the H-reflex, even after initial stages of fatigue, for the endurance athletes compared to sedentary population. The authors suggested that endurance trained athletes may have different strategies to control motoneuron pool excitability either by involving lower neural drive to maintain submaximal tasks, or by reducing the co-contraction of other muscles, which enhances economy by reducing the activity of muscles that are not used for the main task. These changes in spinal excitability after fatigue, are interpreted by different authors as altered sensitivity of the muscle spindles or smaller muscle afferents III/IV (Nicol et al. 1996; Walton et al. 2002).

2.4.2 Fatigue in soccer

Different approaches have been previously used to study the effects of fatigue in soccer. Some studies attempted to cause exercise-induced reductions in performance using real soccer matches (de Hoyo et al. 2016; Rampinini et al. 2011; Wollin et al. 2016) while others used laboratory non-specific fatigue protocols (Greig & Siegler 2009; Rahnama et al. 2003; Sangnier & Tourny-Chollet 2007).

The problem with most of the laboratory fatigue protocols is the lack of reproducibility of the match demands such as change of directions and running intensities (Greig & Siegler 2009; Rahnama et al. 2003; Sangnier & Tourny-Chollet 2007). For this reason, soccer specific protocols were created to simulate the intensity and activities performed during a game (Cohen et al. 2014; Coratella et al. 2015; Delextrat et al. 2010; Kellis et al. 2006; Lovell et al. 2008; Marshall et al. 2014; Nicholas et al. 2000; Russell et al. 2011; Small et al. 2009). Russell et al. (2011) was the only research group including shooting, passing and dribbling skills in the fatigue protocol, tasks that are soccer specific (see 2.3.1). Blood lactate and blood glucose concentrations obtained during simulations are comparable to the ones found during simulated matches (blood lactate = 2-10 mmol/L vs 3.8- 6.5 mmol/L; blood glucose = 5-6 mmol/L vs 6.3-6.4 mmol/L) (Nicholas et al. 2000). Similarly, total distance is related (10.1-12.4 km vs 10-13km) (Lovell et al. 2008; Nicholas et al. 2000; Russell et al. 2011).

Due to this apparent more realistic representation of the demands of the game, during the next sections, my focus will be mainly in the studies using either a real match or a soccer specific simulation.

2.4.3 Physical and technical parameters changes during fatigue

Sprint times have shown to increase between 2% and 8% after soccer match (Krustrup et al. 2006; Magalhaes et al. 2010; Rampinini et al. 2011; Russell et al. 2011; Small et al. 2009) partly due to decreases in stride length (Small et al. 2009). In agreement, jumping height, measured using countermovement jumps, has been shown to also decrease about 12-14% and remain reduced after 48h (de Hoyo et al. 2016; Magalhaes et al. 2010). The effect of fatigue on some technical skills has been studied by some researchers who found reductions in kicking performance (Ferraz et al. 2016; Kellis et al. 2006) and some who did not find any alteration on kicking, passing and dribbling (Russell et al. 2011). Interestingly, the group who found no influence of fatigue in the soccer skills performance was the only group that included these skills in the simulation protocol (Russell et al. 2011).

Regarding recovery from soccer activities, numerous studies have been conducted in order to explore this process after a match, especially due to the increasing congested fixtures in modern soccer leagues (Carling et al. 2015; Dupont et al. 2010b; Page et al. 2016a). Page et al. (2016) found no difference in physical performance during a soccer specific protocol when the players rested 48h or 72h in between trials. Similar results were reported by Dupont et al. (2010), who showed that players who performed 2 matches in less than 96 hours did not show significantly decrease in performance when compared with the ones who rested more than 6 days. De Hoyo et al. (2016) also examined the eccentric phase of the countermovement jump and found this parameter recovers faster when compared to the concentric phase.

2.4.4 Muscular performance during fatigue

Numerous research has been made to determine how muscles behave during and after a fatigable soccer match. Peak Torque and angle of Peak Torque are two of the most common parameters used in the literature to assess muscle function and, in this case muscle fatigue (Cohen et al. 2014; Coratella et al. 2015; Delextrat et al. 2010; Wollin et al. 2016). Research concerning soccer fatigue for the past 15 years focused mainly in quadriceps and hamstrings muscles, possible due to the importance of the balance between these two muscle groups for hamstrings injury diagnosis (Yeung et al. 2009).

Previous research showed that quadriceps and hamstrings groups are affected differently during a soccer match (Cohen et al. 2014; Coratella et al. 2015; Sangnier & Tourny-Chollet 2007). Rozzi et al. (1999) used an isokinetic fatigue protocol on soccer and basketball players and measured muscle activity during a single leg drop jump before and after the protocol. They showed a significant increase in the time for the onset of the medial hamstring muscles (ST and SM) activity but not for the lateral hamstring (BF), or any of the quadriceps muscles.

Not only quadriceps are less fatigued than hamstring muscle group during soccer, possibly owing to a higher percentage of fast twitch fibre type in hamstrings (Coratella et al. 2015), but Cohen et al. (2014) found significant decrease in hamstrings eccentric PT while the concentric PT remained unaltered after a stimulated soccer match. Wollin et al. (2016) followed the recovery after a soccer match and found that hamstring muscles recovered from a 17% decrease in the MVIC after 48h. In this same study, range of motion of the ankle, knee and hip were shown to be unaltered after a fatiguing match.

Montini et al. (2016) showed, however, no significant decrease in the knee extensors maximal voluntary force after a soccer match, while the muscle activity of Vastus Lateralis (VL) was decreased by 24%. This seems to indicate that reductions in muscle intrinsic properties may not correlate with performance measures.

Merely one study investigated the effects of a specific soccer fatigue in Voluntary Activation (VA) of the hamstrings and muscle stimulation was used (Marshall et al. 2014). In this study, VA was measured using direct muscle stimulation which has been shown to require “large voltages” and is not recommended in humans in order to obtain M-wave responses. (Gandevia et al.1995, 15). The authors found a significant decrease in the knee flexors VA (7.6%) and the EMG activity of the BF (20.7%) while the medial hamstring muscles weren’t affected. This was accompanied by a maximum voluntary torque decrease of 10% and 15% at half-time and end of the match, respectively. Very recently, Goodall et al. (2017) described the corticospinal changes in the knee extensors after a 120 minutes of a simulated soccer match. They found 11%, 20% and 27% reduction in knee extensors MVIC after 45min, 90min and 120 min respectively. These changes were accompanied by reductions in VA (indication of central fatigue) of 11%, 15% and 17%, respectively, but no changes in the motor evoked potentials or amplitude of the M-wave were found. These reductions in VA were, however, lower when compared to resistance exercises (36%) or 10 sets of sprints (23%) (Babault et al. 2006; Goodall et al. 2015). Rampinini et al. (2011) found even smaller decreases in VA (8%) following a 40 minutes soccer match play. These findings indicate that fatigue resultant from a soccer match is highly related to central drive decreases even though it is lower when compared to other exercises. These discrepancies may be due to differences in the contraction modes, exercise duration and type of exercise.

2.4.5 Effects of short-term fatigue on muscle architecture parameters

To this date, no studies have investigated the effect of acute exercise in the muscle architecture of the hamstrings. For this reason, it will be presented some data from studies done using other muscles. To study muscle architecture in the muscle in vivo, previous research as mainly used 2D ultrasound (Bennett et al. 2014; Blazevich et al.2006; Kellis et al. 2010; Thomas et al. 2015; Timmins et al. 2015; Timmins et al. 2014; Tosovic et al. 2016).

Ishikawa et al. (2006) and Thomas et al. (2015) showed an increases in fascicle length and decreases in pennation angle in the ankle plantar flexor muscles after exhaustive exercise. Ishikawa et al. (2006) showed a significant increase in passive soleus FL, immediately after

the fatiguing protocol. This protocol was similar to the one described by Nicol et al. (1996) which was estimated to last around 2.3 minutes. In exercises with similar duration and intensity, the decreases in force production have shown to be more related to contractile elements (peripheral fatigue) than central sources impairment. On the other hand, research in soccer has showed larger decreases in central drive when compared to muscle contractile properties failure (Goodall et al. 2017; Marshall et al. 2014). In fatiguing exercises where the source of fatigue is, from previous research, more central than peripheral, as in long intermittent isometric contractions (Thomas et al., 2015), FL increased (8-14%) and the PA decreased (7-9%). In this research the muscle tested was the MG and the decrease in force production was around 30% which is comparable to a full time (20%) but not a half time soccer match (10%). Other research was conducted involving the plantar flexors (MG and SOL) where participants walked for 60 min on a treadmill (Cronin et al. 2013). Although the aim of this study was not to assess the effects of fatigue on muscle architecture, the authors found an increase of 6% in the FL of the MG with no changes in the FL of the SOL. Mitsukawa et al. (2009) tested the effect of 60 maximal isometric contraction (2-s hold + 2-s rest between repetitions + 8s rest every 10 repetitions) on the architecture of MG and SOL. The total time of the protocol was estimated to be about 4.6 minutes. The results showed that initial force decreases happened with no changes in FL. At the end of the protocol, increased FL was highly correlated with force decreases only for MG, remaining the SOL unaffected. This evidence seems to support the idea that muscle architecture alterations occur only in late stages of fatigue.

Recently three studies have investigated the effects of sport specific competition and training on muscle architecture. The first research consisted in cycling on a cycloergometer until exhaustion and included 30 athletes from numerous sports (Brancaccio et al. 2007). The authors found significant increases in the rectus femoris and vastus intermedius PA and MT of 12% and 8%, respectively. Secondly, Storey et al. (2012) assessed how VL muscle architecture of weightlifters and resistance trained men changes after two heavy training sessions (10 sets with 2 min rest) the same day (4-6h apart). Despite no differences in the VL anatomical or physiological cross sectional area, the results showed lower baseline force levels for resistance trained men and only this group showed decreases in maximum force

production (12% to 16%). The authors found no correlation between PA or MT of the VL and force decrease. Similarly, a study involving snowboarders also found no changes in PA, FL or MT after a snowboard simulated race (about 36s) with no measures of strength (Vernillo et al. 2017).

Considering the previous literature, exercise induced force reduction seems to alter muscle architecture (Brancaccio et al. 2007; Mitsukawa et al. 2009; Thomas et al. 2015) with some exceptions (Storey et al. 2012; Vernillo et al. 2017).

3 PURPOSE OF THE STUDY

In soccer, fatigue and reduced FL have been shown to be two factors associated with increased incidence of hamstring strain injuries and a match has shown to induce significant fatigue in the knee flexors. Previous research has shown that fatiguing exercise induces changes in different muscle groups' architecture but no studies have focused on soccer or on the BF.

Thus, the aim of this research was to evaluate the effects of a soccer specific protocol on regional differences of the Biceps Femoris long head muscle architecture. A secondary aim of the study was to assess the reliability of Extended Field of View ultrasound technique to measure BF muscle architecture.

The four hypotheses for this study were 1) FL would increase and PA would decrease after the simulated soccer match; 2) middle regions would be more affected by fatigue; 3) fatigue-induced changes in the muscle architecture would be more pronounced when measured at higher contraction intensities; 4) the reliability of PA and MT would be lower than measured for FL.

4 METHODS

4.1 Subjects

Subjects were recruited at the university campus and the local soccer teams. All the students included in a mailing list from the university were contacted to participate in the study and 4 local soccer teams were introduced to the study personally by the main researcher. A total of 17 players showed interest in volunteering in the study but 5 subjects were excluded for not meeting the inclusion criteria. These included: 1) male soccer player (for the experimental group -EG) or physically active (for the control group - CG); 2) age between 18-30 years old; 3) no history of hamstring or anterior cruciate ligament injuries (Heijne et al. 2013), no symptoms of acute illness or medical contra-indication to exercise, 4) participation in regular soccer practices (minimum 2 times a week) for the last 2 years (EG). Twelve amateur level soccer players (mean \pm SD, age (years) = 24 ± 3 , weight (kg) = 76 ± 7 , height (cm) = 177 ± 7 , BMI = 24.2 ± 1.3) and 5 physically active male subjects (mean \pm SD, age (years) = 26 ± 4 , weight (kg) = 72 ± 7 , height (cm) = 178 ± 7 , BMI = 22.8 ± 0.8) volunteered to participate in this study. In the EG, one subject got injured during the fatigue protocol and two others had poor quality data.

Subjects were informed of the experimental protocol and all the associated risks and agree in volunteering in the research by signing a written informed consent. This study was part of a major research project and all the procedures were approved by the ethics committee of the University of Jyväskylä in accordance with the Declaration of Helsinki.

4.2 Experimental design

Subjects in the EG visited the laboratory in 2 different occasions, one for familiarization and one for experimental session (FIGURE 1). The experimental session consisted in strength and muscle architecture measurements pre- and post-45 minutes of an intermittent soccer

aerobic fitness test (SAFT). The CG visited the lab only in one occasion and the same measurements were done pre- and post- 20 minutes of rest.

During the familiarization session, subjects performed similar strength measurements protocols, muscle architecture measurements and experienced 10 minutes of the soccer specific protocol. The subjects were asked to use the bring the same shoes for the experimental session. As previously mentioned, the larger research project, where this study was included, involved measurements of muscle activity, however these are not going to be focused in this thesis. Each measurement lasted between 2 and 3 hours and the post measurements started 1-2 minutes after the end of the protocol and were completed in 10-15 minutes for every subject.

The two sessions were separated by 7 to 14 days to allow subjects recovery from the vigorous exercise. In the 24hours prior the experimental session, subjects did not perform any unfamiliar or vigorous exercise and did not consume alcohol.

Although previous research shows that caffeine may increases aerobic performance (Tallis et al. 2016), when compared to non-caffeine consumers, heavy-caffeine consumers were shown to perform worst in physical and cognitive performances, if undertook acute (2 days before) caffeine withdrawal (Rizzo et al. 1988; Rogers et al. 2013). For this reason, subjects were allowed maintain their regular caffeine consumption and were asked not to ingest any caffeine only 2 hours prior the measurements (Liguori et al. 1997).

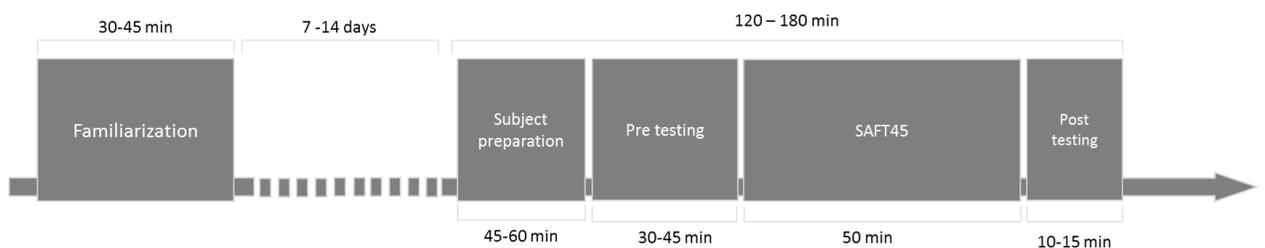


FIGURE 1. Timeline of the research protocol. SAFT45 is an adaptation of the original SAFT90 (Small et al. 2008) consisting of sets of 15 minutes of predetermined intermittent soccer specific protocol.

4.3 Fatigue protocol

An adaptation of the SAFT⁹⁰ protocol (FIGURE 2.) was used to simulate the physiological and muscular demands of a soccer match. The protocol was developed based on time-motion data from the 2007 English Championship, and it was previously validated (Small et al. 2008). In sets of 15 minutes, different commands are given by an audio tape to the subjects, indicating the speed (0 km/h, 5km/h, 10.3 km/h, 15 km/h or 24.4 km/h) and an alternative utility movements (forward, backward or side running) to perform. This set of 15 minutes was then repeated for 3 times to complete 45 minutes of a soccer-specific exercise.

A similar (Woods et al. 2004) and in some cases higher (Ekstrand et al. 2011a) incidence of hamstring injuries have been found between 30 and 45 minutes when compared to the last 15 minutes of a match play. For this reason, it was decided not to use 90 minutes of exercise in order to optimize the total time for the measurements.

Before the fatigue protocol and after the strength measurements, a warm-up protocol involving 2 minutes running (10-12 km/h) and 10 full circuit protocol (40 meters with changes of direction) at increasing speed were completed. The protocol was finished after 45 minutes and care was take to minimize the time between the end of the measurements and the post measurements (1-2 minutes to start the post measurements).

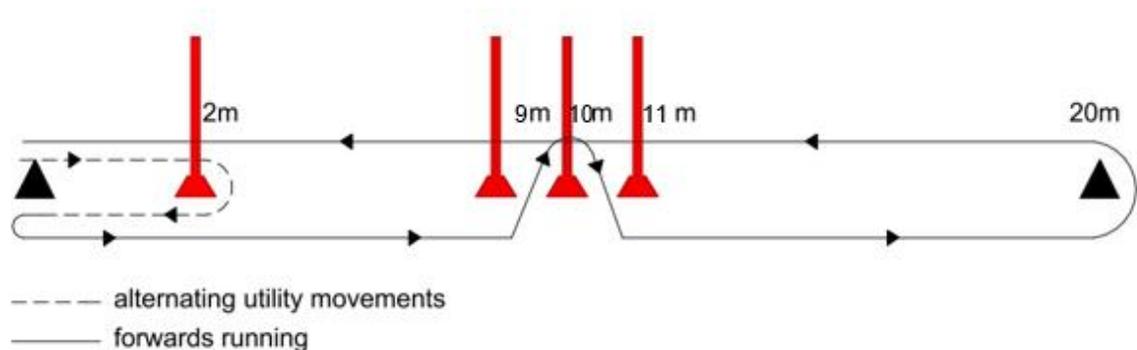


FIGURE 2. Representation of the SAFT protocol. Adapted from (Small et al. 2008)

4.4 Strength measurements

The subject was lying prone with the ankle fixed to a custom-made isometric dynamometer. The hip of the subjects was firmly strapped with a belt to reduce the hip movements. The hip and knee joints were positioned at a 0° and 10° flexion (0=full extension), in a similar position as used in a previous study (Marshall et al., 2014). The dynamometer was calibrated before the subject arrived to the laboratory, using a known load. The force signals were recorded at 1000Hz using an analogue to digital converter (CED Power1401-3A, Cambridge, UK) and a compatible acquisition software (Spike 2, v6, CED, Cambridge, UK).

A standardized warm-up was performed involving 10 submaximal isometric contractions (10%-90% of perceived MVIC) and 3 quasi-maximal contractions (95%-100% of perceived MVIC). After 1 minute rest, 3-5 MVIC attempts were performed with 60 seconds rest between contractions. After the fatigue protocol, only 2 repetitions were performed to minimize the time until the muscle architecture measurements. The repetition with the maximum force production was used for calculating 25%, 50% and 75% of MVIC used for the muscle architecture measurements under contraction.

4.5 Muscle architecture measurements

The muscle architecture properties of the muscle were measured, by the same trained ultrasound operator, using a 2D Ultrasound device (Alpha-10; Aloka, Tokyo, Japan, 7.5-MHz probe, 60 mm field of view). The subject was lying prone with hip in neutral position (0° flexion) and knee at 10° flexion (0° = full extension). A water-soluble gel was used on the probe to ensure acoustic contact and a better image quality. To identify regional differences along the muscle length, an extended field of view (EFOV) function was used as shown in the FIGURE 3.

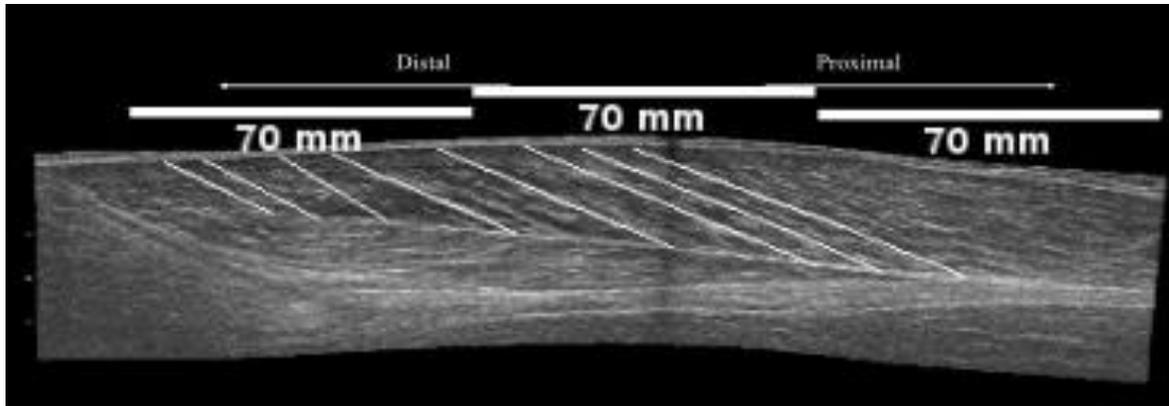


FIGURE 3. Example of an extended field of view (EFOV) 2D ultrasound image. The drawn lines represent the direction of the fascicles. Dark shadow in the middle of the image represents a reflective tape placed over the skin at half point between the ischial tuberosity and the head of the fibula.

Before the muscle architecture measurements, the muscle was scanned longitudinally to find the points along the muscle where it was possible to see the deep and superficial aponeurosis of the BF long head (FIGURE 3). A line was drawn over the skin with permanent marker to last until the end of the measurements. Small strip of reflective tape was placed over the skin at the mid distance between the ischial tuberosity and the head of the fibula (origin and insertion of the Biceps Femoris long head). This stripe was used later in the analysis to define the different regions of the muscle. The ultrasound images were obtained at rest (0% of MVIC) and during isometric contraction (25%, 50% and 75% of MVIC) similar to reported in a previous study (Bennett et al. 2014). The subjects were asked to contract the muscle and maintain constant force at the target level for about 8 seconds. Visual feedback was given to the subject, on a screen, with the target force levels. When the subject achieved the target force level, the longitudinal scan the muscle started. The scan was done at constant speed, in about 4-5 seconds, along the sagittal plane of the muscle. Care was taken to minimize the pressure of the US probe on the skin. Two images from each condition were collected and 60 seconds rest between repetitions was allowed to the subject to minimize fatigue.

4.6 Data management

4.6.1 Force Measurements

The isometric force measurements were analysed using Spike 2 software (v6, CED, Cambridge, UK). The repetition with the highest maximum force production was registered for pre- and post-fatigue conditions.

4.6.2 Muscle architecture parameters

The images were exported from the ultrasound device in TIF format and analysed using an image processing program (ImageJ 1.50b, Wayne Rasband, National Institutes of Health, <http://imagej.nih.gov/ij> Java 1.8.0_60, 64-bit).

Three regions of the muscle were defined using as a reference the shadow of the reflective tape and 4 fascicles for each region were digitized in the image (FIGURE 3). Only distal and middle regions of the muscle were used due to the poor quality of the images. The line was digitized from the superficial aponeurosis and the fascicles were followed until the deep aponeurosis. In case of having images with curved fascicles, they were tracked in a series of connected lines (Bennett et al. 2014). For fascicles where the insertion was not clearly seen, the fascicle was extrapolated until it intersected the upper surface of the deeper aponeurosis. The PA was calculated as the angle between each fascicle and the deep aponeurosis. The MT was calculated as the perpendicular distance between superficial and deep aponeurosis at 50% of the fascicle length. The average of 3-4 measurements of FL, PA and MT for each region was used to describe the muscle architecture for each image. A total of 4-8 fascicles per region per condition were measured. The images were analysed in a random order and the averages and rearrangement was done using an Microfot Excell 2010 Macros to decrease bias during the analysis. For the control group images were taken before and after 20 minutes of rest (sitting position) to calculate the reliability of the measurements.

4.6.3 Statistical Analysis

Two- and Three-way repeated measures ANOVA's were used to compare differences by region (middle or distal), intensity and fatigue stage (PRE and POST) for FL, PA, MT and fascicle length normalized to muscle thickness (FL_{norm}) (Bennett et al., 2014). The assumption of sphericity was verified prior to the analysis of nan interaction using Mauchly's Test of Sphericity. If the sphericity assumption was violated, a Greenhouse-Geisser estimation was used to correct the biased interaction effects.

In cases where an interference effect was found, paired samples t-tests were conducted to identify the conditions in which there was a significant difference. The different statistical tests were done using SPSS software statistical package (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Statistical significance was set at $p < 0.05$.

Effect sizes were calculated for variable with significant differences between conditions using the method described by Lakens 2013). Pearson's correlation coefficients (r) were also calculates using the same software to identify whether any of the muscle architectural changes were associated to force decrease (fatigue) after the soccer simulation.

To assess measurements reliability, interclass correlation (ICC), minimal detectable change (MDC) and typical error (TE) were calculated. Comparison of two images from each contraction level at PRE, were used to calculate intra-condition reliability. Comparison of 2 images for each contraction intensity, for each condition (2 images PRE-and 2 images post POST) were used to calculate inter-condition reliability. ICC's and TE were obtained from an excel spreadsheet developed by Hopkins 2015) and the MDC was calculated by the formula $TE \times 1.96 \times \sqrt{2}$ as used in a previous study (Opar et al. 2013).

5 RESULTS

From the 12 soccer players who volunteered to participate in this study, 9 were analysed. From the 3 players who dropped out, 1 sustained an injury during the fatigue protocol and 2 had poor data from the ultrasound measurements.

After the fatigue protocol, all the subjects showed a significant ($p=0.08$) reduction in isometric force production from PRE ($286.5 \text{ N} \pm 152$) to POST ($253.1 \text{ N} \pm 121.9$). On average the MVIC decreased 9%, however, a large variability was observed (force decrease ranged from 2% to 30%).

5.1 Reliability

The ICC's, %TE and MDC for the FL, PA, MT and FL_{norm} are shown in the TABLE 3.

To assess muscle architecture reliability, the criteria were similar to the ones used by Timmins et al. (2014): Reliability was considered 1) high, moderate or poor based on ICC > 0.90, 0.80 - 0.89, <80, respectively; 2) high or low if %TE ≤ 10 or $>10\%$, respectively.. As shown in the TABLE 1, for all the conditions, FL showed moderate to high reliability, while PA, MT and FL_{norm} showed poor to moderate reliability.

5.2 Effects of fatigue alone on BF muscle architecture

A comparison between different muscle regions showed significant differences in some muscle architecture parameters at distal and middle muscle regions (FIGURE 4). Fascicle length at distal region ($p=0.029$, $\eta^2 = 0.04$, $\eta^2_p = 0.58$) and PA at middle region ($p=0.032$, $\eta^2 = 0.13$, $\eta^2_p = 0.70$) were the only parameters that were significantly affected by the fatigue protocol. Thus, FL was significantly decreased after fatigue for distal but not for middle regions. Inversely, PA was decreased significantly from pre- to post-fatigue at middle regions

but not distal (FIGURE 4 and TABLE 4 and 5). Regarding MT and FL_{norm}, no significant changes were observed for any of the muscle regions.

TABLE 3. Reliability data for the muscle architecture measurements along different contraction intensities. The * represents the conditions that were reliable. ICC = intraclass correlation coefficient; %TE = percentage of typical error; MDC = minimal detectable change;

		ICC	%TE	MDC
FL (mm)	0% *	0.82	8.6	11.2
	25% *	0.86	8.4	13.4
	50% *	0.93	7.2	4.3
	75% *	0.86	6.7	11.3
PA (°)	0%	0.24	12.2	7.0
	25%	0.46	12.0	4.6
	50%	0.67	9.5	5.3
	75%	0.18	11.9	7.6
MT (mm)	0%	0.51	8.1	3.7
	25%	0.11	11.8	19.2
	50%	0.69	9.6	3.7
	75%	0.64	5.7	2.0
FL_{norm} (mm)	0%	0.26	10.1	1.1
	25% *	0.75	9.5	0.7
	50% *	0.88	7.4	0.5
	75%	0.55	6.7	0.7

5.3 Effects of contraction intensity alone on BF muscle architecture

Different changes in the muscle architecture parameters across contraction intensities were observed. FL was significantly decreased from rest to active conditions either at middle ($P < 0.01$, $\eta^2 = 0.16$, $\eta^2_p = 0.71$) and distal regions ($P < 0.01$, $\eta^2 = 0.23$, $\eta^2_p = 0.69$). In contrast,

PA was significantly increased under contraction (middle: $P < 0.01$, $\eta^2 = 0.21$, $\eta^2_p = 0.64$; distal: $P = 0.02$, $\eta^2 = 0.33$, $\eta^2_p = 0.47$). No differences were found across contraction intensities in MT (middle: $P = 0.21$, $\eta^2 = 0.17$, $\eta^2_p = 0.29$; distal: $P = 0.28$, $\eta^2 = 0.06$, $\eta^2_p = 0.19$).

5.4 Interaction effects between fatigue and contraction intensity on BF muscle architecture

Differences in the muscle architecture parameters between PRE and POST fatigue conditions are presented in TABLE 4 and 5, for middle and distal regions of the muscle, respectively. The interaction effect between fatigue and contraction intensity was calculated and no differences were found for any of the muscle architecture parameters ($P > 0.05$, TABLE 4 and 5). In other words, Effect size is considered low, medium or large be based on eta squared value of 0.01, 0.06 or 0.14, respectively (Lakens 2013). The effect size, calculated as eta squared, is showing medium interaction effect between fatigue and intensity of contraction, only for PA at distal region.

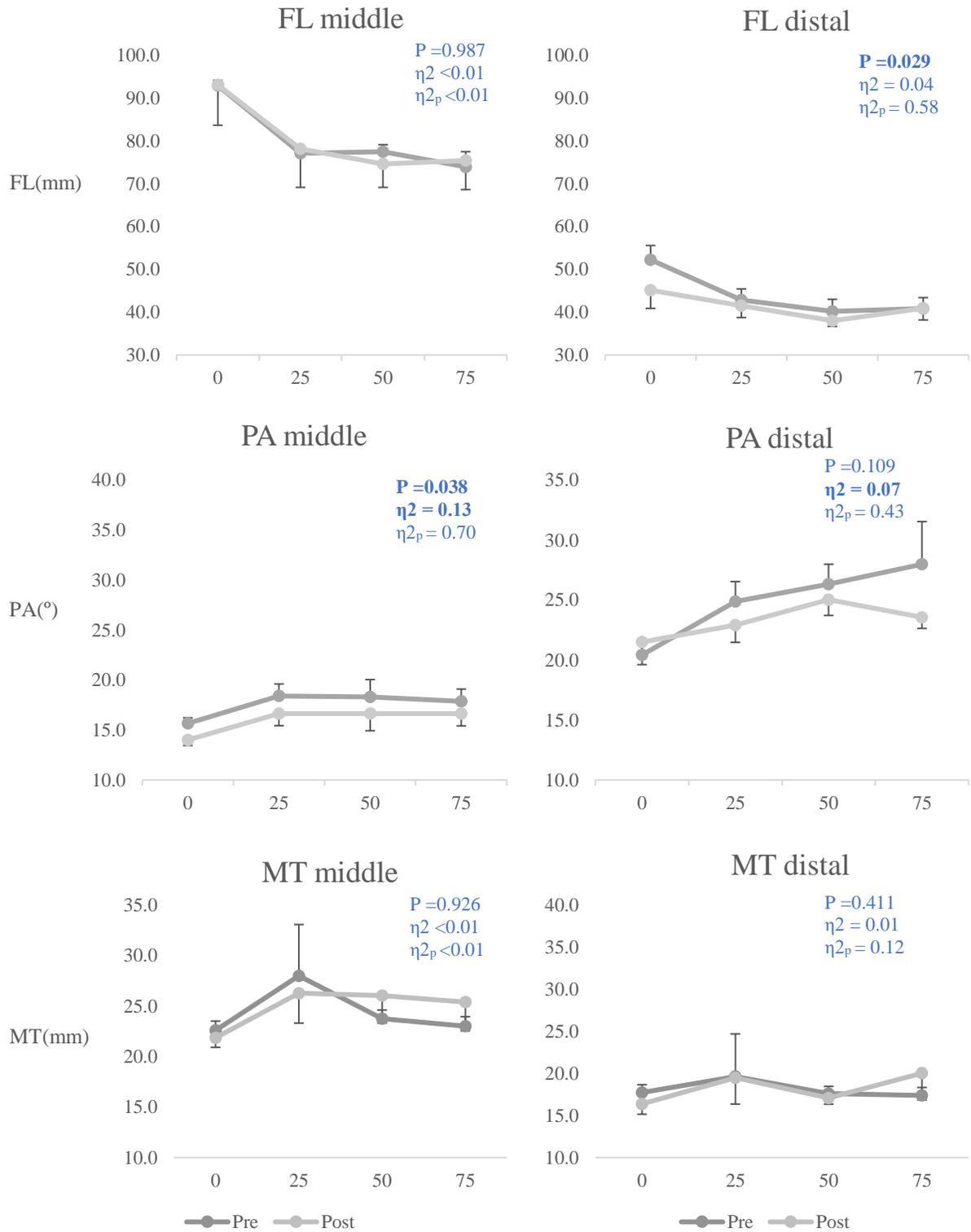


FIGURE 4. Comparison of fascicle length (FL), pennation angle (PA) and muscle thickness (MT) at different contraction intensities and muscle regions, pre-and post-fatigue protocol.

TABLE 4. Interaction effects between fatigue and contraction intensities for middle muscle architecture parameters. Absolute mean (Pre and Post) and standard deviation (SD) values together with absolute changes (and percentage) are presented. p-values based on two-way repeated measures ANOVA.

		Middle Region					
		Pre	Post	Change (%)	p-value	η^2	η^2_p
FL (mm)	0	92.9 ± 8.8	93.1 ± 9.4	0.2 (0.2)	0.62	<0.01	0.11
	25	77.5 ± 8.5	78.1 ± 9.0	1.0 (1.3)			
	50	77.4 ± 7.0	74.6 ± 5.5	-2.8 (-3.7)			
	75	73.9 ± 5.8	75.4 ± 6.8	1.5 (2.0)			
PA (°)	0	15.7 ± 0.6	14.0 ± 1.6	-1.7 (-10.6)	0.99	0.01	<0.01
	25	18.4 ± 1.2	16.6 ± 1.3	-1.8 (-9.6)			
	50	18.3 ± 1.7	16.6 ± 0.8	-1.7 (-9.2)			
	75	17.9 ± 1.2	16.6 ± 0.8	-1.2 (-6.8)			
MT	0	22.6 ± 0.9	21.9 ± 0.9	-0.7 (-3.2)	0.60	0.09	0.05
	25	28.0 ± 5.1	26.3 ± 3.0	-1.7 (-6.1)			
	50	23.8 ± 0.9	26.1 ± 2.7	2.3 (9.7)			
	75	23.0 ± 0.9	25.4 ± 2.8	2.4 (10.4)			
FL_{Norm}	0	4.1 ± 0.3	4.2 ± 0.3	0.1 (2.8)	0.68	0.01	0.09
	25	3.3 ± 0.3	3.3 ± 0.3	0 (0)			
	50	3.3 ± 0.3	3.1 ± 0.2	0.2 (-5.1)			
	75	3.2 ± 0.2	3.2 ± 0.3	0 (0)			

TABLE 5. Interaction effects between fatigue and contraction intensities for distal muscle architecture parameters. Absolute mean (Pre and Post) and standard deviation (SD) values together with absolute changes (and percentage) are presented. p-values based on two-way repeated measures ANOVA.

		Distal Region			p-value	η^2	η^2_p
		Pre	Post	Change (%)			
FL (mm)	0	52.2 ± 3.3	45.1 ± 4.2	-7.1 (-13.6)	0.18	0.04	0.23
	25	42.8 ± 2.6	41.5 ± 2.8	-1.3 (-3.1)			
	50	40.2 ± 2.8	38.0 ± 1.3	-2.2 (-5.4)			
	75	40.9 ± 2.5	40.9 ± 2.7	0 (0)			
PA (°)	0	20.4 ± 1.2	21.5 ± 1.9	1.1 (5.3)	0.38	0.07	0.18
	25	24.9 ± 1.7	22.9 ± 1.4	-2.0 (-7.8)			
	50	26.3 ± 1.7	25.0 ± 1.3	-1.3 (-4.9)			
	75	28.0 ± 3.6	23.6 ± 0.9	-4.4 (-15.8)			
MT	0	17.7 ± 1.1	16.4 ± 1.2	-1.4 (-7.6)	0.35	0.04	0.16
	25	19.6 ± 1.8	19.5 ± 3.1	-0.1 (-0.7)			
	50	17.6 ± 0.9	17.1 ± 0.7	-0.5 (-2.9)			
	75	17.4 ± 1.0	20.0 ± 3.2	2.6 (15.2)			
FL_{Norm}	0	3.0 ± 0.1	2.8 ± 0.2	-0.2 (-7.7)	0.55	0.04	0.11
	25	2.3 ± 0.2	2.4 ± 0.2	0.1 (3.0)			
	50	2.3 ± 0.1	2.2 ± 0.1	0.1 (-1.9)			
	75	2.3 ± 0.1	2.3 ± 0.1	0 (0)			

5.5 Correlations between force decrease and muscle architecture of the BF

Correlations between decrease of force production and muscle architecture changes in the BF muscle after the soccer simulation were calculated and are represented in the TABLE 6. The guidelines presented by Mukaka (2012) were used to interpret the results. Correlations were considered negligible ($r=0.00-0.30$), low ($r=0.30-0.50$), moderate ($r=0.50-0.70$), high ($r=0.70-0.90$) or very high ($r=0.90-1.00$). In general, poor to moderate correlations were found between decrease in force and architectural changes after the simulated match (TABLE 6). The MA parameter that better correlates with force decreases were MT at 75% MVIC ($r=0.91$). All the parameters (FL, PA, MT and FL_{norm}) at 25% MVIC showed moderate to high correlation to force decrease ($r=0.66$ to 0.86). with the exception of FL and PA in middle region ($r= -0.33$ and 0.29 , respectively).

TABLE 6. Correlation between muscle architecture variables (first column) and force decrease after a fatigue protocol. Pearson's correlation coefficients (r) is shown and was interpreted based on the guidelines from Mukaka (2012). *represents moderate correlation (± 0.50 to ± 0.70); ** represents high correlation (± 0.70 to ± 0.90); *** represents very high correlation (± 0.90 to ± 1.00)

	0	25	50	75
FL middle	-0.27	-0.33	-0.52*	-0.52*
FL distal	0.27	-0.71**	0.14	-0.30
PA middle	-0.59*	0.29	0.20	0.15
PA distal	-0.25	0.80**	0.18	0.07
MT middle	-0.19	0.86**	0.55*	0.91***
MT distal	-0.20	0.81**	0.08	0.91***
FL_{norm} middle	-0.27	-0.66*	-0.57*	-0.67*
FL_{norm} distal	0.35	-0.69*	-0.05	-0.60*

6 DISCUSSION

To my best knowledge, this was the first study evaluating the effects of a simulated soccer match on muscle architecture. As main finding from this study, the results show regional differences in the BF muscle architecture adaptations to fatigue. Furthermore, the results show that, despite significant decrease in FL and PA at distal and middle regions, respectively, these changes were not relevant due to the large MDC found for these parameters.

6.1 Effects soccer simulation on BF muscle architecture

The data from this study shows that SAFT was effective simulate the muscular demands of a soccer match. Similar reductions in force production were previously found after 45 minutes of a simulated soccer match (Marshall et al. 2014; Goodall et al. 2016), however, when compared to a 90-minute match, the degree of force reduction was lower in the present study (17% vs 9%) (Wollin et al. 2016). Regarding fatigue-induced changes in muscle architecture, the soccer simulation resulted in decreased FL at distal (-7.1 mm/13.6%) and PA at middle regions (-1.8°/9.6%). However, the reliability data suggests that these changes were smaller than the calculated MDC for each variable (FL=13.4 mm; PA = 7.6°) showing this way that the significant changes were not relevant. This way, these results show a slight but not pertinent decrease in FL and PA which is in agreement with previous literature showing no muscle architecture changes after a snowboard race (Vernillo et al. 2017), high intensity resistance training (Storey et al. 2012) or during initial stages of isometric induced fatigue (Mitsukawa et al. 2009). Some conflicting results have, however, been found after exhaustive exercise performances, where FL has shown to, either decrease (Ishikawa et al. 2006) or increase (Mitsukawa et al. 2009; Thomas et al. 2015), depending on the research protocol. Cronin et al. (2013) also showed differences in muscle architecture changes that after walking 60 minutes on a treadmill, however, FL increased 6% in the MG but no changes were found in the soleus muscle. Therefore, it seems clear from the literature that, when using protocols where the aim was to exercise until exhaustion or muscle failure (Ishikawa et al. 2006; Mitsukawa et al. 2009; Thomas et al. 2015; Brancaccio et al. 2008), muscle

architecture seems to acutely adapt. However, if a sport's specific or submaximal fatiguing protocol is performed instead (Vernillo et al. 2017, Mitsukawa et al. 2009), as in the present experiment, literature seems to suggest that muscle architecture remains unaltered.

One possible explanation may be the fact that 45 minutes of a soccer match are not yet enough to induce changes in muscle architecture. According to Mitsukawa et al. (2009), during initial observations of fatigue, the force decrease is not accompanied by changes in the muscle architecture while, when force reduces further, FL alterations may correlate with force impairments. This is in line with the results of this study where most of the MA parameters do not correlate with force reductions but some do. This possibly indicates that after 45 minutes of a soccer match, changes in FL and PA are starting but they are too small to be detected with EFOV technique. Not only could the exercise protocol be insufficient to alter muscle architecture but also the subjects could have recovered from the exercise. Csapo et al. (2011) found a FL and PA recovery to baseline levels 5 and 15 minutes, respectively, after exhaustive leg press exercise. In this situation, the measurements were completed in about 10-15 for all subject which also may explain the tendency but not significant decrease in FL and PA.

Furthermore, a large variability in the data may be explain why no overall changes were observed. The decrease of force production (2% to 30%), changes in FL (-23% to 32%) and PA (-45% to 17%) were shown to have large variability probably due to the heterogeneity of the athletes' physical fitness, as one can observe by the differences in the initial maximal isometric force from all the subjects (ranging from 165.1 N to 568.7 N). This shows that some players were better trained than others and, according to Storey et al. (2012), this effects the degree of fatigue and muscle architectural changes.

Other justification may be that, architectural changes resultant from a single task, do not affect every muscle in the same way (Mitsukawa et al. 2009; Cronin et al. 2013), which explains for the confounding findings between the present research and previous studies that assessed other muscle groups. As shown by Schuermans et al. (2014), the ST muscle has higher metabolic activity after exhaustive knee flexions which indicates that, similarly to what happens in the triceps surae (Cronin et al. 2013), the BF muscle architecture may not

be affected by the onset of fatigue but other hamstring muscles may be. Supporting this idea of load distribution between the hamstring muscles, Chumanov et al. (2011) showed, using biomechanical models, that SM muscle performs more negative work during running than the BF or the ST. In this study, the absence of muscle architecture changes for the BF implies that its force may have remained similar to the pre-match situation (Mitsukawa et al. 2011) being the reductions in force production likely related to reductions in ST or SM muscle function. For this reason, further research should focus also on the architectural changes of other hamstring muscles after fatigue.

6.2 Regional differences in BF muscle architecture

As expected and corroborating previous literature (Bennett et al. 2014; Kellis et al. 2010), regional MA differences were found at pre and post fatigue. The results suggest longer FL and lower PA at the middle region of the muscle when compared to the distal region. Muscle thickness and FL_{norm} were also greater at middle region. The FL for values middle regions found in this study (74mm to 93mm) are in line with the results from previous studies (63mm to 83mm) but slightly shorter in the distal region (41mm to 53mm vs 63mm to 70mm) (Kellis et al. 2010; Tosovic et al. 2016). Bennet et al. (2014) did not present the values from the measured fascicle lengths, still, from the graphs presented in the paper it is possible to estimate that the measured FL was between 90mm (distal/middle region) and 130mm (proximal/middle region). This equally seems to be similar to the results found in the present study.

Regarding the effects of fatigue on regional differences in muscle architecture, despite no relevant changes (as explained in the previous sub-section), there was a trend towards a decrease in FL and PA at distal and middle muscle regions, respectively. This is the first study to investigate regional muscle architecture at different regions after a fatigue protocol. The results of a smaller decrease in FL at distal regions seem to adjust to previous literature that indicates that shorter fascicles have previously been associated with greater risk of injury (Timmins et al. 2015) and more injuries occur at the distal region of the muscle (Ekstrand et al. 2016a). In future research, these regional differences could be addressed by comparing

different scanning methods or by comparing dynamic to isometric testing to assess whether any of these techniques is more sensitive than others to detect regional architectural differences in the BF.

6.3 Correlations between fatigue and muscle architecture

In this study, for muscle architecture seems to poorly correlate to force reductions which has previously been found in other studies (Storey et al. 2012; Mitsukawa et al. 2009). This lack of associations may indicate that either BF is not affected and other muscles were fatigued (Schuermans et al. 2014), resulting in the decrease in the overall knee flexion force production, or the other parameters of the BF were affected such as MTU stiffness (Marshall et al. 2015; Ishikawa et al. 2006) or neural input drive (Marshall et al. 2014; Goodall et al. 2017).

6.4 Ultrasound to measure BF muscle architecture

The secondary aim of the present study was to test the reliability of EFOV ultrasound technique to observe changes in the BF muscle architecture. The current data confirms the hypothesis that FL (ICC= 0.82-0.93%) measures are more reliable than PA (ICC= 0.18 -0.67) and MT (0.11-0.69). On the other hand, the hypotheses of increased FL, larger changes in the muscle architecture at middle regions and at higher contraction intensities were rejected owing to the large MDC found during the reliability analysis. This measure is of extreme importance when researchers aim to assess the practical and see true significance of their results. As it is clearly shown in this study, the fact that significant changes are found (i.e. FL at distal and PA at middle regions), does not necessarily imply that the results valuable.

The reliability measures are in line with previous research analysing different regions of the muscle (Bennett et al. 2014; Kellis et al. 2010; Tosovic et al. 2016) while they are lower than the ones obtained using a single muscle site (Timmins et al. 2014). This might indicate that, even though a we can observe the total fascicle length when using EFOV imaging, the image resolution may be compromised possibly creating larger errors during the analysis. Timmins et al. (2014) estimated FL based on a formula developed for vastus lateralis muscle

(Blazevich et al. 2006). When FL between the two studies is compared, it is observed that these calculations might overestimate the true FL, when compared to direct measures (Kellis et al. 2010; Tosovic et al. 2016). This has been shown before by Reeves and Narici (2003) comparing directly tibialis anterior's FL with estimated mathematically. In this case, the authors found 2.4% increased FL when estimated, compared to directly measured. However, tibialis anterior seems to have shorter fascicles than BF (80mm vs 90mm), thus, the over estimation of FL when measuring BF could be much higher.

The main strength of this study compared to previous literature was the use of EFOV imaging to scan the muscle and, with this, the direct determination of FL of the BF instead of extrapolating it. Additionally, a standardized soccer specific protocol was used in order to apply a similar stimulus to all the subjects. In further research projects, it is definitely advised to compare both (static and EFOV) techniques to have a more detailed perspective on the differences between methods. Also, comparing a soccer match play to a simulation should be considered to assess if there are mechanical adaptations that are specific from a match play that cannot be reproduced during a match such as opposition or complex decision making.

6.5 Relationship between muscle injury and fascicle length changes

As suggested by Timmins et al. (2014), shorter BF fascicles result in increased risk of HSI. Chumanov et al (2011) also identified increased eccentric load during high speed running as mechanism for muscle injury, especially under fatigue situations where knee and hip range of motion seems to be affected by fatigue (Small et al. 2009). Moreover, Espinosa et al. (2015) suggested that a training protocol more focused on eccentric contractions reduces the risk of injury. The authors suggested that this “protective adaptation” was not by related to an increase in muscle strength but to neural and mechanical changes in the neuromuscular system. Thus, one would expect a decrease in the FL at late stages of a match as a possible explanation to increased HSI risk as fatigue sets in.

However, the current study's results show no relevant changes in the BF fascicle length after a soccer simulation. Thus, one can speculate that BF muscle architectural changes after an

acute soccer match might not be a mechanism for increased injury rates. However, other mechanical properties such as increased stiffness (Marshall et al. 2015), decrease in the angle of PT (Cohen et al. 2014), increases in the muscle electromechanically delay (Conchola et al. 2015) or changes in the aponeurosis and tendon properties (Fiorentino & Blemker 2014) can be suggested as mechanisms for increased HSI rates.

It would be relevant for future research to focus also on other MTU properties and to follow-up mechanical BF changes, after several matches or a season period, since accumulated fatigue has shown to be a risk factor for strain injuries (Bengtsson et al. 2013).

6.6 Limitations

The main limitation of this study is the small sample size ($n=9$) which can be observed by the low effect sizes (η^2) presented (Lakens, 2013). Despite having a small sample size, it was similar other studies where effects of fatigue on muscle function was compared (Cohen et al. 2014; Ishikawa et al. 2006; Marshall et al. 2014).

7 CONCLUSION

A trend was found towards decreases in FL at distal and a decrease in PA at middle muscle regions of the biceps femoris muscle after 45 minutes of soccer match simulation. However, the methods were not sensitive enough to find relevant differences, i.e. the minimal detectable change associated with the used technique was greater than the degree of change in FL and PA.

The use of EFOV ultrasound technique was shown to be highly reliable to measure FL but poor to measure PA and MT. This way it is a valid method to measure directly FL of the BF in future research.

The findings of this study suggest that, in amateur level soccer players, 45 minutes of a simulated soccer match result in no muscle architectural changes in the BF muscle. Consequently, these changes may not be a mechanism to explain increased HSI rates after 45 minutes of a soccer match.

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