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Is the dimension of the proximal aponeurosis of biceps femoris long head a risk factor for a strain injury?

Dissertação elaborada com vista à obtenção
do Grau de Mestre em Treino de Alto Rendimento

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À minha mãe, pelo apoio incondicional diário,

nesta longa jornada.

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LIST OF ABBREVIATIONS

BF – Biceps femoris

BFlh – Biceps femoris long head

BFlhApo – Biceps femoris long head proximal aponeurosis

FIFA – Fédération Internationale de Football Association

HSI – Hamstring strain injury

MRI – Magnetic resonance imaging

MTJ – Musculotendon unit joint

SM – Semimembranosus

ST – Semitendinosus

UEFA – Union of European Football Associations

Título: Será a dimensão da aponevrose proximal da longa porção do bicípite femoral um fator de risco de rotura?

Resumo

Objetivo: Determinar: i) a fiabilidade de um método de rastreamento semiautomático para quantificar o tamanho da BFlhApo (aponevrose proximal da longa porção do bicípite femoral) com base em imagens de ressonância magnética; e, ii) examinar se o tamanho da BFlhApo (i.e. área de interface, largura média, volume e comprimento) de futebolistas de elite com história de lesão da BFlh (longa porção do bicípite femoral) difere em comparação com um grupo de controlo, sem historial de lesão da BFlh.

Método: Quarenta sujeitos realizaram ressonância magnética em ambas as coxas (31 sem história de lesão da BFlh e 9 com história de lesão da BFlh) durante o período de pré-época. Para medir com mais precisão o tamanho da BFlhApo, um método de rastreamento semiautomático foi desenvolvido e testado (fiabilidade intra e inter examinador). Comparações do tamanho da BFlhApo entre as coxas com história de lesão da BFlh (grupo experimental) e coxas não lesionadas (do grupo controlo) foram estabelecidas, bem como dentro dos grupos (lesionado vs. não lesionado; esquerdo vs. direito).

Resultados: A análise da fiabilidade do método de rastreamento semi-automatizado mostrou uma boa fiabilidade intra examinador e interobservador (ICC entre 0,75 e 0,9, com intervalo de confiança de 95%). Não foram encontradas diferenças estatisticamente significativas (P <0,05) no tamanho da BFlhApo em relação a todas as comparações estabelecidas.

Conclusões: Um procedimento de medição fiável foi capaz de quantificar melhor as dimensões da BFlhApo entre indivíduos com e sem histórico de lesão da BFlh. Indivíduos com histórico de lesão da BFlh sugerem não apresentar diferenças significativas nas dimensões da sua BFlhApo relativamente a indivíduos sem este histórico. Assim, parece desajustado afirmar que uma menor dimensão da BFlhApo seja fator de risco independente para desenvolver uma lesão da BFlh.

Palavras chave: junção miotendinosa; ressonância magnética; lesão dos isquiotibiais; matlab; fator de risco; futebol; área de interface; largura média; volume; comprimento.

TITLE: Is the dimension of the proximal aponeurosis of biceps femoris long head a risk factor for a strain injury?

Abstract

Purpose: To determine i) the reliability of a semi-automated tracking method to quantify the BFlhApo (biceps femoris long head proximal aponeurosis) size (i.e area interface, average width, volume, and length) based on MRI (magnetic resonance imaging) data; and, ii) to examine if the BFlhApo size of elite footballers with history of BFlh (biceps femoris long head) injury differed compared to matched controls without a previous BFlh injury.

Methods: Forty individuals performed a MRI in both thighs (31 with no BFlh strain history and 9 with a BFlh strain history) during the preseason period. To measure more precisely the BFlhApo size, a semi-automated tracking method was built and tested (intra- and inter-rater reliability). Comparisons of the BFlhApo size between thighs with history of BFlh injury (experimental group) and non-injured thighs (control group) were set, as well as in between groups (injured vs non injured; left vs right).

Results: The analysis of the reliability using the semi-automated tracking method showed a good intra-rater and inter-rater reliability (ICC between 0.75 and 0.9, at 95% confidence interval). No statistically significant differences ($P < 0.05$) were found in the BFlhApo size regarding to all thighs comparisons.

Conclusions: A reliable measurement procedure was able to better quantify BFlhApo dimensions between individuals with and without history of BFlh injury. Individuals with history of BFlh injury suggest no significant differences in their BFlhApo dimensions compared to individuals without this history. Thus, it seems inappropriate to state that a smaller BFlhApo size is an independent risk factor for developing a BFlh injury.

Key words: myotendinous junction; magnetic resonance imaging; hamstring injury; matlab; risk factor; soccer; interface area; average width; volume; length.

CHAPTER I – INTRODUCTION

1.1. State of Art

Hamstring strain injuries (HSI) in elite football (i.e soccer) are still a continuing issue, since their rates have remained unaltered over the years, and even slightly increased (Jan Ekstrand, Waldén, & Hägglund, 2016). To date, they are the most prevalent time loss injury in football, involving negative impact on the player, team performance and club finance (Jan Ekstrand, 2013; Martin Hägglund, Waldén, Magnusson, et al., 2013). Given these implications, a significant body of research has emerged in recent years in an attempt to identify risk factors and thereby, develop proper prevention and rehabilitation strategies (Buckthorpe et al., 2018). Despite of some well described, there is not a clear understanding of why HSIs happen, since appears they do not operate in isolation, but instead as a complex web of determinants (Bittencourt et al., 2016). Among all hamstrings, the BFlh (biceps femoris long head), and particularly his proximal muscle–tendon unit (MTU) (aponeurosis), is the most frequent injury location, so that research has arisen to study whether or not this structure morphology and behavior, sustains a risk factor (De Smet & Best, 2000; Evangelidis, Massey, Pain, & Folland, 2015a; Fiorentino, Epstein, & Blemker, 2012a; Koulouris & Connell, 2003; Malliaropoulos et al., 2010; Rehorn & Blemker, 2010a; Slavotinek, Verrall, & Fon, 2002). Primary findings based on magnetic resonance imaging (MRI) and computational modelling suggested that a disproportionately small BFlh proximal aponeurosis (BFlhApo) (i.e. muscle fiber attachment site), may be a potential risk factor for strain injury, since peak muscle fiber strains appear to be higher within this region, especially during active lengthening (Fiorentino & Blemker, 2014a; Fiorentino et al., 2012a; Rehorn & Blemker, 2010b). Later, in addition to aponeurosis width measurements, Evagenlidis et al. quantified the interface area between the muscle and aponeurosis to better analyze the concentration of mechanical strain at this interface, as well as to establish a relationship with muscle size (i.e. maximal anatomical cross-sectional area and volume) and knee flexor strength (isometric and eccentric) (Evangelidis et al., 2015a). They found that BFlhApo interface was highly variable between individuals, and it was not related to BFlh volume or knee flexor maximal strength. These data supported the hypothesis that a relatively small BFlhApo could be subject to greater mechanical strain in the muscle tissue surrounding the aponeurosis and lastly predispose them to a BFlh injury (Evangelidis, Massey, Pain, & Folland, 2015b). However, all this authors have followed the same (or equivalent) BFlhApo measurement method, who carried some limitations, since very subjective criteria with no inter-rater agreement (i.e. very low ICC) were used to perform it (Evangelidis et al., 2015b; Fiorentino et al., 2012a; Handsfield, Fiorentino, & Blemker, 2010a) . Beyond this major limitation, which do not allow for any valid comparison between subjects, none of this evidence tested the BFlhApo dimensions between athletes with BFlh injury history from those without

1.2 Study aim and hypothesis

Thereby, the aim of the present study was: i) to determine the intra- and inter-rater reliability of a new and objective semi-automated tracking method to quantify the BFlhApo dimensions (i.e area interface, average width, volume, and length) based on MRI data; and, ii) to examine if the BFlhApo size of elite footballers with history of previous BFlh injury differed compared to matched controls without a previous BFlh injury. We hypothesized that: i) a semi-automated tracking method would be a reliable option to measure the BFlhApo dimensions (i.e area interface, average width, volume, and length) and; ii) athletes with previous BFlh injury would present smaller BFlhApo dimensions compared to their matched controls.

CHAPTER II – STATE OF ART

2.1. Football Injuries

2.1.1. Epidemiology

To date, injuries in football represent a continuing problem across all genders, age groups, and performance levels (Klein, Henke, & Platen, 2018). Over professional football, this is major concern once overall risk of injury is about 1,000 times higher compared with industrial occupations, generally looked as high risk (J. Ekstrand, 2008). Bearing this is mind, knowledge regarding which injuries are the main priority according to injury risk, including its incidence[1], severity[2], and burden[3], as well as how they occur, enables the identification of promising prevention areas (e. g. training, rehabilitation or politics), important stakeholders (e. g. trainers, physicians, referees, and sport politicians) (Jan Ekstrand et al., 2018) and development of relevant content for detailed preventive measures (Klein et al., 2018).

The governing bodies of football, Fédération Internationale de Football Association (FIFA) and the Union of European Football Associations (UEFA), have expressed their concerns regarding this topic, especially because of the high demands that adult professional football impose (J. Ekstrand, 2008; J. Ekstrand, Hägglund, & Waldén, 2011). Since 2001, Ekstrand and his colleagues have implemented an injury survey among Champions League clubs, with the aim of reducing injuries (Jan Ekstrand, 2013). Over the last 16 years, the UEFA Champions League Injury Study has included close to 50 teams from 18 different countries that have participated at some point during these seasons (Jan Ekstrand, Hägglund, Kristenson, Magnusson, & Waldén, 2013; Jan Ekstrand et al., 2016; Martin Hägglund, Waldén, Magnusson, et al.,

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[1] Number of injuries per 1000 hours of player exposure [Σ injuries/Σ exposure hours) x 1000]

[2] Number of days lost from the date of injury to the date of the player’s return to full participation.

[3] Number of injury days lost per 1000 hours of exposure (i.e. the cross-product of severity and incidence).

2013; Waldén, Hägglund, & Ekstrand, 2013). Using standardized forms with all participating clubs, training and match injury data, individual player exposure (in minutes) and attendance reports have been collected every day and sent to the study group. Besides of the periodically feedback sent to the teams to help them reviewing his performance, this study group has been systematically publishing in the scientific literature (Bahr, Clarsen, & Ekstrand, 2018; J. Ekstrand, 2008; Jan Ekstrand et al., 2013, 2018, 2016; J. Ekstrand et al., 2011; Martin Hägglund, Waldén, Magnusson, et al., 2013; Waldén et al., 2013). To date, a professional football team can expect near 40 injuries that cause time-loss from play each season, which equates at least one injury per player per season (seasons 2016/2017) (Jan Ekstrand, 2017). Researchers have reported a match injury incidence average of 19.8 per 1000 hours of exposure, and training injury average of 2.3 per 1000 hours of exposure, with individual rates ranging from 7.1 to 37.6, and 0.1 to 4.2, respectively (Jan Ekstrand, 2017). Overall, on average, it is expected that 12% of the squad is unavailable to train or play due to injury at any point during sports season calendar (Jan Ekstrand, 2017).

Regarding the injured body location, injuries of the lower limbs are still the main problem in elite football (87%), especially knee, ankle and thigh muscle injuries (J. Ekstrand et al., 2011; Klein et al., 2018). Thigh strains are the single most common, representing 17% of all injuries, with hamstrings, by far more common than quadriceps strains (J. Ekstrand et al., 2011). In fact, hamstring injuries are itself the most common injury in male football, with a substantial severity associated (see figure 1, to a wider comprehension of the relationship between the severity and incidence of most frequently reported injury types in UEFA Champions League) (Bahr et al., 2018; Jan Ekstrand, 2017; J. Ekstrand et al., 2011). To date, this type of injury represents about 12% of all football related injuries, of which up to 30% reoccur within the same season after return to play (Jan Ekstrand, Hägglund, & Waldén, 2011; J. Ekstrand et al., 2011)

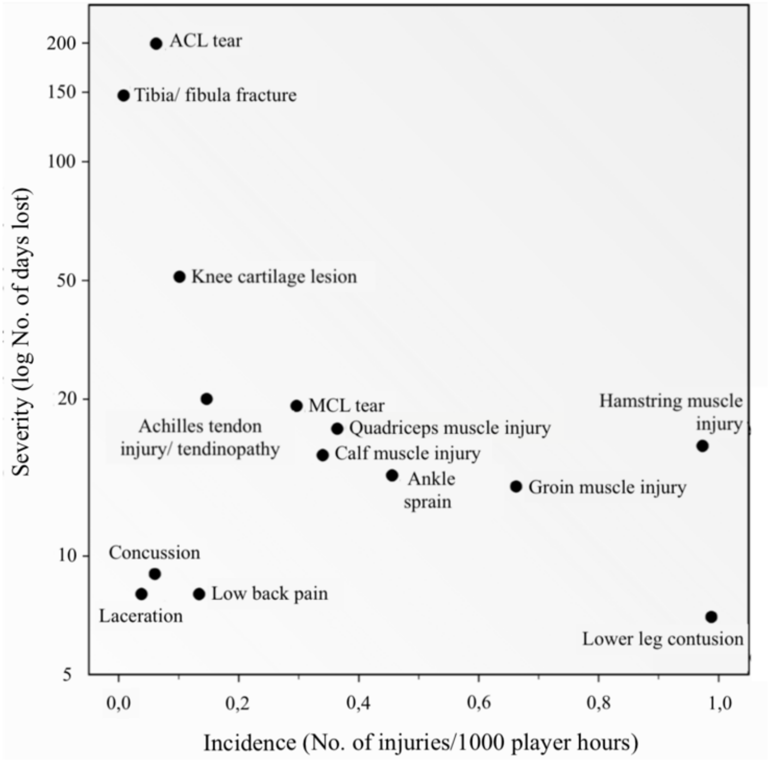

Figure 1. Incidence and severity for each of the 14 most common types of time-loss injuries in UEFA Champions League football, including hamstring strains (data from the UEFA Elite Club Injury Study). In this risk matrix, severity is expressed as the average number of days lost from training and competition (log scale), while incidence is shown as the number of injuries per 1000 hours of total exposure (match and training combined), for each injury type. Dots located in darker areas of the gray shade graph suggest a injury type with a greater burden, and a bigger priority should be given to his prevention. Adapted from (Bahr et al., 2018)

2.1.2. Sports and financial impact

Given that a team of 25 players can expect about 6 hamstring injuries per season, each with an absence from sports of 80 days and an average of 14 missed matches (Jan Ekstrand et al., 2011), this data evidences bothersome repercussions not only on the individual player, but just as much at team and club level (Bahr et al., 2018; Eirale, Tol, Farooq, Smiley, & Chalabi, 2013; Martin Hägglund, Waldén, Magnusson, et al., 2013). In fact, professional football teams with lower season injury rates, win more matches and have higher final league ranking in the European cups administered by UEFA (Martin Hägglund, Waldén, Magnusson, et al., 2013). This is particularly sensible for those teams with less injuries causing high burden, like hamstrings strains (Martin Hägglund, Waldén, Magnusson, et al., 2013). The hamstring injury epidemic manifests itself not only with aforementioned high incidence rates but as much at the youth, amateur and female divisions (Klein et al., 2018). More so, since 2001 hamstring injuries has remained high and even increased by 4% annually in men's european professional football (Jan Ekstrand et al., 2016). This increase only happened during training sessions, but there was not a significant increase during matches (Jan Ekstrand et al., 2016). Distinct opinions point for these high incessant (re)occurrence rate like:

1. insufficient load/preventive management, related to higher intensive and (un)protective training sessions as match preparation method (Bahr, Thorborg, & Ekstrand, 2015);
2. possible better clinical performance by cautiously removing the athlete from training for recovery, without affecting the availability to play during matches (Eirale, 2018);
3. incomplete rehabilitation/recovery times (de Visser, Reijman, Heijboer, & Bos, 2012; Opar, Williams, & Shield, 2012);
4. and, as previously mentioned, (*and more probably*) increasing demands in sports performance (Bradley et al., 2015).

Despite the efforts already done, research teams and clinical staff are still lacking enough evidence concerning the exact cause of the particular vulnerability of the hamstring within this athletic population, and how this should be addressed preferably. After all, due to this worrying epidemiological numbers and substantial amount of uncertainty involved, other problems like high expenses in health care and financial loss arise to this issue (Jan Ekstrand, 2013). Notwithstanding of the limited to publicly available information, Shakhtar Donetsk CEO affirms that the average cost of a first-team player being injured for 1 month is calculated to be around €500 000 (Jan Ekstrand, 2013). Similar concerns, from Australian Football League, indicate that each club, on average, loses the equivalent of one athlete's yearly salary (Hickey, Shield, Williams, & Opar, 2014). In other words, clubs are paying an average athlete's yearly salary for no on-field return for their investment, without even considering the medical expenses (like doctor consults, medical imaging, or rehabilitation costs) (Hickey et al., 2014). Directly to the players, being out for prolonged periods of time has a detrimental influence on performance, overall physical health and psychosocial wellbeing, so that hamstring injuries cannot be overlooked and the urgent need for better prevention is beyond dispute (Appaneal, Levine, Perna, & Roh, 2009; Verrall, Kalairajah, Slavotinek, & Spriggins, 2006)

2.2. Hamstring Strain Injuries

2.2.1. Site of injury

Among all thigh and hamstrings muscles, the BFlh is the most predominantly affected by structural or functional lesions in football (C. M. Askling, Tengvar, Saartok, & Thorstensson, 2007a; C. M. Askling, Tengvar, & Thorstensson, 2013; De Smet & Best, 2000; William E. Garrett, Ross Rich, Nikolaou, & Vogler, 1989; Hallén & Ekstrand, 2014; Koulouris & Connell, 2003; Malliaropoulos et al.. This is particularly shown by Hallén et al. (2014) who analysed, via MRI, the injury sites over 6 seasons of male professional football from the top european divisions (Hallén & Ekstrand, 2014). It was reported that BFlh was affected in 83% of the total hamstrings strains and the highest affected by re-injuries within this muscular group (Hallén & Ekstrand, 2014). Regarding to the second most injured muscle (and with a much lesser expression, <10%), distinct opinions point between the semitendinosus (ST) (C. M. Askling et al., 2007a; De Smet & Best, 2000; Slavotinek et al., 2002) and the semimembranosus (SM) (Hallén & Ekstrand, 2014; Koulouris & Connell, 2003; Malliaropoulos, Isinkaye, Tsitas, & Maffulli, 2011). This asymmetric injury occurrence, may probably rely on the type of activity that determined the muscle involved (C. Askling, 2006; C. M. Askling et al., 2013; C. Askling, Tengvar, Saartok, & Thorstensson, 2000). In fact, Askling et al. has proposed two distinctly different types of acute hamstring strains: 1) during high-speed running and mainly involving the BFlh (72% of the cases); 2) during movements leading to extensive lengthening of the hamstrings, primarily involving the free proximal tendon of SM, such as, high kicking, sliding tackle and sagittal split (occurring in 28% of the times) (C. M. Askling, Malliaropoulos, & Karlsson, 2011; C. M. Askling et al., 2007a; C. M. Askling, Tengvar, Saartok, & Thorstensson, 2007b; C. M. Askling et al., 2013). Nevertheless, previous studies do not present data to fully explain why these muscles are injured at different conditions (see section 2.2.2).

Deepening in the precise location where strains occur, several authors advocate muscle tissue adjacent to the myotendinous joint (proximal or distal) as the main site, based on both animal and human experiments (De Smet & Best, 2000; Fiorentino et al., 2012a; William E. Garrett et al., 1989; Koulouris & Connell, 2003; Malliaropoulos et al., 2010; Slavotinek et al., 2002; Tidball & Chan, 1989). Despite of the uncertainty why strain injuries take place near the myotendinous joint, it has been hypothesized that sarcomeres close to it are stiffer compared to central sarcomeres, so that less compliant to an applied force (Noonan & Garrett, 1992). Moreover, a larger muscle and/or narrower proximal myotendinous joint (aponeurosis) dimensions, may also concentrate a higher mechanical strain on the surrounding myotendinous joint tissue (Fiorentino & Blemker, 2014a) and therefore be a risk factor for sustain an injury (please see section 2.2.2). Recalling to the hazardous numbers of BFlh strains, research is in accordance with this injury location (C. M. Askling et al., 2007a; Fiorentino & Blemker, 2014a; Fiorentino, Epstein, & Blemker, 2012b).

2.2.2. Mechanism of hamstrings strain injury

Accordingly to Ekstrand et al. (Jan Ekstrand et al., 2012), non-contact events are the condition under which the hamstrings gets injured most often (95%), while contact occurrences only are reported in 5%, based on 23 european professional teams analysed between 2007 and 2011. The same study also shown that 70% of the times HSI occur during sprinting or high-speed running, followed by overuse, stretching/sliding movements (each 5%), shooting, twisting/turning actions (each 4%) or passing and jumping activities (each 2%) (Jan Ekstrand et al., 2012).

In correlation to this incidence, Barnes et al. investigated the evolution of physical and technical performance from seasons 2006/2007 to 2012/2013 in the English Premier League, and found a 30% increase in high-intensity running distance and actions, and a 35% increase in sprint distance and number of sprints over the years (Barnes, Archer, Hogg, Bush, & Bradley, 2014). They also found a significantly higher proportion of explosive sprints during the season 2012/ 2013 compared with 7 years earlier (Barnes et al., 2014). Consequently, an increase in the hamstring injury rate seems to be natural if such actions were increased during the play since, as previously referred by Ekstrand et al., 70% occurred during sprinting or high-speed running (Jan Ekstrand et al., 2012). To date, once each player from a team only has, on average, 1 minute (53.4±8.1s) of ball possession per match, it just highlights that running/sprinting constitutes the main player activity, involving the highest physical volume and intensity compared to the other previous mentioned activities (Carling, 2010). Moreover, based on 51 players of the Swedish first league, HSI have been analysed by with little difference between the dominant and the non-dominant leg, supporting the symmetric and cyclic nature of sprinting biomechanics as main task for football, compared to the remaining asymmetrical demands (J. Ekstrand et al., 2011; Svensson, Eckerman, Alricsson, Magounakis, & Werner, 2018; Woods et al., 2004).

Several studies have been performed on the biomechanics of running to better understand the concrete mechanism under which hamstrings develop strain injuries (Chumanov, Heiderscheit, & Thelen, 2011; Schache, Dorn, Blanch, Brown, & Pandy, 2012; Thelen, Chumanov, Best, Swanson, & Heiderscheit, 2005; Yu et al., 2008). During high speed running, hamstrings play a key role not only to propulse through explosive concentric contraction from mid stance to back swing (Yu et al., 2008), but also to quickly decelerate leg movement towards hip flexion and knee extension throughout front swing, by a forceful and crucial eccentric muscle effort (Chumanov et al., 2011; Schache et al., 2012; Thelen, Chumanov, Best, et al., 2005; Yu et al., 2008). Whilst the stance phase is a possible period of susceptibility to HSI (due to peak knee flexion and hip extension external moments that are generated by the ground reaction force) it involves much shorter hamstring lengths compared with terminal swing and thereby, it has been interpreted to have a lower risk (Chumanov, Heiderscheit, & Thelen, 2007; Picerno, 2017; Thelen, Chumanov, Best, et al., 2005; Thelen, Chumanov, Hoerth, et al., 2005; Yu et al., 2008). In fact, results of previous evidence with animal models suggested that muscle strain injuries are highly associated with eccentric contractions due to the magnitude of strain, and the higher the activation level of a muscle during eccentric contraction, the more mechanical energy the muscle absorbs prior to strain injury (W. E. Garrett Jr, Safran, Seaber, Glisson, & Ribbeck, 1987; Lieber & Fridén, 1993; Lovering, Hakim, Moorman, & De Deyne, 2005). Keeping this in mind, more recent sprinting biomechanics research has been clear: peak hamstring stretch and force occur in the late swing phase prior to foot contact, when the thigh starts to extend backward but the leg is still rotating forward due to motion-dependent torque (Chumanov et al., 2011; Schache et al., 2012). Liu et al. refers that in order to pull the leg backward and downward prior to ground contact, the hamstring muscles contract intensely, creating an acceleration that causes a quick eccentric to concentric change (Liu, Sun, Zhu, & Yu, 2017). The author reveals that the largest muscle torques occur at the end of the swing phase, almost simultaneously with the largest hip extension and knee flexion muscle torques (Liu et al., 2017). This muscle dependent torque is argued primarily to counterbalance the stretching effect of the motion dependent torque during the swing phase. Besides this, Liu et al. highlights that high load on the hamstrings is caused by the motion dependent torque, since the muscle dependent torque functions to counterbalance it, in order to control the rapid limb rotation during the swing phase (Liu et al., 2017). Accordingly, to the author, the major component of the motion dependent torque at both knee and hip is the motion-dependent torque due to the acceleration of the leg. These findings contribute to the hypothesis why the hamstrings are stretched to their maximum length and why the muscle force reaches its maximum value in the late swing phase, as observed by others (Chumanov et al., 2011; Schache et al., 2012; Thelen, Chumanov, Hoerth, et al., 2005). When comparing the various hamstrings, it has been found that BFlh experiences the greatest musculotendon strain with respect to upright stance (6,7,34,40) and develops the greatest peak musculotendon force and electromyographic activity just before ground contact (Chumanov et al., 2007, 2011; Schache et al., 2012; Schache, Dorn, Wrigley, Brown, & Pandy, 2013).

Nevertheless, assuming that excessive muscle strain in the late swing phase of sprint running is the direct, if not exclusive, cause of HSI might be daring since, other possible factors may contribute. Muscle strain injuries produced by eccentric actions in animal studies (including the aforementioned) are typically induced by strains beyond the optimal muscle fiber length, in a well-controlled, isolated contraction of an in situ muscle (W. E. Garrett Jr et al., 1987; Lieber & Fridén, 1993). On the other hand, hamstrings injuries in sprint running engage a more complex framework, where the maximum hamstring lengths are close to the muscle optimal length (Wan, Qu, Garrett, Liu, & Yu, 2017). Therefore, there may actually be no eccentric, but rather an isometric action of the hamstrings during the swing phase in high-speed running, witch rather than experiencing an eccentric action during every swing phase, the hamstrings may only sporadically experience an eccentric muscle action (Ruan, 2018). Indeed, a loss of coordinative control of the pelvic area may increase the distance between the attachment points and hence cause an eccentric muscle action, leaving the muscle vulnerable to injury (Ruan, 2018). Despite that muscle fibers may shorten while the muscle tendon unit is elongating, there are no experimental studies on fascicle lengths of the hamstring muscles in sprint running. The cited theoretical studies of Thelen et al. (Thelen, Chumanov, Hoerth, et al., 2005) and Chumanov and co-workers (Chumanov, Schache, Heiderscheit, & Thelen, 2012), suggesting a lengthening of the hamstrings in the swing phase, cannot be taken as direct evidence of hamstring fascicle lengthening because hamstring slack lengths are not known with certainty (Ruan, 2018). Studies investigating the muscle tendon unit behavior in vivo during sprint running, and studies focusing on injury mechanisms based on injury trials, are needed.

Nevertheless, if an acute and abnormal repeated exposure to frequent and intense eccentric loading bouts occur, hamstrings may develop microscopic lesions (i.e. micro tears) that cause the muscle-tendon tract to become less compliant and less stretch tolerant (C. L. Brockett, Morgan, & Proske, 2001; Schmitt, Tim, & McHugh, 2012; Thorborg, 2012). This decrease in muscle-tendon compliance is caused by an increase in connective tissue viscosity (embedded within the muscle fibers and tendon cells) and alterations in mechanical behavior of the muscle-tendon unit, caused by these changes in connective tissue characteristics. This entails that the connective tissue will allow less deformation/elongation within the muscle for the same amount of force/stress and muscle-tendon lengthening imposed on it and thus, will be prone to failure and structural damage prematurely. When not encountered and corrected for, these structural changes and microscopic lesions could ultimately lead to macroscopic strain injury (Chumanov et al., 2007, 2011, 2012; Ono, Higashihara, Shinohara, Hirose, & Fukubayashi, 2015).

To summarize, hamstrings muscles reach their peak activation during the late swing phase, with the BFlh experiencing the longest length of all, which is the place where the muscles most fail functionally and/or structurally. More research regarding this issue is needed by acknowledging detailed hamstring anatomy and the structural particularities of each one of these muscles, particularly the BFlh, since it will influence their function and injury risk.

2.2.3. Classification systems

Given the impact of HSI, prognostic information is crucial for medical teams to address questions from players, coaches and other important stakeholders regarding return to play. Muscle injuries can arise in several types, locations, severities and sizes, making the prognosis a challenge about healing times and rehabilitation. Thereby, a successful implementation of a muscle injury classification system is mandatory (Hamilton, Alonso, & Best, 2017). While a wide range of classification and grading systems have been validated, including the Munich consensus system, the British athletics system or the Barcelona system, limited evidence or consensus on how to either describe a specific muscle injury between them, or determine the prognosis of any given injury, as arised (Hamilton et al., 2017). Each of these systems has unique strengths, weaknesses, and ability to be incorporated into widespread use. However, the inconsistencies in approach to muscle injury description currently available, continue to thwart a universal approach to addressing muscle injury prognostication and management effectively (Hamilton et al., 2017). Bearing this in mind, Valle et al. has recently proposed a classification system capable of describing the injury, with useful clinical application, a quick learning curve, and the potential to provide prognostic value, solving all previous issues found in past systems (Valle et al., 2017). Although this classification was designed with the aim of being applied to any muscle group, it was initially described to the hamstring muscles. This evidence-informed and expert consensus-based classification system is based on a four-letter initialism system: MLG-R, respectively referring to the mechanism of injury (M), location of injury (L), grading of severity (G), and number of muscle re-injuries (R) (see table 1).

Table 1 Summary of the muscle classification system

Mechanism of injury (**M**)	Locations of injury (**L**)	Grading of severity (**G**)	No. of muscle re-injuries (**R**)
Hamstring direct injuries			
T (direct)	**P** Injury located in the proximal third of the muscle belly **M** Injury located in the middle third of the muscle belly **D** Injury located in the distal third of the muscle belly	0-3	0: 1st episode 1: 1st reinjury 2: 2nd reinjury …
Hamstring indirect injuries			
I (indirect) plus sub-index **s** for stretching type, or sub-index **p** for sprinting type	**P** Injury located in the proximal third of the muscle belly. The second letter is a sub-index p or d to describe the injury relation with the proximal or distal MTJ, respectively **M** Injury located in the middle third of the muscle belly, plus the corresponding sub-index **D** Injury located in the distal third of the muscle belly, plus the corresponding sub-index	0-3	0: 1st episode 1: 1st reinjury 2: 2nd reinjury …
Negative MRI injuries (location is pain related)			
N plus sub-index **s** for indirect injuries stretching type, or sub-index **p** for sprinting type	**N** p Proximal third injury **N** m Middle third injury **N** d Distal third injury	0-3	0: 1st episode 1: 1st reinjury 2: 2nd reinjury …

Grading of injury severity	
0	When codifying indirect injuries with clinical suspicion but negative MRI, a grade 0 injury is codified. In these cases, the second letter describes the pain locations in the muscle belly
1	Hyperintense muscle fiber edema without intramuscular hemorrhage or architectural distortion (fiber architecture and pennation angle preserved). Edema pattern: interstitial hyperintensity with feathery distribution on FSPD or T2 FSE? STIR images
2	Hyperintense muscle fiber and/or peritendon edema with minor muscle fiber architectural distortion (fiber blurring and/or pennation angle distortion) ± minor intermuscular hemorrhage, but no quantifiable gap between fibers. Edema pattern, same as for grade 1
3	Any quantifiable gap between fibers in craniocaudal or axial planes. Hyperintense focal defect with partial retraction of muscle fibers ± intermuscular hemorrhage. The gap between fibers at the injury's maximal area in an axial plane of the affected muscle belly should be documented. The exact % CSA should be documented as a sub-index to the grade
r	When codifying an intra-tendon injury or an injury affecting the MTJ or intramuscular tendon showing disruption/retraction or loss of tension exist (gap), a superscript (r) should be added to the grade

CSA cross-sectional area, *FSE* fast spin echo, *FSPD* fat saturated proton density, *MRI* magnetic resonance imaging, *MTJ* myotendinous junction, *STIR* short tau inversion recovery. Adapted from (Valle et al., 2017).

2.2.4. Risk factors

The analysis of the underpinning risk factors that lead to a hamstring injury is crucial, once it will give to the related football professionals the insights to decrease their rate. The HSI is multifactorial in nature and involve intrinsic as much as extrinsic factors. The intrinsic risk factors are those that relate to the individual/athlete (e.g. muscle strength and flexibility), while the extrinsic risk factors are related to the environment (e.g. the game conditions, other athletes, climate etc.). These factors are naturally blended since, for instance: progressive increases in chronic workload with high sprint training may provide protective adaptations from hamstrings strains, and therefore decrease the risk of injury (Malone, Hughes, Doran, Collins, & Gabbett, 2019; Malone, Roe, Doran, Gabbett, & Collins, 2017); but a limited/improper training and recovery time or a high demanding sports calendar with consecutive matches, may lead players to experience large and rapid increases in those distances (above their yearly session average) and thereby increase the odds of HSI (Duhig et al., 2016).

Thus, setting a single risk factor that prompt to injury can be daring, since an injury is the result of the accumulation of a number of risk factors in a complex combination with exposure to high risk conditions and lastly, an inciting event (Bittencourt et al., 2016; Buckthorpe et al., 2018).

Over this section it will be overviewed several of the main non-modifiable and modifiable risk factors proposed in the literature, including increasing age, previous injury, strength imbalances, flexibility or fatigue. Many other potential risk factors have been argued, yet the majority are lacking robust scientific evidence to support them (Buckthorpe et al., 2018). Finally, the anatomy of BFlh, including is aponeurosis as risk factor, will be explored taking into account the aim of this review and based on the available literature.

2.2.4.1. Non-Modifiable

2.2.4.1.1. Age

Age has been consistently been reported as an independent risk factor for HSI in football (Henderson, Barnes, & Portas, 2010). In fact, a football player older than 30 years can expect a 14-18 times greater risk than a 20-year old, with each year of age been reported to increase the risk by as much as 1.8-fold (OR; 95% CI: 1.2-2.7) (Arnason et al., 2004; Henderson et al., 2010). Importantly, all studies that report age as a significant risk factor have utilized regression or multivariate analysis to conclude that increasing age increases the risk of sustaining an HSI independently of confounding variables such as previous injury (Arnason et al., 2004; Belinda J. Gabbe, Bennell, & Finch, 2006; B. J. Gabbe, Bennell, Finch, Wajswelner, & Orchard, 2006; M. Hägglund, Waldén, & Ekstrand, 2006; Henderson et al., 2010; Orchard, 2001; Woods et al., 2004)

Despite of remaining unclear why older athletes are predisposed to strain injuries, it is argued that older athletes (≥25 years) have increased body mass and reduced hip flexibility compared to younger athletes (≤20 years), which are independent risk factors for strain injury in the older athletes (Belinda J. Gabbe et al., 2006).

2.2.4.1.2. Previous Injury

It has been consistently described that athletes with a previous HSI hold a remarkable factor for a new strain injury, which may be up to 2-5 times more likely to occur (Arnason et al., 2004; Engebretsen, Myklebust, Holme, Engebretsen, & Bahr, 2010; Belinda J. Gabbe et al., 2006; Martin Hägglund, Waldén, & Ekstrand, 2013; M. Hägglund et al., 2006; Orchard, 2001). Silder and colleagues revealed that scar tissue might be found adjacent to the injury location up to 23 months after an HSI, and thereby it was suggested as a possible consequence factor to an increase in the stiffness of the tissue (SilderHeiderscheit, Thelen, Enright, & Tuite, 2008). Thus, due to the existence of inelastic scar tissue, the muscle fibers would need to lengthen more for a given change in musculotendon unit (MTJ) length than before the injury. Among subjects with a previous proximal BFlh strain injury and healthy controls, Silder et al. using CINE phase contrast imaging calculated strains near the proximal BFlh MTJ under eccentric loadings. They concluded to be greater on the first group and the greater localized strains observed possible reflected the limited stretch capacity of the scar tissue present, despite of cannot be implied that these subjects exhibited a stiffer aponeurosis-tendon complex before the injury (Silder, Reeder, & Thelen, 2010). Moreover, the mentioned injured athletes revealed a 10% BFlh atrophy compared to their uninjured leg, while no atrophy was seen in healthy controls (Silder et al., 2008). Remarkably, for at least one month before enrolling in this study, the injury group not only had received a supervised rehabilitation program but also had fully restarted to their normal sport activities. Despite strength was not assessed, H:Q (hamstrings:quadriceps) strength imbalances and reduced knee flexor strength would be a consequence of the BFlh muscle atrophy, which, on the other hand, are deemed as risk factors for HSI (see section 2.2.4.2.1). Curiously, some of the mentioned injured athletes not only presented BFlh atrophy but also revealed a hypertrophy in BFsh, proposing an adaptive response to compensate for the lower BFlh strength capacity. This muscle hypertrophy may also propose a subjacent BFlh neuromuscular inhibition, despite the greater knee flexor loading that commonly occurs during rehabilitation (Fyfe, Opar, Williams, & Shield, 2013).

Nevertheless, a shift to shorter and favorable muscle length, might be involved since, other evidence refers that peak concentric (Camilla L. Brockett, Morgan, & Proske, 2004) and eccentric (Croisier & Crielaard, 2000; Proske, Morgan, Brockett, & Percival, 2004) torque changes after a HSI towards more flexed knee joint angles. A more favorable shorter length indicates that hamstrings will work at their descending part of their force-length curve, with a more extended knee joint angle. According to the hypothesis proposed by Morgan et. al which states that stretch induced muscle damage results from very non-uniform lengthening of sarcomeres when active muscle is stretched beyond optimum length, some sarcomeres might be stretched beyond their actin-myosin filament overlap, at the descending part of the force-length curve (Morgan, 1990). If these sarcomeres are the weakest along the muscle fiber, then an additional stretch at the moment of an eccentric contraction may lead to microscopic muscle fiber damage, as a result of an uncontrolled lengthening of these sarcomeres. Additionally, the gradual gathering of such microscopic damage may eventually lead to an HSI, as proposed by Brockett et al. (C. L. Brockett et al., 2001). Notwithstanding, a shift in the angle of peak torque noticed in aforementioned injured subjects remains unclear as pre-existed or product of the injury. Evidence from Opar and colleagues in recreational athletes pointed a 18-20% reduced electromyographic activity during eccentric contractions for a previously injured BFlh, yet not for the ST and SM, compared to the uninjured leg (Opar, Williams, Timmins, Dear, & Shield, 2013a). Once more, all subjects went through rehabilitation, as well as were only allowed to return to play at least 2 months after the injury, at the moment of data collection. Besides the neural activity decline, 10-11% lower eccentric strength was also found compared to the uninjured leg (Opar et al., 2013a). As stated by Opar et al. a previously injured BFlh may be more vulnerable to a future injury since the reactivity to eccentric training is lower, critical as preventive and treatment tool (Heiderscheit, Sherry, Silder, Chumanov, &

Thelen, 2010). Additionally, in slow eccentric contractions, lower knee flexor rate of torque development and reduced impulse at 50 ms and 100 ms after the contraction onset were also described in another research by the same group, along with close reductions in BFlh (but not the ST and SM) (Opar, Williams, Timmins, Dear, & Shield, 2013b).

Nevertheless, more prospective research is mandatory, since the previous mentioned evidence does not clarify if a reduced electromyographic activity of BFlh is the origin or the effect of a HSI. Altogether, HSI arise as consequence of a neuromuscular and functional shift, which may be identifiable even in the long term after a player returned to play, making it susceptible to future recurrent strain episodes.

2.2.4.2. Modifiable

2.2.4.2.1. Strength Imbalances

In order to evaluate knee joint muscle strength imbalances, knee extensors or flexors strength have been commonly assessed by comparing the strength between the two sides (bilateral imbalances) and/or by calculating the relative strength of the knee extensors and flexors (H:Q ratio) unilaterally. Originally, the H:Q ratio was calculated from the concentric peak torque of the knee extensors and flexors, known as the conventional ratio. Later, the dynamic strength ratio (Dvir, Eger, Halperin, & Shklar, 1989) or functional ratio (Aagaard, Simonsen, Magnusson, Larsson, & Dyhre-Poulsen, 1998; Aagaard, Simonsen, Trolle, Bangsbo, & Klausen, 1995) was introduced, which calculates the ratio of hamstrings peak eccentric to quadriceps peak concentric torque, and it is thought to better reflect the reciprocal antagonistic function of these muscles during athletic activities such as sprinting and kicking.

Despite the widespread use of the H:Q ratio, there are no objective cut-off ratio limits due to differences in isokinetic dynamometers and exercise protocols used, so that its controversial to predict HSI in professional football players with these strategy (Croisier, Forthomme, Namurois, Vanderthommen, & Crielaard, 2002; Dauty, Menu, & Fouasson-Chailloux, 2018; Green, Bourne, & Pizzari, 2018). Some studies found that isokinetic testing was weakly associated or could not predict HSI (Henderson et al., 2010; van Dyk et al., 2016), while other studies found some predictive ability of isokinetic testing (Lee, Mok, Chan, Yung, & Chan, 2018).

Croisier et al. found that the most affected functional parameters in previously injured individuals were the hamstrings eccentric bilateral strength and the hamstrings eccentric to quadriceps concentric strength ratio (functional H:Q ratio) (Croisier et al., 2002). In a large prospective study (n= 462) that examined the relationship between strength imbalances and injury risk, Croisier et al. recorded 35 hamstrings injuries and found that professional footballers with preseason strength imbalances that were left untreated, either bilateral hamstrings strength deficits >15% and/or a low conventional (<0.47-0.49) or functional (<0.80-0.89) H:Q strength ratio), had >4-fold increased risk of strain injury during the subsequent season compared to players with no strength imbalances (Croisier, Ganteaume, Binet, Genty, & Ferret, 2008). In addition, players with initial imbalances that were restored (according to statistically defined cut-off criteria) reduced their risk of injury to levels comparable to players with no imbalances.

More recently, Lee et al. (2018) with a sample of 169 professional football players, found that lower eccentric hamstring strength (Ecc 30º/s) and a lower concentric hamstring to quadriceps strength ratio (Con 60/Con 60º/s), were significant risk factors of HSI (Lee et al., 2018). Besides this, the author mentioned that players with a pre-season eccentric hamstring peak torque weaker than 2.44 times his body weight and concentric quadriceps to hamstring strength ratio below 50.5% increased 5.6-fold and 3-fold, respectively, for the risk of HSI. However, the bilateral imbalance of isokinetic hamstring strength or the absolute and relative isokinetic quadriceps strength were not associated with increased risk in this study (Lee et al., 2018).

Contrary to Lee and colleagues, results from van Dyk et al. found a weak association between a lower body weight-adjusted isokinetic eccentric hamstring strength (Ecc 60) or lower quadriceps concentric strength (Con 60) and increased injury risk of HSI with a small effect size ($d < 0.2$) among 614 professional football players, despite different testing protocol and methodology used (van Dyk et al., 2016). Moreover, the study did not identify the H:Q ratio as a risk factor for HSIs.

Given the different cut-offs values usually used in the literature to predict the intrinsic hamstring injury risk, Dauty et al (2018) suggests the prediction has to be assessed by continuous isokinetic muscle strength values and not by isokinetic cut-offs (Dauty et al., 2018). Significant eccentric strength changes across preseason and in-season periods occur (Opar et al., 2015), so that strength testing modalities should have the capacity for repeat measures to be taken in ongoing monitoring and screening practices (McCall, Dupont, & Ekstrand, 2016).

To summarize, a recent systematic review and meta-analysis support moderate or strong evidence for no association between all quadriceps strength measures and future HSI, as well as, small, significant effects for absolute and relative eccentric knee flexor weakness at 60°/s to predict future HSI (Green et al., 2018). In regard to all the 38 isokinetic variables identified, 53% displayed moderate to strong evidence for no association with HSI risk; 36% presented limited evidence for no association; 8% had conflicting evidence for an unknown association, while only 3% of all suggested the potential presence of an association with future HSI (concentric hip extensor 60°/s) (Green et al., 2018). Thus, isokinetic testing may not be best suited to detect the influence of strength in future HSI, but athlete monitoring or profiling in response to training loads, according to the needs of the specific sport, or to individual attributes that may predispose to injury, yes (Green et al., 2018).

2.2.4.2.2. Fatigue

Towards the end of each half period of a football match, nearly half (47%) of the hamstrings strains occur (Woods et al., 2004). This suggests that fatigue may induce changes in muscle strength and sprint mechanics that could contribute to the hamstrings injury susceptibility (Woods et al., 2004). Knee flexor maximal strength has been shown to be significantly reduced in professional and amateur footballers after the completion of laboratory and field-based football-specific exercise (Delextrat, Gregory, & Cohen, 2010; Greco, da Silva, Camarda, & Denadai, 2013; Greig, 2008; Small, McNaughton, Greig, & Lovell, 2010). Moreover, at later stages of a football match, the knee flexors have a decreased capacity to absorb energy during the late swing phase of sprinting which may increase the risk of a strain injury (Schache et al., 2012). Changes in sprinting mechanics have also been observed due to fatigue (Pinniger, Steele, & Groeller, 2000; Small, McNaughton, Greig, Lohkamp, & Lovell, 2009). Pinniger et al. reported a reduced hip and knee flexion, and reduced thigh and leg angular displacement during swing phase after a fatiguing protocol involving isolated knee flexion and 40-m repeated maximal sprints (Pinniger et al., 2000). These changes were accompanied with changes in neural activation patterns with the rectus femoris activation ceasing earlier while the hamstrings were activated earlier during the swing phase. The authors suggested that the observed kinematic changes may be protective mechanisms to reduce the fast-eccentric action of the fatigued hamstrings during the late swing phase and, therefore, the stress and strains within the hamstrings. Similarly, the earlier activation of the hamstrings and their increased duration of activation may compensate for their reduced force production capacity, providing more time to the weaker hamstrings to successfully decelerate the shank before ground contact (Pinniger et al., 2000). In contrast to Pinniger et al., Small et al. found a reduced hip flexion but increased knee flexion and lower limb velocity after a football-specific field protocol (Pinniger et al., 2000; Small et al., 2009). Small et al. also reported an increased anterior pelvic tilt and suggested that these changes in sprint kinematics may predispose the hamstrings to strain injuries, as an increased anterior pelvic tilt would increase the hamstrings stretch and strain (Small et al., 2009). Forced lengthening of hamstrings to greater lengths, combined with the reduced eccentric capacity of hamstrings due to fatigue, could potentially result in a strain injury. Also, an increased anterior pelvic tilt suggests an increase in the hamstrings MTU length which again may predispose to a strain injury.

2.2.4.2.3. Flexibility

Current findings concerning the relationship between hamstrings flexibility and risk of strain injury are conflicting. Three prospective studies in professional footballers have found that decreased flexibility of hip and knee flexors increases the risk of hamstrings strain injury (Bradley & Portas, 2007; Henderson et al., 2010; Witvrouw, Danneels, Asselman, D'Have, & Cambier, 2003), while other studies did not find any association (Arnason et al., 2004; Engebretsen et al., 2010; Belinda J. Gabbe et al., 2006; van Doormaal, van der Horst, Backx, Smits, & Huisstede, 2017; Yeung, Suen, & Yeung, 2009).The rationale for the hypothesis that hamstring flexibility and hamstring injuries are related is found in the kinematic process of the sprint in which the hamstrings endure high forces in a stretched position (van Doormaal et al., 2017). However, as previously mentioned in section 2.2.2., there is no supporting evidence that the hamstrings are maximally stretched during the last swing phase in a sprint, and thereby may not be the reduced hamstring flexibility that is responsible for a hamstring injury but the reduced eccentric hamstring strength of a football player (van Doormaal et al., 2017). While it is unclear why this discrepancy in the results exists, it may be also partly due to the different methods used and the difficulty in differentiating hamstrings flexibility from flexibility in the lumbar spine and pelvis (Dallinga, Benjaminse, & Lemmink, 2012; Opar et al., 2013a; Prior, Guerin, & Grimmer, 2009).

2.2.4.2.4. Biceps femoris long head aponeurosis morphology as risk factor

Given the BFlh frequent injury location, research has emerged to study whether or not this structure morphology and behaviour, including his aponeurosis, sustains a risk factor (Evangelidis et al., 2015b; Fiorentino et al., 2012a; Rehorn & Blemker, 2010b). Three studies using computational modelling and dynamic MR imaging, calculated higher localised tissue strains for individuals with a narrow proximal BFlh aponeurosis and they suggested that a disproportionately small BFlh proximal aponeurosis may be a potential risk factor for strain injury (Fiorentino & Blemker, 2014a; Fiorentino et al., 2012a; Rehorn & Blemker, 2010b). Initially, (Rehorn & Blemker, 2010b) using a finite element model approach and based on MR images, examined the influence of BFlh proximal and distal aponeurosis dimensions on stretch distribution in the muscle during a simulated eccentric contraction and found that a decrease in proximal aponeurosis width by 80% resulted in 60% increase in peak stretches along the proximal proximal myotendinous junction (MTJ).

The findings of that study were confirmed by an in vivo study from the same laboratory that used CINE dynamic MR imaging to measure the BFlh strains during active and passive lengthening in 13 individuals (Fiorentino et al., 2012a). Specifically, they found that individuals with a narrow BFlh proximal aponeurosis experienced the highest strains near the aponeurosis during active lengthening compared to individuals with a wider aponeurosis. Furthermore, it was suggested that localized tissue strains near the proximal aponeurosis were higher during active lengthening as compared to passive lengthening, which would result in increased injury potential (Fiorentino et al., 2012a).

Later, performing in vivo measurements for muscle and tendon dimensions over a range of individuals, and assessing what impact measured physiological variability has on local tissue strain during sprinting based on finite element computational meshes (Fiorentino & Blemker, 2014b), corroborated what was previously speculated: a larger muscle and/or narrower proximal aponeurosis make an individual more susceptible to injury by increasing peak local muscle tissue strain, especially adjacent to the proximal aponeurosis. These three studies provided the first biomechanical rationale that aponeurosis size may contribute to hamstrings strain injuries, and that individuals with a narrow aponeurosis may be at an increased risk.

The aforementioned research works were followed by Evangelidis et al. (Evangelidis, Massey, Pain, & Folland, 2015c) study, who examined the relationship of BFlh proximal aponeurosis area and with muscle size (i.e. maximal anatomical cross sectional area and volume) and knee flexor strength (isometric and eccentric); since higher torques can often be achieved eccentrically, which likely explains the high risk of BFlh MTJ strains during eccentric actions, like sprinting (Evangelidis et al., 2015b). They found that proximal aponeurosis size was highly variable between individuals, and it was not related to muscle size or knee flexor maximal strength, reinforcing the idea that individuals with a relatively small aponeurosis could be subject to greater mechanical strain in the muscle tissue surrounding the aponeurosis, which could predispose them to HSI (Evangelidis et al., 2015b). It is important to note that all previously described aponeurosis data have followed the same (or equivalent) measurement method, as initially suggested by a preliminary report (Handsfield, Fiorentino, & Blemker, 2010b). This method carried some limitations that do not fully reflect the extent of aponeurosis size variability or allows for any valid comparisons

CHAPTER III – METHODS

3.1 Study design

A cross-sectional study design was set for the study purpose.

3.2 Participants

An elite male football player convenience sample, from a portuguese first league team, took part in this study. The number of participants was determined by using a G*Power 3.0.10 software. With the use of an alpha of .05, a power of 0.8 and an estimated effect size of 1, a total sample of 40 participants was determined (ie, 31 for the control group with no BFlh strain history and 9 for the BFlh strain history group). After explaining the aims, benefits and potential risks, subjects provided written informed consent according to the Declaration of Helsinki of 1975 (Carlson, Boyd, & Webb, 2004; “Declaration of Helsinki,” n.d.). This study was approved by the Ethics Committee of the Faculdade de Motricidade Humana, Universidade de Lisboa. All data was collected face-to-face during pre-season player evaluations, including the anthropometric data (age, height and body mass) and the BFlh strain history. The BFlh strain history was based on MRI, obtained at the time of injury and its report, saved in the clinical information of the club's medical department, or from the previous athlete club, after successful contact in getting this information. Despite of different exam origins and staff who performed them, all BFlh strain history was brought together and interpreted by the same group. This team staff was 20 years experienced and board certified in sports medicine (nutritionist, physiotherapist and medical doctor). Only BFlh strains with a diameter equal or bigger than a muscle fascicle/bundle and visible in high resolution MRI records were accepted for the BFlh strain history group. From those with a BFlh strain history longer than 3 years, allocation was set at the control group (see figure 2). All participants were healthy, however with the possibility of other previous history of musculoskeletal problems or injuries of the lower back, pelvis, or legs. At the time of scanning, all participants were free from lower extremity injuries, as it could compromise the MRI visualization of the soft tissues.

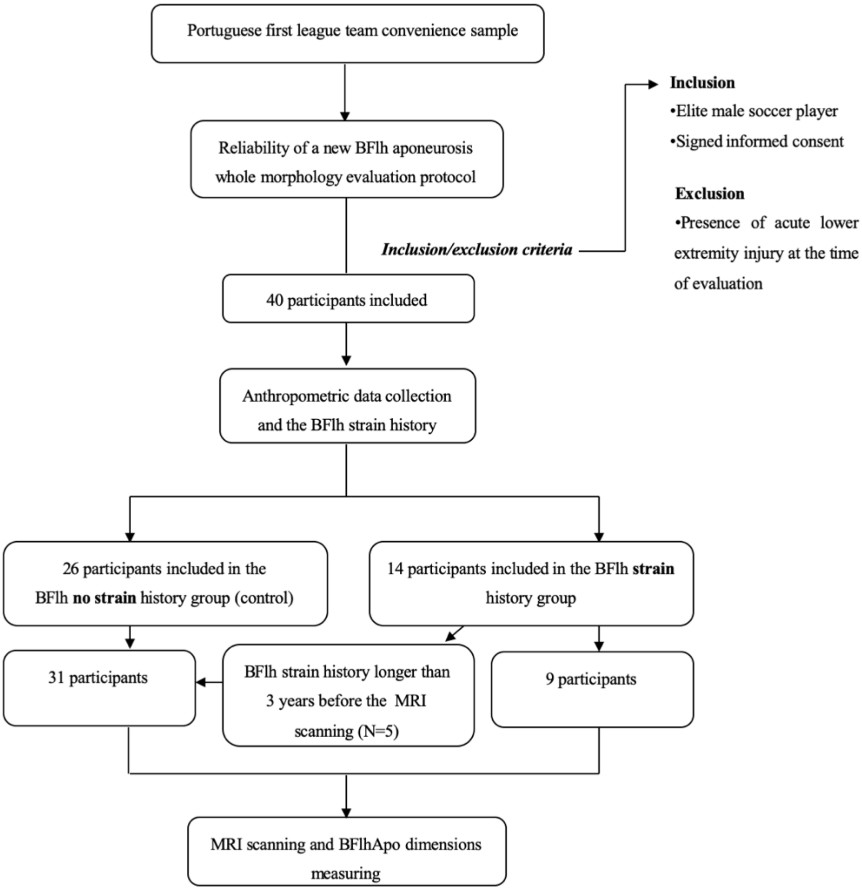

Figure 2. Study design

3.3 Protocol

In the same day and after the clinical interview to register the aforementioned data, participants visited the laboratory. A 3-T MRI scanner (Siemens Trio 3T MR; Erlangen, Germany) was used to scan both tights in the prone position with the hip and knee joints extended. T1-weighted non-fat suppressed axial plane images were acquired by a more than 10-year experienced radiologist, from the anterior superior iliac spine to the knee joint space in two blocks. Oil-filled capsules were placed on the lateral side of the participants' thigh to help with the alignment of the blocks during analysis. The following imaging parameters were used: imaging matrix, 512 512; field of view, 260 mm 260 mm; spatial resolution, 0.508mm 0.508 mm; slice thickness, 5 mm; interslice gap, 0 mm; time of repetition, 500ms and eco time, 10ms. After completing, participants name was blinded, and MRI images were analyzed with Osirix (version 9.0; Pixmeo, Geneva, Switzerland) and MatLab (version R2016b; MathWorks, Inc, Massachusetts, United States) to objectively quantify the BFlhApo dimensions (interface area, average width, volume, and length)

3.4 Data Processing

A semi-automated tracking method was built for the study purpose using Matlab. The routine created (see appendix_1) directly measured, in each axial-plane image the interface slice width (mm) (i.e. interface length between the BFlhApo and the BFlh muscle adjacent to it), as well as the BFlhApo slice area. The BFlhApo was recognized by first identifying the proximal BFlh tendon, and then scrolling through MRI slices from proximal to distal, untill visualized muscle adjacent to the proximal tendon (figure 3A). At that point, BFlhApo interface slice width and BFlhApo slice area measurements started, and for all slices where this structure was beside or inside the BFlh muscle belly, it was measured. To define BFlhApo slice area, a precise criterion was set to outline it, since aponeurosis morphology color scheme ranges between black and dark grey, making hard to decide objectively the interface between BFlhApo and surrounding structures. To solve this, an evident small portion of aponeurosis (darkest color area identifiable over epicenter - figure 3B) and muscle area (more or less in grey - figure 3C) were selected, in each slice, with a Matlab polygon tool. A percentage (i.e. 60%) relative to the difference of the mean grayscale between muscle-area and aponeurosis-area was calculated, enabling Matlab to automatically localize and outline his morphology (aponeurosis slice area, mm2 - figure 3D). This means that if a mean grayscale intensity area of a muscle was A, B for the aponeurosis area, and P for the percentage, then selected pixels were the ones that had the intensity below (A+P*(A-B)/100). To find this ideal percentage cut off value, a consensus among 7 experienced specialists in musculoskeletal identification via MRI was previously studied (see appendix 2, part 1).

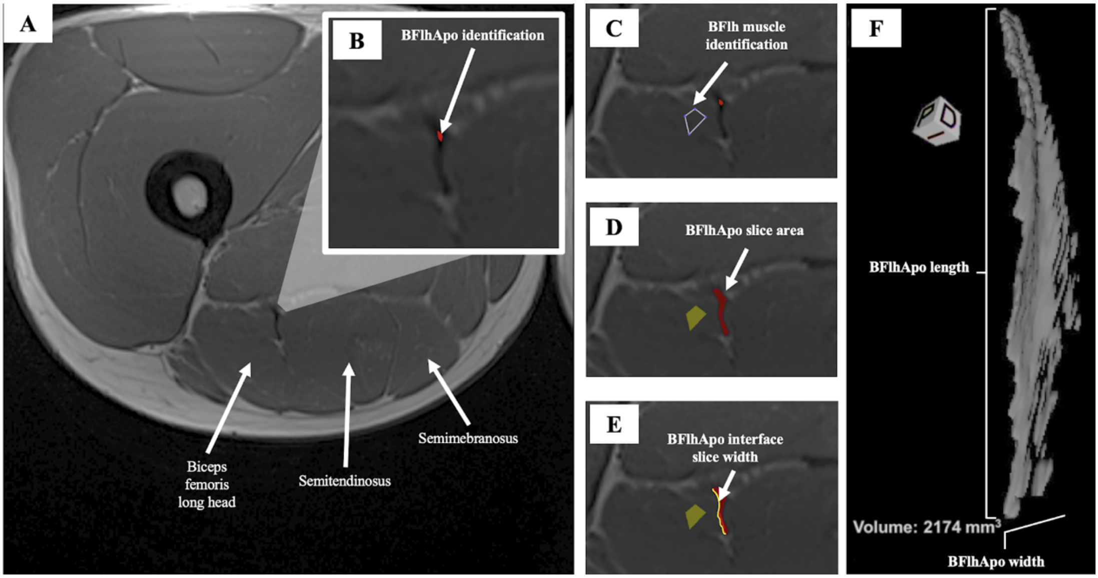

Figure 3. Matlab routine procedure overview from A to E; A. Uploaded MRI slice in .dicom format and anatomical identification of the hamstrings; B. Zoom to define the BFlhApo region of interest; C. Zoom to define the BFlh muscle region of interest; D. Automatic calculated slice area with the anatomical threshold criteria at 60% manually set; E. BFlhApo interface slice width, set by a curvilinear line between the BFlhApo and the BFlh muscle belly (yellow); F. Osirix three dimensional view image of the BFlhApo with volumetric value (P - posterior side; D - right side; I - inferior side)

After defined aponeurosis slice area, aponeurosis interface slice width was acquired by determining the length (mm) of a curvilinear line drawn over external (adjacent to the BFlh muscle belly - figure 3E) and internal part of the proximal aponeurosis (inside the BFlh muscle belly, if the case).

Finally, to test this semi-automated tracking method, intrarater and interrater reliability was performed with two trained and blinded observers over 10 MRI slices from a randomly selected participant, accordingly with the procedure above mentioned, computing intraclass correlations with absolute agreement. The BFlhApo measurements were processed two times, in two different days, to evaluate the reliabilities of the BFlhApo slice area, BFlhApo interface slice width procedures.

The BFlhApo length (figure 3F) was calculated as the sum of the slices where BFlhApo was identifiable, multiplied by the slice thickness (5 mm). As aforementioned, BFlhApo first slice was defined when visualized muscle adjacent to the proximal tendon at the axial-plane MRI images. Last aponeurosis slice was defined accordingly to a cutoff BFlhApo thickness mean value (i.e. 0,72mm), gathered among the same specialist panel used to set the threshold criteria of BFlhApo slice area (see appendix 2, part 2).

To measure the BFlhApo volume and the BFlhApo interface area, the sum of all BFlhApo slice areas and the sum of all BFlhApo interface slice widths were respectively multiplied by the slice thickness (5mm) using an excel sheet. All manual segmentation measurements were completed by the same investigator, blinded to the strain results.

3.5 Statistical analysis

Statistical analysis was performed using the SPSS software (v24, Chicago, USA). Normality of all variables was tested using the Shapiro-wilk test. The intra-rater and inter-rater reproducibility of the BFlhApo Matlab measurements was determined by calculating the intraclass correlation coefficient (ICC2) with absolute agreement. ICC values less than 0.5 were indicative of poor reliability, values between 0.5 and 0.75 indicated moderate reliability, values between 0.75 and 0.9 indicated good reliability, and values greater than 0.90 indicated excellent reliability (Koo & Li, 2016). Descriptive statistics (i.e. mean and standard deviation) were calculated for both groups and in between groups. The coefficient of variation (%CV) of the BFlhApo dimensions was also calculated as a measure of dispersion. If normality and homogeneity of variances assumed, a T-Test for independent samples was used to compare morphological differences of BFlh proximal aponeurosis (i.e. area interface, average width, volume, and length) between the BFlh strain history group and the BFlh without strain history group. The same test was also used to compare the same morphological differences of BFlh proximal aponeurosis between left and right thighs among the control group, as well as between injured and non-injured thighs among BFlh strain history group. The significance was set at $P < 0.05$.

CHAPTER IV – RESULTS

4.1. Demographic and clinical characterization of the sample

The demographic and clinical characteristics of the subjects are described in table 2. Among the 40 participants, 31 took part of the control group (62 thighs), while 9 (18 thighs) joined the BFlh strain history group (4 subjects with a previous BFlh left injury and 5 subjects with a previous BFlh right injury).

In the BFlh strain history group, a higher age ($P < 0.05$, 28.6±5.05 vs 23.3±4.26) and body mass ($P < 0.05$, 83.29±6.53 vs 76.71±7.73) was observed compared to the control group. From the clinical point of view, all subjects with previously injured thighs had on average 1.41±1.04 years distance between the date of testing and the injury date.

Table 2. Demographic and clinical characterization of the sample

Variables name	BFlh strain history group	Control group	P	Total
N	9	31	-	40
Age (years)	28.6 ± 5.05	23.3 ± 4.26	0.00[a]	24.5 ± 0.55
Height (m)	1.82 ± 0.09	1.81 ± 0.08	0.50[b,c]	1.82 ± 0.01
Body mass (Kg)	83.29 ± 6.53	76.71 ± 7.73	0.02[a]	78.19 ± 0.89
Injured thighs				
Left	4 (44.44)	-	-	-
Right	5 (55.55)	-	-	-
Time lost due to injury (days)	28.9 ± 12.87	-	-	-
Time between testing and injury (years)	1.41 ± 1.04	-	-	-
Athletes with BFlh recurrence in last 5 years	3 (33.33)	-	-	-

BFlh: biceps femoris long head; N: number of participants; Quantitative variables: Mean ± standard deviation; Qualitative variable: frequency (%); P: Statistically significant differences between groups; P <0.05 according to the [a]T-Test for independent samples and the [b]Mann-Whitney-Wilcoxon Test ([c] see appendix 3, section 1 for parametric statistical results)

4.2. Comparison between the previously injured thighs of BFlh strain history group and the control group

Table 3 shows no statistically significant differences (P <0.05) between the injured thighs of BFlh strain history group (N=9) and the thighs of the control group (N=62), regarding to the BFlhApo volume, length, interface area and average width. The biggest dispersion of sizes was found in the BFlhApo volume (3266.56 ± 1710.54, CV% 52.37 vs 2345.18 ± 921.66, CV% 39.30) and interface area (1998.97 ± 775.87, CV% 38.81 vs 1586.29 ± 519.56, CV% 32.75).

Table 3. Comparison between the injured thighs of BFlh strain history group and the control group

Variables name	Group				
		N	**M±SD**	**CV%**	**P**
BFlhApo volume (mm^3)	PIT	9	3266.56 ± 1710.54	52.37	0.15[a]
	Control	62	2345.18 ± 921.66	39.30	
BFlhApo length (mm)	PIT	9	175.56 ± 39.09	22.26	0.28[b]
	Control	62	162.58 ± 32.35	19.90	
BFlhApo interface area (mm^2)	PIT	9	1998.97 ± 775.87	38.81	0.16[a]
	Control	62	1586.29 ± 519.56	32.75	
BFlhApo average width (mm)	PIT	9	9.66 ± 2.04	21.12	0.06[b]
	Control	62	11.07 ± 2.15	19.42	

BFlh: biceps femoris long head; BFlhApo: biceps femoris long head proximal aponeurosis; CV%: coefficient of variation (%); PIT: previously injured thighs of the BFlh strain history group; N: number of thighs; Quantitative variables Mean ± standard deviation; P: Statistically significant differences between groups; P <0.05 according to the [a]Welch's t-test and the [b]T-Test for independent samples

4.3. Comparison between the previously injured thighs of BFlh strain history group and the control group

Comparing the BFlhApo dimensions (volume, length, interface area and average width) between the injured (N=9) and non-injured (N=9) thighs of BFlh strain history group, no statistically significant differences ($P < 0.05$) were found, as well as between the left (N=31) and right (N=31) thighs of the control group.

Table 4. Comparison between thighs of BFlhApo dimensions among the BFlh strain history group and the control group

	BFlh strain history group		**Control group**	
	Injured limb	Non injured limb	Left limb	Right limb
N	9	9	31	31
BFlhApo volume (mm^3)	3266.56 ± 1710.54	3692.11 ± 2638.37	2274.13 ± 798.72	2416.23 ± 1038.68
P	0.86[a,b]		0.55[c]	
BFlhApo length (mm)	175.56 ± 39.09	176.67 ± 50.37	162.10 ± 32.91	163.06 ± 32.32
P	0.96[c]		0.91[c]	
BFlhApo interface area (mm2)	1998.97 ± 775.87	1956.44 ± 934.63	1523.13 ± 516.20	1649.45 ± 523.62
P	0.92[c]		0.34[c]	
BFlhApo average width (mm)	11.07 ± 2.15	10.71 ± 2.33	9.32 ± 2.10	10.00 ± 1.95
P	0.74[c]		0.19[c]	

BFlhApo: biceps femoris long head proximal aponeurosis; N: number of thighs; Quantitative variables: Mean ± standard deviation; P: Statistically significant differences between groups; $P < 0.05$ according to the [a]Mann-Whitney-Wilcoxon Test ([b] see appendix 3, section 2 for parametric statistical results) and [c] the T-Test for independent samples

4.4. Intra-rater and inter-rater reliability for BFlhApo measurements

The analysis of the reliability using the Matlab routine with 10 MRI slices selected from a randomly chosen participant thigh, showed a good intra-rater and inter-rater reliability (ICC between 0.75 and 0.9, at 95% confidence interval), both for the BFlhApo slice area and interface slice width measurements.

Table 5. Intra-rater and inter-rater reliability for BFlhApo measurements using the Matlab routine

	N	ICC	ICC 95%		P
			Lower bound	Upper bound	
Intra-rater reliability					
BFlhApo slice area	10	0.88	0.75	0.95	0.00
BFlhApo interface slice width	10	0.82	0.63	0.92	0.00
Inter-rater reliability					
BFlhApo slice area	10	0.79	0.35	0.94	0.01
BFlhApo interface slice width	10	0.75	0.28	0.93	0.02

BFlhApo - biceps femoris long head proximal aponeurosis; N: number of MRI slices selected from a randomly chosen participant thigh; ICC: intraclass correlation coefficient; ICC 95%: 95% confidence interval; P: Statistically significant differences for $P < 0.05$

CHAPTER V – DISCUSSION

The primary goal of this study was to investigate if athletes with a previous BFlh injury would present smaller BFlhApo dimensions compared to their matched controls (without a BFlh strain history). The main finding was that elite footballers with previous BFlh injury in the last 3 years, showed no differences ($P < 0.05$) to the control group regarding to all the BFlhApo dimensions (volume, length, interface area and average width), which challenges the hypothesis proposed, as well as the reported data by Evangelidis et al (2015) and Fiorentino et al (2012), as a possible independent risk factor for injury (Evangelidis et al., 2015a; Fiorentino & Blemker, 2014b).

Despite of this conflicting information, attention should be given to the methodology used by the aforementioned authors, since measurement procedures were similar, based on a preliminary report (Handsfield et al., 2010b). This method carries some limitations, like measuring BFlhApo interface slice width at one arbitrary point (rater dependent) and without considering his extension transversely into the muscle (internal aponeurosis). Thereby, it does not fully reflect the BFlhApo size variability or allows for any valid comparison between subjects. Beyond this, aponeurosis interface slice width measurement alone seems to be a poor reflection of the whole BFlhApo size and interindividual variability. To overcome this issue, a more detailed description of BFlhApo dimensions (volume, length, interface area and average width), was developed via a semi-automated tracking method, providing an insight to assess the link between this structure and the muscle's strain injury susceptibility. Compared to the previous studies, this objective procedure proved to have a better reliability (intra-rater and inter-rater), built from a consensus set by a specialist panel in observing musculoskeletal MRI (see appendices 1,2). As an algorithm-based routine procedure, not only it was possible to be less operator dependent, but also capable of precisely outline this structure, or others hereafter, when their morphologies are difficult to determine. An interesting observation made during the analysis between the injured thighs of BFlh strain history group and the control group, was that all

BFlhApo dimensions were highly variable between individuals (e.g. 52.37-39.30 CV% for volume), as in accordance to what it was previously stated by Evangelidis and colleagues (2015) (Evangelidis et al., 2015a).

The rationale behind the hypothesis that previous BFlh injury individuals would present smaller BFlhApo dimensions compared to their counterparts, was based on the smaller the size, the greater local deformation at the interface between BFlh and the BFlhApo (Fiorentino & Blemker, 2014b). Accordingly, to finite element simulations, the BFlh cross sectional area is larger in the middle between the two aponeuroses and smaller adjacent to the proximal aponeurosis, so that a given amount of muscle activation may generate more stress in the middle than near the proximal BFlhApo (Fiorentino & Blemker, 2014b).. However, to balance the difference in stress, the muscle tissue near the BFlh proximal aponeurosis must stretch more than adjacent tissue, and thereby may be more vulnerable to a strain injury (Fiorentino & Blemker, 2014b). Although the dimensions of BFlhApo with BFlh muscle size and muscle function data were not measured to fully answer the proposed hypothesis, the highly variable BFlhApo dimensions among subjects and the lack of his relationship with a previous BFlh injury help to discard this assumption.

Notwithstanding, the results of the present study must be cautiously interpreted by its cross-sectional and retrospective model. The admitted football players were recruited from a small convenience sample (9 in the BFlh strain history group) so the results cannot be generalizable to individuals in other contexts, as well as the potential selection bias cannot be disregarded. At the same time, the images were collected in a static position, when in muscle contractions, the BFlhApo may adopt another morphology (Raiteri, 2018). Furthermore, although the size of the aponeurosis was similar between the injured and uninjured legs of the players, the fact that the size of the aponeurosis was measured after the injury, which by definition affects the part of the measured anatomy, secondary alterations cannot be disregarded to the injury and, therefore, does not necessarily represent the pre-injury state (Silder et al., 2008). Finally, the presence of individuals with higher age and body mass in the group with BFlh strain history than in the control group may have influenced the results in the study (Belinda J. Gabbe et al., 2006; Henderson et al., 2010). However, it is important to highlight that the presence of this characteristics are common, since they are independent risk factors for strain injury, which supports the external validity of the results (Belinda J. Gabbe et al., 2006; Henderson et al., 2010).

CHAPTER VI – CONCLUSION

To summarize, the present study showed: (1) no differences regarding to the BFlhApo dimensions (volume, length, interface area and average width) between a team of professional football players with and without a BFlh strain history. This data seems to debunk prior hypotheses of proximal aponeurosis size as a possible independent risk factor to sustain a BFlh injury. (2) In the background, our results arise from a good reliability measuring procedure based on purposed built algorithm, inserted in a semi-automated tracking method, able to quantify properly the dimensions of this structure.

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ATTACHEMENTS

Appendix 1

Matlab Routine used to quantify the BFlhApo interface slice width and the BFlhApo slice area.

```
[filename,user_canceled] = imgetfile;
%only for png files
%I=rgb2gray(imread(filename)\\

info=dicominfo(filename);
pixelSpacing=info.PixelSpacing;
scale=pixelSpacing(1);
I=dicomread(filename);
imin=min(min(I));
imax=max(max(I));
I=uint8(255.0*double(I-imin)/double(imax-imin));
factor=6;
I=imresize(I, factor*size(I));
f=figure;
imshow(I, 'InitialMagnification', 250);
axis off; % Turn off axis numbering

%ask for aponeurosis
BW1=roipoly;
[L,n]=bwlabel(BW1);
RGB=label2rgb(L, 'autumn', 'black', 'shuffle');
imshow(I, 'InitialMagnification', 250);
hold on;
himage = imshow(RGB);
```

```
himage.AlphaData = 0.3;
drawnow;
%ask for muscle
BW2=roipoly;
mask=BW1;
mask(BW2)=2;
[L,n]=bwlabel(mask);
RGB=label2rgb(L, 'autumn', 'black', 'shuffle');
imshow(I, 'InitialMagnification', 250);
hold on;
himage = imshow(RGB);
himage.AlphaData = 0.3;
drawnow;

%apoCoordinates=[840 538; 866 532; 898 512; 902 496; 896 472; 874 496;
840 538];
%musCoordinates=[632 544; 676 546; 750 478; 760 396; 612 426; 632
544];
%BW1 = poly2mask(apoCoordinates(:,1), apoCoordinates(:,2), size(I,1),
size(I,2));
meanApo=mean(I(BW1));

%BW2 = poly2mask(musCoordinates(:,1), musCoordinates(:,2), size(I,1),
size(I,2));
meanMus=mean(I(BW2));

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
thresholdPct=51; %pct
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
threshold=meanApo+double(meanMus-meanApo)*thresholdPct/100.0;
aponeurosisTh=I<threshold;
```

```
global aponeurosis
aponeurosis=BW1 | aponeurosisTh ;
[L,n]=bwlabel(aponeurosis);
indx=find(BW1==1);
aponeurosis= L==L(indx(1));
aponeurosis(BW2)=2;
[L,n]=bwlabel(aponeurosis);
RGB=label2rgb(L, 'autumn', 'black', 'shuffle');
% Initial Image
hold on;
himage = imshow(RGB);
himage.AlphaData = 0.3;
%SLIDER
b = uicontrol('Parent',f,'Style','slider','Position',[81,54,419,23],...
            'value',thresholdPct, 'min',0, 'max',100);
bgcolor = f.Color;
bl1 = uicontrol('Parent',f,'Style','text','Position',[50,54,23,23],...
             'String','0','BackgroundColor',bgcolor);
bl2 = uicontrol('Parent',f,'Style','text','Position',[500,54,23,23],...
             'String','100','BackgroundColor',bgcolor);
bl3 = uicontrol('Parent',f,'Style','text','Position',[240,25,100,23],...
             'String',sprintf('Threshold %2.2f',
thresholdPct),'BackgroundColor',bgcolor);

b.Callback = @(hObject, event) sliderCallback(hObject, event, meanMus,
meanApo, I, BW1, bl3, BW2) ;
%QUANTIFY
btn = uicontrol('Style', 'pushbutton', 'String', 'Quantify',...
        'Position', [20 600 50 20],...
        'Callback', @(hObject, event) quantifyCallback(hObject, event, I, BW2,
scale, factor, filename)  );
```

Appendix 2

This appendix overviews the consensus set by a 7 specialist panel (i.e. 3 medical doctors, 2 physiotherapists and 2 doctorates in Biomechanics) in observing musculoskeletal MRI to:

1. define the percentage relative to the difference of the mean greyscale (255 pantone scale) between muscle-area and aponeurosis-area, in order to objectively limit the BFlhApo from the surrounding structures in axial plane images (step 1);

2. define the minimum observable important BFlhApo thickness (mm) in order to limit the last distally axial plane image identifiable (step 2).

Step 1.

1. Specialists were contacted by email to manually outline the BFlhApo perimeter (using the free form from shapes) over 10 randomly MRI axial plane images (sent in a .pptx file), according to the criterion they considered most appropriate based on their knowledge and expertise.

2. After successfully receiving the 70 images, every .jpeg file was uploaded to imageJ software (NIH, v1.47, USA) to analyse the average grayscale color range of:

   A - the area of the BFlh belly (muscle tissue);
   B - the area of the BFlhApo;
   I - the interface area that outlines the BFlhApo

3. - Using the equation: P = (I - A) / ((A-B)/100), a percental grayscale interface (P) was determined for each image. The final threshold criteria were set by finding the average P value over the 70 processed images. Thus, a 60% average P value was set among the specialist panel.

Step 2.

1. In a second moment, the same specialist panel was once again contacted by email, to manually mark (using arrows from shapes) the starting and ending points of the BFlhApo interface slice width, over 10 randomly MRI axial plane images (sent in a .pptx file), according to the criterion they considered most appropriate;

2. After successfully receiving the 70 images, every .jpeg file was uploaded to imageJ software (NIH, v1.47, USA) to measure the BFlhApo thickness (mm) of the proximal and distal points using a straight-line tool.

3. A 0,72 mm thickness criteria was set as cut off value to observe important BFlhApo, based on the average width of the 140 locations measured. Once moving from proximal to distal, the BFlhApo fades, getting too small and thin, this precise criterion solved the issue.

4. Using Osirix software, the minimum observable important BFlhApo slice was recognized by first identifying the proximal BFlh tendon, and then scrolling through MRI slices from proximal to distal, till find the last BFlhApo slice with a minimum of 0,72mm, measured with a straight-line tool.

Appendix 3

This appendix presents parametric statistics for:

1. height comparisons between the BFlh strain history group and the control group (section 1);
2. BFlhApo volume comparisons between the injured and non-injured tights of the BFlh strain history group (section 2).

Section 1.

1. Assumption of normality of the dependent variable (height)

Table 6. Sample height normality test

	Shapiro-Wilk		
	N	Statistic	**P**
Height			
BFlh strain history group	9	0.88	0.02
Control group	31	0.97	0.13

BFlh: biceps femoris long head; N: number of participants; P: Statistically significant differences between groups;

2. Assumption of homogeneity of variance

Table 7. Sample height equality of variances test

		Levene	
		F	**P**
Height	Equal variences assumed	0.21	0.65

F: test statistic; P: p-value

3. Independent sample T-test

Table 8. Independent sample T-test for height

		T-test for equality of means		
		t	df	**P**
Height	Equal variences assumed	0.39	78	0,70

t: t-statistic; df: degrees of freedom; P: p-value

Section 2.

1. Assumption of normality of the dependent variable (BFlhApo volume)

Table 9. BFlhApo volume normality test

	Shapiro-Wilk		
	N	Statistic	**P**
BFlhApo volume			
Injured thighs of the BFlh strain history group	9	0.92	0.38
Non injured thighs of the BFlh strain history group	9	0.80	0.02

BFlh: biceps femoris long head; N: number of thighs; P: Statistically significant differences between groups;

2. Assumption of homogeneity of variance

Table 10. BFlhApo volume equality of variances test

		Levene	
		F	**P**
BFlhApo volume	Equal variences assumed	0.39	0.54

F: test statistic; P: p-value

3. Independent sample T-test

Table 11. Independent sample T-test for BFlhApo volume

		T-test for equality of means		
		t	df	**P**
BFlhApo volume	Equal variences assumed	-0.41	16	0,69

t: t-statistic; df: degrees of freedom; P: p-value