Protect her knees - Exploring the role of football specific fatigue on dynamic knee stability in female youth football players

A project funded by the UEFA Research Grant Programme

CONTENTS

Executive Summary

Context:

It is well recognised that females appear to have a greater relative risk of non-contact ACL injury compared to males when hours of athlete exposure are taken into account. A number of non-modifiable (anatomical and hormonal) and modifiable (muscular and neuromuscular functioning) risk factors have been proposed for this sex difference. Limited epidemiological information suggests that this relative risk may peak in the pubertal / immediately post pubertal years when the influence of growth and maturation are most prevalent. Injury incidence data also shows an increased risk of injury towards the end of matches, when fatigue is most likely to be present. Currently our knowledge of the effect that football specific fatigue has on the muscular and neuromuscular functioning of female youth footballers, through the pubertal years, is limited. Therefore, this study aimed to examine the effect of football specific fatigue on muscular and neuromuscular variables associated with dynamic knee stability in elite youth female footballers.

Methods:

36 elite youth academy female footballers (U13, U15 and U17 age groups) were recruited from an FA Women's Super League club. Anthropometric variables including maturation determined as off-set from peak height velocity and 'Quadriceps Angle' (Q angle) were determined for each age group. Relative leg stiffness, Functional Hamstring/Quadriceps (FH/Q) ratio, and Electromechanical Delay (EMD) were determined pre and post an age appropriate simulated football match using the SAFT90 protocol.

Key Findings:

Football specific fatigue had little influence on muscular stability of the knee, as determined by the FH/Q, although the ratio was reduced post fatigue close to full knee extension. Fatigue had a detrimental effect on neuromuscular stability with significantly longer EMD recorded post fatigue for all age groups. However, these negative effects were significantly greater in the U13 age group. Fatigue influenced the U15 group the greatest with a reduction in FH/Q and increase in EMD post fatigue. Neuromuscular functioning was diminished the most in the U13 age group with reduced feedback responses (EMD) as well as reduced feed-forward responses (lower leg stiffness). The U17 age group increased muscular stability (FH/Q ratio) and feed-forward mechanisms (leg

stiffness) post fatigue and this may be a compensatory mechanism for the reduction in neuromuscular feedback mechanisms (EMD) when fatigue is present.

Implications for practice: Key recommendations

Our findings suggest that the following need to be considered in the further development of neuromuscular conditioning programmes for elite female youth footballers:

1. That a neuromuscular conditioning programme is focused on being related to fatigue resistance and is undertaken in the middle or end of training sessions rather than solely during the warm-up when fatigue is not present

2. That muscular conditioning includes and focuses on the portion of the movement that is towards full knee extension (in particular the first 15° of knee flexion) – this should include training aimed at improving landing performance

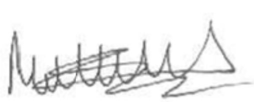
3. That training includes fast velocity movements as well as more controlled slower velocity movements

4. That training programmes are especially effective in enhancing neuromuscular functioning as a primary goal as well as improving torque production as a secondary goal

5. That training is both age group and maturational stage specific. Specific neuromuscular training should be recommended, implemented and developed as early as possible

6. That training in younger age groups focuses on the development and enhancement of neuromuscular feed-forward mechanisms in response to fatigue. This training should also include fundamental movement skill development

7. That training during puberty is enhanced and individualised to focus on both muscular and neuromuscular qualities



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1.1 Acknowledgements

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Finally I would like to thank UEFA for funding this project and contributing to our increased knowledge of the effect football specific fatigue has on dynamic knee stability in young girls.

Review of literature

2.1 Incidence of injury in football

There is a growing epidemiological evidence base mapping out the incidence of football related injury. These data are still predominantly based on males and from adult populations (Ekstrand et al., 2009). Nevertheless there is growing recognition for the need to map injury rates in female players and in particular female youth player who may be at greater risk of injury due to numerous factors including growth and maturation. There are a number of injury incidence studies available on female soccer players from a wide range of age groups, using a range of sample sizes (n = 45 -), but importantly the majority of data is from adult professional players (Hagglund et al., 2009; Lilley et al., 2002; Walden et al., 2010). There is limited information regarding the incidence of injury in youth female professional players (Schiff et al., 2010; Rumpf and Cronin, 2012). There are a whole range of issues surrounding injury incidence data and it is beyond the extent of this report to explore all of these in detail. It should be noted however that limitations in our current knowledge of injury incidence in female youth footballers may be attributed to one or more of the following limitations: a) Definition and severity of injury; b) method of assessment and reporting of injuries; c) reporting of injury rates.

In possibly the most comprehensive injury incidence study in male professional footballers Ekstrand et al. (2009) reported data for 7 consecutive seasons from the first team squads of 23 squads selected by UEFA. Based on hours of athlete exposure the data suggests that injury incidence in this population is 8 injuries per 1000h of exposure and was significantly greater during match play compared with training. Importantly, the findings demonstrate an increasing injury tendency with time in both the first and second halves of matches. This would suggest that injury rates are higher when players are more fatigued towards the end of match play. Studies have shown that fatigue is prevalent towards the end of a game and the amount of high intensity work and technical performance is lowered (Bangsbo et al., 2007). A number of studies have attempted to explore the sex difference in injury incidence rate in football with conflicting findings. Hagglund et al. (2009) reported higher injury incidence rates for Swedish professional male compared to female players during both training (4.7 Vs 3.8 injuries/1000h) and match play (28.1 Vs 16.1 injuries/1000h). However, there was no sex difference when incidence data were expressed in relation to moderate to severe injury. Earlier data suggested that females have a higher injury rate than males (12 injuries/1000h compared with 5 injuries per 1000h) in professional players from the same country (Sweden) (Engstrom et al., 1991). The 7 year study by de Loes et al (2000) also reported a higher incidence rate in females compared with males (9.5 Vs 7.5 injuries/1000h) More recently Lilley et al. (2002) conducted a 5 year retrospective injury survey of Australian female players and reported a mean incidence rate of 12 injuries per 1000h. Interestingly they also reported that the injury incidence fell when exposure almost doubled (to as low as 5 injuries/1000h) and tentatively suggest that exposure to more training and match play helps to develop strategies to prevent injuries. Despite the recorded reduction in injury incidence there was a concomitant increase in the number of major injuries.

There are far fewer studies on which to judge the injury incidence of exposure to athlete hours in youth players, and even less focusing on youth female players. Rumpf and Cronin (2012) recently undertook a review of literature examining injury incidence data in 6-18 year old footballers. They reported that injury in the youth players was: a) more likely to occur during match play rather than during training; b) 'low skill' and less experienced players are more likely to sustain an injury compared with 'high skill'/experienced players; c) a greater training load, higher quality training and appropriate warm up are related to fewer injuries; d) Defenders and midfielders have a higher injury rate than strikers and goalkeepers; e) that maturity status is not related to injury risk, but the severity of injury is greater in late compared with early maturers; f) that girls show a 2-fold greater injury risk than boys. They suggest that total injury incidence in relation to chronological age is 8.0 injuries/1000h in 9-12y-olds, 65.8 injuries/1000h in 13-15y-olds and 8.4 injuries/1000h in 16-18y-olds. These data would suggest that there is a significant age effect in the incidence of football related injury during childhood and that children aged 13-15y are most at risk. Given this finding it is surprising that the authors have not concluded that there may be a link between maturational stage and injury risk. Even given that elite youth performers tend to be early maturers this age range would coincide with progression through puberty and all of the associated developmental changes that occur during this time period. More studies are needed that focus on injury incidence in relation to maturational status as well as chronological age given these data. It should be noted that these suggestions are based on limited empirical data and more studies are needed to support these initial findings. Some of these suggestions are further supported by another recent review of the literature, exploring the epidemiology of ACL injury in football from a gender related perspective (Walden et al., 2011). Walden et al. (2011) conclude that females have a 2-3 times higher ACL injury risk compared with their male counterparts and that injury is more likely to occur during match play. Importantly they reported that females tend to sustain their ACL injury at a younger age than males (19 V 27y).

There appears to be only 2 studies that have focused exclusively on the incidence rate of injury in female youth footballers (Schiff et al., 2010; Lislevand et al., 2011). Schiff et al. (2010) explored different injury surveillance methods to examine injury rates in 12-14y-old girls. They reported an injury rate of 4.7 injuries/1000h with the majority of these injuries occurring during match play rather than during training. A recent injury incidence study on female youth footballers in Africa during a 2 day tournament reported a very high injury incidence ratio (93.3 injuries/1000h) (Lislevand et al., 2011). However, most of the injuries reported were considered minor and allowed players to continue to play. Interestingly there was an increase risk of injury in the 13-16y-old age group compared to players over the age of 16y. Importantly there are both personal and financial costs associated with injuries in female youth players, including higher risk of osteoarthritis in adulthood (Quatman and Hewett, 2009), cost of medical treatment (de loes et al., 2000), as well as psychosocial factors (Elliot et al., 2010).

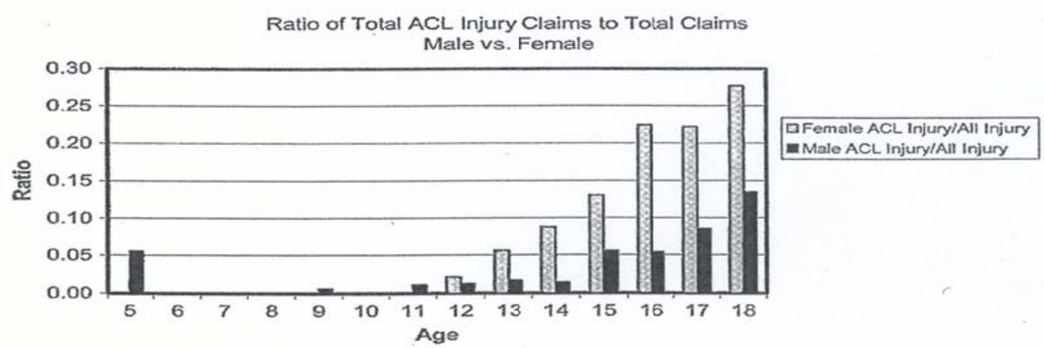
2.1 Incidence of ACL injury

Incidence quantifies the occurrence of new cases of a given injury in a population (Marshall et al., 2007). In the population-based prospective cohort study conducted by Parkkari *et al.* (2008), results showed that 'the general risk for a cruciate ligament injury of the knee is relatively low among adolescents and young adults, but participation to organised sports increases the risk significantly' (p.424). They also stated that young active women, participating in sport are at high risk of suffering such an injury.

Yu (2002, cited in Marshall *et al.,* 2007) used data from the American Board of Orthopaedic Surgeons to report the ACL injury incidence by sex and age. They demonstrated that ACL injuries have a peak of occurrence in the 16-18 year-old age group, and that males sustain more ACL injuries that females in every age group except age 15 year. An obvious limitation to this study is that these data relate to reconstructions, which are not the same as incident injuries. If an injury presents with no instability or need for reconstruction and require non surgical rehabilitation, then these data do not highlight these cases. It is likely therefore that the Yu (2002) data underestimates in incidence of ACL injury.

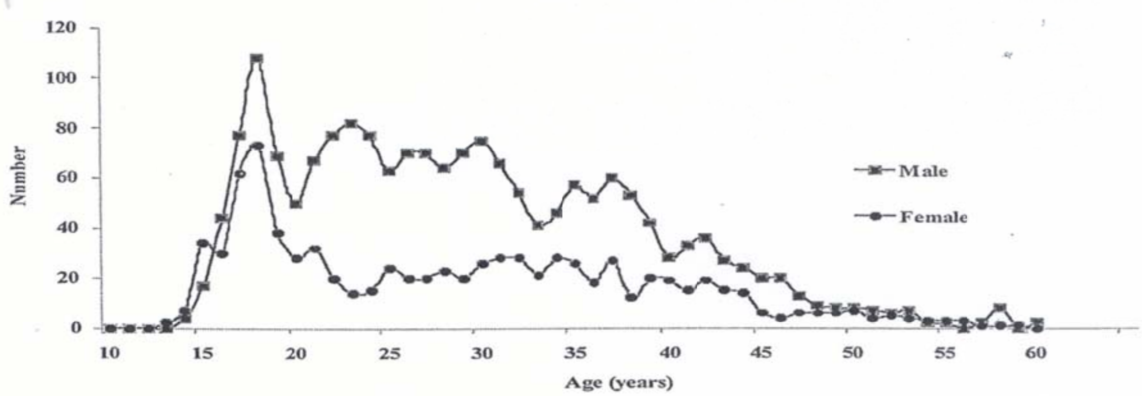
Shea and colleagues (2004, cited in Marshall *et al.*, 2007) obtained insurance data from an insurance agency that covers over 1 million youth football players (half of them female) annually in the United States to analysis trends on ACL injury by age and sex. They also reported that the 16-18 year-old age group is the most at risk group where most injuries are likely to occur (see figure 2.1). However, in contrast to the findings by Yu (2002), females have nearly twice as many ACL injury claims as males. Moreover, in every age group, from 12 years through to 18 years, ACL injury claims made by females account for a higher proportion of all injury claims than those made by males. Another limitation of these data is that not all injuries that occur would result in claims to the insurance company. Despite the small number of studies and limitations to the empirical data, the data consistently shows that there seems to be an increased risk of ACL injury between 13-18 years of age and that this risk is greater in females than males when hours of exposure are taken into account.

A number of studies have reported the frequency of ACL tears in different types of sports activities. The highest incidence is seen in adolescents playing sports that involve pivoting, such as football, basketball and team handball (Lohmander *et al.*, 2007). These competitive team sports are known to require lower extremity dynamic stability to withstand the demands of cutting, decelerating, and jumping situations (Mandelbaum *et al.*, 2005). Alongside the increase in women's participation in sports the incidence of noncontact ACL injuries in women has risen in the last 30 years. In fact, ACL injuries have been extensively researched in female athletes and there is a consensus that they have a relatively higher risk than male athletes (Knowles, 2010).



Incidence of claims for ACL injury in youth soccer, as a percentage of all claims.

Reprinted, by permission, from K.G. Shea et al., 2004, "Anterior cruciate ligament injury in pediatric and adolescent soccer players: An analysis of insurance data," *Journal of Pediatric Orthopedics* 24(6): 623-628.



Number of ACL reconstructions performed by candidates for certification before the American Board of Orthopedic Surgeons in 2000.

Figure 2.1: Incidence data from the studies of Shea et al (2004) and Yu (2002)

As highlighted by Marshall *et al.* (2007), "the fact that males account for more injuries than females in the general population is almost certainly due to their greater exposure to athletic tasks that predispose on to ACL injury, such as cutting and jumping, and to contact sport" (p.11).

Research has shown that the long-term risk of suffering premature osteoarthritis (OA) is considerably greater among ACL injured athletes in comparison with athletes who do not suffer these severe injuries (Hewett *et al.*, 2009). ACL injuries place any person at a greater risk of suffering from knee OA later in life (Quatman and Hewett, 2009). The cost of this injury is estimated to be $17,000 including surgical and rehabilitation costs, and these costs can be considerably higher over the athlete's life span with long-term disability, sick leave, and the possibility of additional surgical procedures associated with the increased risk of re-injury (Hewett *et al.*, 2009).

2.2: Risk factors for ACL injury

Increased female sports participation has generated substantial discussion and investigation into the relative risk of injury, and research studies have increasingly examined sex differences or focused exclusively on female athletes (Knowles, 2010). Understanding the risk factors for ACL injury is essential to help develop strategies for identifying athletes at risk and developing injury prevention programs (Brophy *et al.*, 2010b). Briefly, risk factors for sport injury are traditionally described as extrinsic and intrinsic, referring to factors outside the body or factors inside the body (Knowles, 2010). Multiple internal and external risk factors may be associated with ACL injury, particularly in the competitive athlete (Brophy *et al.*, 2010b). For both sexes, sport is the most extrinsic factor for injury. It is clear that injury patterns can be sport-specific, and based on varying rules and regulations of sports. Unfortunately, no sport is risk-free and researchers are increasingly identifying distinct patterns of injury within an increasingly broad range of sports (Knowles, 2010). It is generally acknowledged that risk factors associated with ACL injury are multifactorial and complex and may be different in male and female populations, as well as sport specific (Hewitt et al., 2007). The ability to understand the mechanisms involved in ACL injury risk is further confounded in paediatric populations where issue relating to growth and maturation are relevant and complex. A recent editorial by Professor Sandra Shultz (2008) suggests that ACL injury in the female athlete is a multifactorial problem that remains poorly understood. Numerous reviews have outlined the main pre-disposing factors that contribute to the relative risk of injury in female youth footballers, however, most have ignored the complex interaction of growth and maturation on this relative risk (Mandelbaum and Putukian, 1999).

Regarding the intrinsic or internal risk factors, there are generally 3 theories to explain the differences in female and male knee injury rates: anatomical, neuromuscular and hormonal factors (Hewett, 2000). Because of anatomical differences, women are often thought to be more susceptible to injury than men. The reasons given for this include the altered hip- and knee-loading resulting from the wider female pelvis and greater genu valgum, less muscle mass and greater body fat content (Bartlett, 1999). The Quadriceps angle (Q-angle) has been studied as a possible explanation for sex difference in ACL injury rates, with the rationale that high Q-angles may be associated with an excessive valgus loading of the knee (Hewett *et al.*, 2009). Valgus angles at the knee are often coupled with decreased knee and hip flexion, and pronation at the subtalar joint in the female population (Brophy *et al.*, 2010a). Brophy et al. (2010c) have also reported, using 3D kinematics and EMG analysis that during kicking actions females exhibit lower activation of the hip abductors and greater hip adduction of supporting limbs than males. The Q angle is determined as the angle described by a line drawn from the anterior superior iliac spine through the centre of the patella and a line drawn from the tibial tubercle through the centre of the patella (see figure 2.2). It is an important measurement as it intends to predict the quadriceps forces acting on the patellofemoral joint. However, there are currently no standardised methods for assessing the Q angle but available methods range from using simple goniometers, to radiography and three-dimensional kinematics. A recent study by Stensdotter et al. (2009) using four different techniques in standing and supine positions has demonstrated significant differences between techniques and participant positioning. Therefore comparison of Q angles between studies that have used different techniques and positioning should be avoided. Stensdotter et al (2009) conclude that 3D kinematic analysis is the most reliable technique but that Q angle can vary depending upon the projection plane used. To date there are no studies that have examined changes in the Q angle during growth and maturation, which may be a contributing factor to the increased incidence rates during adolescence.

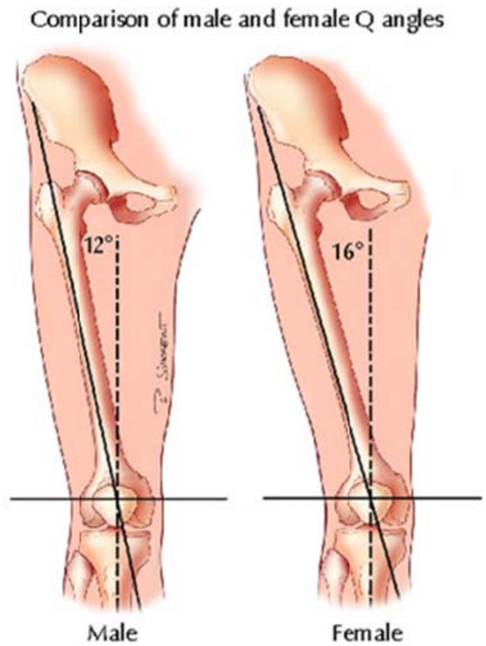


Figure 2.2: Sex differences and determination of the Q angle (The Bone Architect, 2011)

Evidence also suggests that female sex hormones may have significant effects on the female neuromuscular system. Oestrogen is known to both directly and indirectly affect the female neuromuscular system (Hewett, 2000). Unfortunately, even though the anatomical and hormonal components are useful in terms of understanding the phenomenon; they cannot be modulated, hence not offering any opportunity for intervention in this area (Hewett, 2000, Yoo *et al.*, 2010). However, linking changes in hormonal status during growth and maturation may help us to elucidate changes in the relative risk in female youth footballers during growth and maturation. This understanding may help us to identify best practice in terms of pre-habilitation at a given chronological age or maturation stage.

According to Bartlett (1999), sports participants with excessive ligament laxity, previous ligament injury, or poor muscle strength are particularly vulnerable to ligament injury. Regarding the neuromuscular theory, training and strength differences may account for only a portion of the higher incidence of knee injury in female athletes, but lowering these figures by even a small percentage could have a significant effect on the number of knee injuries in female athletes (Hewett *et al.*, 2005). Pre-participation training of the musculature which stabilises the knee joint may decrease the relative injury incidence in females (Hewett, 2000). Therefore, the neuromuscular background that renders the female athletes more susceptible to ACL injuries is a more attractive topic for research, because it can be improved by preventative training (Yoo *et al.*, 2010). Understanding these risk factors is important to develop intervention strategies and to identify those at increased risk of ACL injury who may especially benefit from targeted interventions (Brophy *et al.*, 2010b).

2.3: *Injury mechanisms*

Mechanically, ACL injury occurs when an excessive tension force is applied on the ACL (Yu and Garrett, 2007). There are two types of mechanisms that lead to an ACL injury: contact and non-contact. Only 30% of cases are caused by some kind of contact from an outside force; in a sporting environment this could be an opposing player, a goalpost or another object on the field/court. The remaining 70% of all reported ACL injuries are non-contact in origin (Silvers and Mandelbaum, 2007). A non-contact ACL injury occurs when a person themselves generates large forces or moments at the knee that apply excessive loading on the ACL (Yu and Garrett, 2007). The mechanism of a sports related non-contact ACL injury in football contexts commonly involves a one-step/stop deceleration, cutting movements, sudden change of direction, landing from a jump with inadequate knee and hip flexion (at or near full extension), or a lapse of concentration (resulting from a unanticipated change of direction of play) (Silvers and Mandelbaum, 2007).

The injury appears to predominantly occur in two particular types of movement: landing from a jump on one leg and rapid change of direction initiated on one leg, such as a side-cutting and cross-cutting manoeuvre (Hughes and Watkins, 2006). Both landing and pivoting are very common mechanisms of ACL injury, though they vary by sport (i.e., landing injuries may occur more often in basketball and cutting injuries may occur more often in football) (Hewett *et al.*, 2008). Non-contact ACL injuries usually occur during a deceleration manoeuvre combined with a change of direction while the foot is in a closed-chain position.

Given the individualised timing of growth and maturation of female youth footballers, there continues to be an urgent need to identify modifiable risk factors and better understand injury mechanisms (Knowles, 2010). Even though the mechanisms associated with female knee injuries may be multifactorial (i.e. hormones, anatomy, length of sports participation), training may be able to neutralise the effects of a number of these factors (Hewett, 2000).

2.4: *The anatomy of the knee*

It is beyond the scope of this report to describe fully the anatomy of the knee joint. However, understanding its articulation is important, given the growth in long bone during biological growth and maturation. The knee joint is a modified hinge joint formed by the articulation of the distal femur and the proximal tibia (Perrin, 1993). The knee travels principally from extension in the anatomical position, to flexion which brings the foot closer to the thigh (Kingston, 1996). It is a modified hinge joint in the sense that extension is accompanied by outward rotation of the tibia, and flexion occurs together with inward rotation of the tibia (Perrin, 1993). The knee is also the most complex synovial joint in the human body (Grimshaw *et al.*, 2006) and no fewer than 12 muscles cross the knee joint, contributing to both its stability and function. Knee extension is accomplished primarily by contraction of the quadriceps femoris muscles, which consist of the rectus femoris, vasti medialis, intermedius and lateralis muscles (Perrin, 1993); and has a major role in sprinting, jumping and ball-kicking (Lehance *et al.*, 2009). Knee flexion is produced by the hamstring muscle group, which consist of biceps femoris, semitendinosus and semimembranosus muscles (Perrin, 1993) and contributes hugely to stride power (Lehance *et al.*, 2009).

The ACL is one of the main supporting ligaments of the knee and it is responsible for supporting the knee in a movement known as anterior tibial translation, where the tibia is moved anteriorly (forward) with respect to the femur. In addition, the ligament also provides a degree of rotational stability to the joint (Grimshaw *et al.*, 2006, Hughes and Watkins, 2006). During everyday activities, the ACL is stressed in the first 45º of knee flexion then relaxes as the knee flexes further (Coombs and Garbutt, 2002). The ligaments, together with the muscles, provide joint support and stability and injury to these ligaments of the knee can seriously affect a player's career (Grimshaw *et al.*, 2006).

ACL injury occurs as a result of a lack of stability provided by the dynamic and passive stability mechanisms of the knee (Hughes and Watkins, 2006). Passive stability is provided by the non-contractile structures of the knee. These structures are the joint capsule, lateral and medial menisci and four extracapsular ligaments: lateral ligament, medial ligament, ACL and the posterior cruciate ligament (PCL) (Hughes and Watkins, 2006). Dynamic stability is related to muscular and neuromuscular quadriceps and hamstring actions at the knee (Ahmad *et al.*, 2006). Current evidence suggests that the greater incidence of ACL injury in females is due to sex differences in the dynamic stabilising structures rather than the passive stability structures (Hughes and Watkins, 2006).

2.5: Muscle, Tendon and Leg stiffness

Functional stability is provided by static and dynamic restraints within the joint and relies on feed-forward and feedback systems (Wikstrom *et al.* 2006). Feed-forward systems include anticipatory muscle actions occurring before the sensory detection of a disruption (Bonci 1999; Riemann and Lephart 2002). Conversely, feedback is the stimulation of a corrective response of the neuromuscular system after sensory detection. Riemann and Lephart (2002) found that joint stability and protection against injury relied on adequate feedback and feed-forward systems to improve muscular stiffness during functional tasks. Therefore, it is useful for club physiotherapists and sports therapists involved in injury prevention or rehabilitation to be able to accurately measure the amount of joint stiffness induced by the surrounding muscles. The use of neuromuscular biomechanical modelling to understand knee ligament loading and subsequent knee joint stability has emphasized the importance and effectiveness of the muscles in providing this stabilization (Lloyd *et al.* 2005). During ground contact, when potential to injure the ACL exists, the response of the neuromuscular system is critical. The ACL can provide up to 86 % of the resistance to anterior tibial translation. However it is well recognized that the internal and external forces incurred at the knee during landing can stress the passive ligament structures beyond their capacity. Muscular forces are crucial in maintaining joint stability predominantly by increasing joint stiffness through co-contraction of antagonistic muscles. *In vivo* exploration of hamstring or quadriceps co-contraction suggests that it can double or even triple joint stiffness and decreases joint laxity by up to 50% (Russell *et al.* 2007). Data from adults indicate that sidestepping and cutting movements are most likely to load the ACL and subsequently increase the relative risk of injury (Lloyd *et al.* 2005). There are no comparative data on children. However, it is likely that such movements in children demonstrate a similar pattern of ligament loading to that of adults. It may be that due to the development of kinematic motor control during childhood the growing child may be predisposed to additional ligament loads compared to adults. However, this suggestion requires further investigation, utilizing biomechanical modelling to explore ligament loading during childhood and during various dynamic tasks. It is beyond the scope of this Chapter to explore the effect of altering movement mechanics on ACL loading but adult studies seem to demonstrate that reduction in peak tibial shear force is possible through changes in knee flexion angle, shank angle and foot location during landing (Myers and Hawkins 2010).

The ability of the muscles to resist movement within the tibiofemoral joint (maintain a particular joint angle) refers to muscle stiffness. The stiffness of the whole limb is affected by both muscle and tendon stiffness as well as the elastic properties of joint structures and stiffness arising from muscle actions. Activation of agonist muscles play a substantial role in setting limb stiffness for 2 reasons: 1. Increases in muscle activation increase muscle specific stiffness (its resistance to stretch) because there are more cross bridges strongly bound; 2. Tendons increase in stiffness as they are stretched because the tendon-elongation relationship is non-linear, so increases in muscle force increase tendon stiffness simply because tendons are stretched (Blazevich et al. 2011). The greater the ability of the muscles of the knee to prevent tibiofemoral shear movement, the less likely the passive structures of the knee, such as the ACL, will be put under strain (Hughes and Watkins, 2006). If the strength of the quadriceps significantly exceeds the strength of the hamstrings, then both the hamstrings and anterior cruciate ligament (ACL) become more susceptible to injury (Holcomb *et al.*, 2007). Therefore, muscle stiffness is an important factor in preventing ACL injury (Hughes and Watkins, 2006) as co-activation increases as limb stiffness increases. This co-activation has been shown to be important for limb stiffness prior to jump landings from a height (Horita et al. 2002). Within the fields of sports physiology and biomechanics, leg stiffness is accepted as a recognised measure of lower limb SSC function (Kuitunen et al., 2002). Established correlations have been reported between higher levels of leg stiffness and greater running velocities and stride cadence (Farley and Gonzalez, 1996; $r > 0.8$), and economy of running gait (Dalleau et al., 1998; $r > 0.8$), all of which are important for football performance. Studies of lower extremity stiffness are becoming more commonplace within the fields of sports biomechanics and physiology as researchers attempt to gain a better understanding of the complexities of lower limb mechanics and its relationship to performance and injury prevention. Not only have high levels of leg stiffness been associated with increased metabolic economy whilst running sub-maximally (Dalleau et al., 1998), but it has also been considered as a critical component of maximal performance during explosive activities (Shorten, 1987; Walshe et al., 1996) and sprint performance (Bret et al., 2002). Interestingly, whilst there is a growing interest in determining leg stiffness the focus has predominantly on the role of leg stiffness in performance rather than injury prevention. This seems somewhat surprising given that high leg stiffness is required in order to absorb ground reaction forces, as well as to store and reutilise, elastic energy (Kuitunen et al., 2002).

Greater leg stiffness will enable a faster rebound when sprinting, reflected by shorter ground contact times at faster running velocities. Harrison et al. (2004) reported higher leg stiffness values in a cohort of sprinters ($5.86\ N{\cdot}m^{-1}$) when compared to a group of endurance runners ($3.38\ N{\cdot}m^{-1}$), thus highlighting the requirement of effective rebounding properties for high velocity running. This has also been highlighted in the paediatric literature, where drop jump performance was significantly related to both 30 metre and 100 metre sprint times (Hennessy and Kilty, 2001), which are important performance parameters in football. Additional research by Kram (2000) detailed the relationship between shorter ground contact times (which are associated with faster running velocities) and increased vertical ground reaction forces. Padua et al. (2006, 2005) highlighted several biomechanical parameters that control leg stiffness including; muscle activation and force, reflex contribution, antagonist muscle co-activation, lower extremity kinematics during ground contact and stiffness properties of the muscle and joint complex. It is the total measurement of these interacting components that enables researchers to determine the ability of an individual to perform SSC functions.

In terms of human performance, stiffness can be viewed within an isolated muscle or more commonly, the entire body acting as a mass and spring. Whilst recent studies have attempted to highlight the stiffness of a joint or tendon (Kubo et al., 2007; Kubo et al., 2006; Hortia et al., 2002), stiffness is typically viewed as the combination of independent values of a number of different components: muscle, joint, tendon, ligament, cartilage and bone (Latash and Zatsiorsky, 1993).

Lower limb stiffness has previously been measured via vertical jumping and hopping tests (vertical stiffness [kvert]), running and jumping protocols (leg stiffness [kleg]), or isolated joint movements to calculate the torsional correlate at an isolated joint (joint stiffness [kjoint]). When running, humans exert both a vertical and horizontal force component and therefore both factors are required to be considered in unison. Most studies have focused on whole limb stiffness but a recent study by Troy Blackburn et al. (2009a,b) has explored sex difference in hamstring structural and material properties, as a contributing factor in women's greater ACL injury risk. They reported that females have less hamstring stiffness compared to males and a reduced capacity for resisting changes in length imposed via joint perturbation from a structural perspective. Whether this structural sex difference in hamstring stiffness is evident during childhood remains to be investigated.

During repeated SSC activity, the human body is essentially acting as a spring, continually rebounding against the ground. Consequently researchers developed what is referred to as the spring-mass model (Cavagna, 1975). The model is deemed the simplest means of understanding the mechanics of human locomotion, whereby the musculoskeletal system symbolizes a single linear spring, and the total mass of the performer denotes a single point, acting through the centre of gravity (Farley and Gonzalez, 1996). Crucial to effective leg stiffness function in human locomotion is the relative stiffness of the series elastic component (SEC) (Horita et al., 2002).

The significance of leg stiffness parameters in augmenting dynamic movements has recently been highlighted (Kubo et al., 2007). Following research analysing the influences of tendon stiffness, joint stiffness and electromyographic activity on jump performance it was purported that tendon elasticity and an increased pre-activation level of muscle were both influential mechanisms for stabilising the joint prior to ground contact. This feed-forward mechanism is poorly understood in the growing child and to our knowledge there are no studies exploring this mechanism in youth female footballers. Additionally it is essential to know if these feed-forward mechanisms are compromised when fatigue is present as this may increase the relative risk of injury. If feed-forward mechanisms are influenced by fatigue then this may mean that neuromuscular feed-forward ability to stabilise the joint prior to ground contact (from either a jump, during a pivoting movement etc) is reduced.

There are a few studies that have explored the effects of fatigue on leg stiffness in adults, using a range of fatigue protocols, and the results are conflicting (Dutto and Smith 2002; Comyns et al. 2006; Girard et al. 2011; Morin et al. 2006). Some studies have shown an increase in stiffness after fatigue, some show a decrease in stiffness (Dutto and Smith 2002; Comyns et al. 2006) and some show no change in stiffness (Girard et al. 2011; Hunter and Smith, 2007; Morin et al. 2006). It is difficult to attribute these differing findings to mechanistic structures but they could be influenced by the nature of the fatigue protocol, and the methods used to calculate leg stiffness. Hunter and Smith

(2007) also state that the response of fatigue to leg stiffness may be very individualised and in their study some participants increased stiffness, some remained the same and some decreased stiffness. Data has also shown that differences in stiffness between children and adults are related to the speed of the movement (Oliver and Smith, 2010) and therefore this needs to be taken into consideration when comparing data from different studies. To our knowledge there are no studies that have explored the effects of fatigue on leg stiffness in children. Given data indicating that non-contact ACL injury is most likely to occur when fatigue is present, and that young girls appear to be an at risk group, it is somewhat surprising that there is no empirical evidence base exploring the role of feed-forward mechanisms in the neuromuscular control of knee stability when fatigue is present.

It is important to note that when exploring differences in leg stiffness between groups that normalisation procedures are used. This is particularly important in paediatric populations where issues of growth and maturation are paramount. Thus when normalizing leg stiffness it is necessary to determine data relative to both body mass and leg length (McMahon and Cheng, 1990), however the influence of leg length is often ignored. This is a pertinent issue when examining stiffness in children, owing to the non-linear variation in leg length development. Paediatric literature has revealed that children use a significantly slower hopping frequency, and produce corresponding lower levels of leg stiffness than adults (Oliver and Smith, 2010), however it is currently unknown how these variables differ between different age groups throughout childhood and adolescence.

The stiffness of the muscles and tendons are also influenced the delay between neural excitation of a muscle and rise in force (Electromechanical delay [EMD]). A greater compliance of the series elastic component (SEC) increases EMD and reduces rate of force development. Another important mechanism that influences movement co-ordination and injury risk is the influence of altering afferent feedback (feedback from muscle and tendon based receptors) on muscle tendon stiffness. This feedback includes afferent information from muscle spindles about muscle lengths and their rate of change, which are essential for the detection of limb position. Therefore differences in SEC stiffness based on age, maturation or influenced by fatigue could all influence individuals movement co-ordination and subsequent joint positioning to either increase or decrease the relative risk of injury.

It is expected that increases in body mass as a result of puberty will lead to increases in absolute leg stiffness in order to maintain true spring-mass model behaviour during ground contact (Granata et al., 2002). Additionally, alterations in limb length will also change the kinematics of rebounding against the ground (McMahon and Cheng, 1990). However, owing to the limited published literature for paediatric leg stiffness measures, whether the same trend exists for relative stiffness is unclear. Previous literature revealing growth-related increases in tendon elastic properties of the vastus lateralis (Kubo *et al.*, 2001) and age-related increases in musculotendon stiffness (Lambertz et al., 2003) suggest that SSC function is likely to be affected by biological maturity.

Owing to the neural regulation of SSC function, it is necessary to consider the potential implications that growth and maturity may have on neuromuscular adaptations. When considering the neural control of SSC function, mechanoreceptors (Golgi tendon organs and muscle spindles) present within the musculotendon unit are implicit in the recoil nature of the specific muscle action. Previous research has suggested that Golgi tendon organs undergo a process of desensitization, thus reducing agonist inhibition and enabling an increased pre-stretch of the muscle during the eccentric phase of the SSC, and a reduction in intramuscular Golgi tendon organ density (Ovalle, 1987). Grossett et al. (2007) also suggest the potential for age-related developments in intrafusal muscle spindles, thus increasing muscular contraction recoil velocity. Development of these properties result in increases in both the magnitude and velocity of the pre-stretch component, both of which are variables deemed to influence SSC function (Cronin et al., 2002). As a result of the development in intrinsic neuromuscular properties during childhood, the neural regulation of SSC is suggested as becoming more efficient as evidenced by increased musculotendon stiffness (Lin et al., 1997), muscle fibre twitch time enhancement (Lin et al., 1997) and rate of torque development (Grosset et al., 2005). Recent research has identified a number of neuromuscular properties were responsible for the longer ground contact times and greater angular displacements at the knee joint by children compared to adults (Lazaridis et al., 2010).

One factor that may influence muscle stiffness is muscle strength. Lower levels of muscle stiffness exhibited by females compared with males may be due, at least in part, to lower levels of muscle strength (absolute and relative) (Hughes and Watkins, 2006). Sex differences in strength are consistent, although small, through childhood and the transition into adolescence. Thereafter, the differences become increasingly larger so that at the age of 16 years and later only a few girls perform at the same level as the average boy (Wood and De Ste Croix, 2011). It could be argued that lower levels of strength may increase the relative risk of ACL injury (Hughes and Watkins, 2006). However, very few studies have examined the age and sex associated changes in limb stiffness and there appear to be no studies that have explored the role of fatigue in inducing changes in stiffness during childhood. Those data that are available indicate that both passive and active stiffness increases with age and can in part be attributed to the to the age related development in muscle force production (Grosset et al, 2005, 2007, Lambertz et al., 2003). There is an urgent need for more studies exploring the role of feed-forward mechanisms in the dynamic control of knee stability for injury prevention in children. Additionally how this mechanism is influenced by fatigue in female youth footballers of differing ages and maturational status may help us to describe the associated relative risk of injury in this population and contribute to the appropriate design and implementation of pre-habilitation strategies.

2.6: The role of the hamstring and quadriceps muscles (Muscular stability)

Quadriceps and hamstring muscle groups are involved in several important motor abilities such as running and jumping in sports such as volleyball and basketball (Bamac *et al.*, 2008). When performing dynamic movements such as landing and cutting (side-stepping), dynamic stability in the form of muscle activity is essential to provide adequate joint stability (Hughes and Watkins, 2006). Muscular contribution is very important to maintain joint stability and it is also helpful in the prevention of injuries.

At the knee joint, muscular stability is provided by the quadriceps (extensors) and the hamstrings (flexors) and the contribution of both muscles groups is crucial for the stability of the knee joint (Grimshaw *et al.*, 2006). As the quadriceps and hamstring muscles contract, they act in a way to increase the joint contact forces and limit shear movement within the tibiofemoral joint (Hughes and Watkins, 2006). The activity of the knee flexors and extensors should, ideally, result in a zero shear load on the proximal tibia, and, therefore, minimal strain should be caused on the knee ligaments. However, if the shear load exerted by the quadriceps is greater than the one exerted by the hamstrings, a resultant anteriorly directed shear force may be exerted on the proximal end of the tibia, which will increase ACL strain (Hughes and Watkins, 2006).

Agonist/antagonist muscle groups, such as the quadriceps and hamstrings, work together to control the joints that they cross (Osternig, 2000), in this case the knee joint. The agonist muscle is defined as the muscle that contracts while another muscle resists or counteracts its motion (i.e. the antagonist). The antagonist muscle is the one that offers a resistance during the action of the agonist muscle (Grimshaw *et al.*, 2006). The capacity of each muscle to produce adequate force to balance its antagonist is considered essential for joint stability (Osternig, 2000).

This muscle force production can take the form of both concentric and eccentric actions. Concentric action happens when the muscle tension is developed and the muscle shortens (Grimshaw *et al.*, 2006). In other words, when the muscles develop sufficient tension to overcome the resistance of the body segment, the muscles shorten and cause joint movement (Pitman and Peterson, 1989). Eccentric action is when muscle tension is developed and the muscle lengthens (Grimshaw *et al.*, 2006); or as Pitman and Peterson (1989) explained "when a muscle cannot develop sufficient tension and is overcome by the external load, it progressively lengthens instead of shortening" (pp.97). One purpose of the eccentric action is to decelerate the motion of a joint (Pitman and Peterson, 1989), for example the quadriceps works eccentrically when descending the stairs. As

Holcomb stated “an eccentric muscle action occurs when a muscle is required to lower a load with a controlled movement in the direction of the force of gravity” (2000, pp.229).

The eccentrically co-acting hamstrings have a dynamic role in maintaining the stability of the knee during forceful knee extension (Coombs and Garbutt, 2002). In fact, Aagaard *et al.* (1998) concluded that the hamstring muscles possess the capacity to provide significant dynamic joint stability during fast and forceful knee extension. This capacity for muscular knee joint stabilization is progressively augmented at gradually more extended knee joint positions and increasing angular velocity. Reduced function of the antagonist hamstrings due to activities that emphasize loads on the knee extensors may result in muscular imbalances between the hamstrings and quadriceps, thereby possibly predisposing athletes to injury (Rosene *et al.,* 2001).

2.7 Isokinetic dynamometry: Measurement of concentric and eccentric torque

Isokinetic dynamometers are used extensively within many forms of human movement for measuring concentric and eccentric torque, for example within sport and exercise settings to target training to develop specific muscles or muscle groups, and within rehabilitation and medicine settings following injury or muscle wasting (Grimshaw *et al.*, 2006). Regarding the knee joint, isokinetic testing can be used to evaluate quadriceps and hamstring muscle strength, providing a determination of the torque generated (Bamac *et al.*, 2008). The term isokinetic is a word used to describe muscle action when the rate of the movement (velocity) is held constant (Grimshaw *et al.*, 2006). Isokinetic testing has become more popular with paediatric populations and it is beyond the scope of this report to explore the role of isokinetic testing in children. It is however, important to note that as long as sound habituation/familiarisation techniques are in place, combined with appropriate protocol and equipment adaptation the isokinetic testing with paediatric populations if valid and reliable. The current available literature base suggests that extension movements are more reliable than flexion movements and that concentric actions are more reliable than eccentric actions. For further detail regarding issues of isokinetic testing with paediatric populations the reader is directed towards the reviews of De Ste Croix (2007) and De Ste Croix et al. (2003).

2.7.1 Conventional Hamstring/Quadriceps ratio (CH/Q)

The most frequently reported strength ratio of muscles of the knee has been the concentric hamstring-quadriceps ratio (CH/Q). Even though, there seems to be little consensus of a normative value for this CH/Q ratio, 0.6 appears to have gained some acceptance (Coombs and Garbutt, 2002). The CH/Q ratio is calculated by dividing maximal concentric knee flexor (hamstring) moment by the maximal concentric knee extensor (quadriceps) moment obtained at a given joint angular velocity (Aagaard *et al.*, 1998). However, as Coombs and Garbutt (2002) stated the use of peak moment ratios to describe normal knee function accounts for the main muscle groups of the thigh but has two major limitations. Firstly, the concentric quadriceps moment is normally compared with the concentric hamstring moment. This situation does not arise during functional movements. Secondly, usually they are cited irrespective of the joint angle at which they occur, ignoring the effect of muscle length. In conceptual terms, the CH/Q ratio implies that concentric (or eccentric) contraction would take place for the knee extensors and flexors simultaneously. However, true knee joint movement only allows eccentric hamstring muscle action to be combined with concentric quadriceps muscle contraction (during extension) or vice versa (during flexion) (Aagaard *et al.*, 1998).

2.7.2: Functional Hamstring/Quadriceps ratio (FH/Q)

During forceful muscle actions, antagonist muscles such as the quadriceps and hamstrings often co-activate, with one group providing directional movement via concentric action while the opposing group provides control and joint support via simultaneous eccentric action (Osternig, 2000). In other words, co-activation of these muscle groups is known to occur and takes place through opposing action modes. During leg flexion, the hamstrings contract concentrically (H_{con}) and the quadriceps eccentrically (Q_{ecc}); whereas when extending the leg, the quadriceps contract concentrically (Q_{con}) and the hamstrings act eccentrically (H_{ecc}) (Coombs and Garbutt, 2002). Therefore, the H/Q ratio should be described either as a H_{ecc}/Q_{con} ratio representing knee extension, or an H_{con}/Q_{ecc} ratio representing knee flexion (Coombs and Garbutt, 2002). As Aagaard *et al.* (1998) explained, the FH/Q ratio for knee extension is calculated by expressing maximal eccentric hamstring moment relative to maximal concentric quadriceps moment obtained at given angular velocity. Coombs and Garbutt (2002) stated that a higher H/Q ratio (conventional or functional) would represent greater balance, and of particular interest would be H_{ecc}/Q_{con} due to the high quadriceps moment production observed during knee extension. According to Coombs and Garbutt (2002), a H_{ecc}/Q_{con} ratio of 1.0 would be the recommendation.

2.7.3: Age and maturation effects on the H/Q ratio

There are few studies examining age and sex associated changes in FH/Q ratio. This may in part be due to the small number of studies that have determined eccentric forces in children. At least for the adult population there is an abundance of literature on isokinetic ECC torque production but fewer studies are available on children, especially including females (De Ste Croix *et al.* 2003). It is possible that the limited information on ECC strength capabilities of children may have arisen from the concern that ECC testing, with its potential for high muscle force production, might predispose children to higher risk of muscle injury. However, there is no reason to expect greater muscle injury with ECC actions in children compared to adults, provided they are given sufficient warm-up and familiarization (Blimkie and Macauley 2001).

As dictated by the force-velocity relationship the FH/Q ratio has been shown to increase as angular velocity increases in pubertal children, teenagers and adults for both sexes (De Ste Croix *et al*. 2007; Kellis and Katis 2007). The FH/Q ratio was significantly higher at 3.14 rad/s (1.12) compared to 0.52 rad/s (0.8). This increase in the ratio at higher velocities is due to the significant anterior tibial translation or shear at high quadriceps forces, and increase in internal rotation of the tibia in relation to the femur (Gerodimos *et al.* 2003). Irrespective of age or sex, the increase in co-activation of the hamstrings during high velocity movements significantly contributes to counterbalance this tibial shear or rotation. These data reinforce the notion that the FH/Q ratio is a more relevant estimate of the capacity for muscular knee joint stabilization than conventional ratios in children. It is important therefore that when making age and sex associated comparisons of the FH/Q ratio that movement velocity is taken into account. Meaningful interpretation of the FH/Q ratio in relation to age and sex has been problematic due to the order of action cycling in the isokinetic protocol. For example, during eccentric/concentric cycles theoretically the eccentric action may potentiate the following concentric action. Only one study appears to have examined angle specific FH/Q ratio in a small group of 13 year-old boys (Kellis and Katis 2007). Kellis and Katis (2007) reported that the FH/Q ratio increases near full knee extension due to the knee flexors' greater relative eccentric torque capability compared with the extensors' concentric torque. This suggests that young boys have reduced injury risk during fast velocity movements near full knee extension.

It cannot be assumed that the relationship between concentric and eccentric actions is the same across ages during childhood and puberty because children have immature neuromuscular systems as evidenced by the incomplete myelination of nerve fibres during childhood (Brooks and Fahey 1987). The few studies that have measured the FH/Q ratio in children have generally found significantly higher eccentric compared to concentric strength, depending upon movement velocity with ratios ranging between 0.84 at the slowest velocity to 1.47 at the highest velocity (De Ste Croix *et al.* 2007; Forbes *et al.* 2009; Iga *et al.* 2009; Kellis and Katis 2007). As can be seen in table 2.1, very few studies have directly measured the FH/Q ratio in children and there appears to be only one study that has included girls (De Ste Croix *et al.* 2007).

Gerodimos *et al.* (2003) reported a non-significant age effect on FH/Q ratios between 12-17 year-old trained male basketball players. Conflictingly, a cross sectional study of prepubertal children, teenagers and adults has reported a significant age effect for the FH/Q ratio at a relatively high angular velocity of 3.14 rad/s with 9-10 year-olds producing a significantly lower ratio (0.97) than teenagers (1.23) and adults (1.19) (De Ste Croix *et al.* 2007). Interestingly, the FH/Q ratio at the slower velocity (0.52 $rad.s^{-1}$) did not demonstrate a significant age effect. The functional ratio found below 1.0 for prepubertal children may be attributed to the inability of 9-10 year-old children to recruit their entire motor unit pool during eccentric actions (De Ste Croix *et al.* 2007). Kawakami *et al.* (1993) demonstrated a flattening of the force-velocity curve in the eccentric condition and hypothesized that boys might be too immature to recruit their entire motor unit pool compared to adults. This seems plausible as Weltman *et al.* (1986) reported that gains from resistance training in boys must be due to factors other than hypertrophy, such as reduced neuromuscular inhibition. The speculation that there might be a more pronounced force suppression in prepubertal children to protect their immature musculoskeletal systems is supported by the findings of the study of De Ste Croix *et al.* (2007).

Table 2.1: Paediatric Functional Hamstring/Quadriceps Ratio Findings

Evidence to support the fact that neuromuscular inhibition is present not only in eccentric actions but also in slow velocity concentric actions in children is provided by Seger and Thorstenson (2000) who found that EMG amplitude decreased with decreasing velocity of the concentric muscle actions, especially in prepubertal boys. Although there was no significant age difference in this measure, there was a trend for the largest decline in electromyographic (EMG) activity with decreasing velocity to occur in the boys (21%) and the smallest for the men (7%). No such trend was found in females. These findings suggest that at a velocity of 0.52 $rad{\cdot}s^{-1}$ there would also be inhibition during concentric actions as well as eccentric actions which could account for the non-significant difference in H/Q_{func} ratios from pre-puberty to adulthood during slow velocity movements.

Conflicting data are available, and a large cross-sectional study of elite male footballers from 12-18 years has demonstrated significant age affects in the FH/Q ratio with significantly lower ratios in 18 year olds (0.84) compared with 12 year olds (1.01) (Forbes *et al.* 2009). This reduction in FH/Q ratio with age is attributed to a relatively greater increase in concentric quadriceps torque compared with the relative increase in eccentric hamstring torque. This age related difference in the studies of De Ste Croix *et al.* (2007) and Forbes *et al.* (2009) may be reflective of limited focus on eccentric hamstring training in youth footballers. Recent work has confirmed that loading patterns experienced during soccer asymmetrically strengthen the muscles about the knee, altering the balance towards quadriceps dominance (Iga *et al.* 2009). Iga *et al.* (2009) demonstrated a training effect in 15 year-old football players on FH/Q ratio with lower ratios in conventionally trained footballers compared with resistance trained footballers and controls These data must be viewed with a degree of caution as the ratio is calculated using eccentric testing at 2.16 rad/s and concentric testing at 1.08 and 4.32 rad/s. The current conflicting findings on the age related changes in FH/Q ratio demonstrate how training status may influence the findings. However, a wider exploration of whether training status influences the FH/Q ratio or if ratios from joints other than the knee show different age and sex associated patterns is required.

Significant age and sex effects have also been observed for the functional quadriceps/hamstrings (Q/H_{func}) ratio during knee flexion (e.g. eccentric quadriceps / concentric hamstrings) at slow and fast velocity movements with adults demonstrating significantly lower ratios than prepubertal children and teenagers (De Ste Croix *et al.* 2007). These data suggest that adults have a reduced capacity for dynamic knee joint stabilization during forceful knee flexion movements with accompanying eccentric muscle actions. This may be attributed to a greater ability of adults to create large forces during eccentric actions due to fully mature musculoskeletal systems. It may also be that adults have different neural mechanisms that control eccentric muscle actions compared to children, but direct evidence to support this notion remains to be established. Although these data are of interest the knee is rarely injured during fast flexion movements when the quadriceps muscles are working eccentrically.

According to Ahmad *et al.* (2006), maturity with associated physiologic and hormonal changes may influence muscle strength and laxity. In their study they examined the effects of gender and maturity on knee laxity and muscle strength to determine the most appropriate time to initiate ACL injury–prevention programs. They observed significant effects of gender and maturity on quadriceps and hamstring strength and strength ratios (figure 2.3).

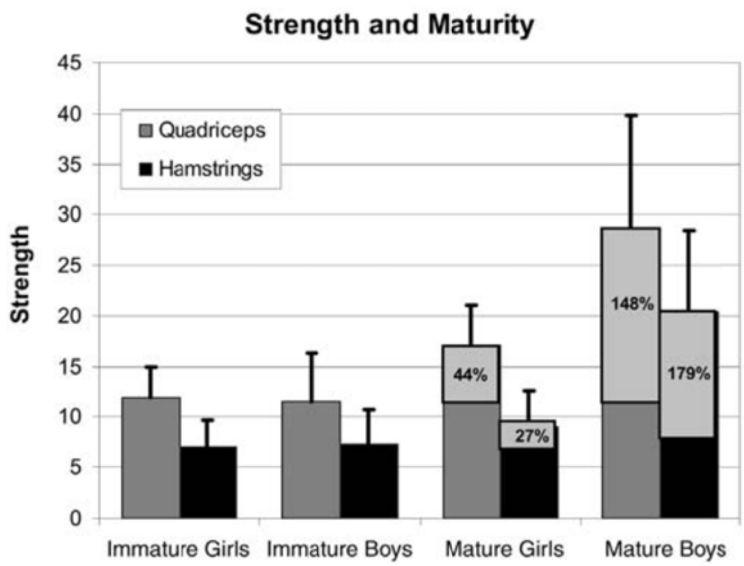


Figure 2.3: Quadriceps and hamstring strength for immature and mature boys and girls. (Ahmad *et al.*, 2006).

Even though the less mature male and female athletes had very similar ratios, mature girls had a greater quadriceps-to- hamstring ratio compared with immature boys, mature boys, and immature girls. They concluded that girls after menarche increased their quadriceps strength greater than their hamstring strength, which may put them at risk for ACL injury.

2.7.4: Sex differences in H/Q ratio – why girls are at risk

It appears that adult females demonstrate a quadriceps dominance, which in turn makes them more susceptible to injury. A high level of quadriceps strength compared to hamstring strength reduces the FH/Q ratio and a ratio of less than 55% may represent a quadriceps dominant athlete. It has been suggested that sex differences in adults in the FH/Q ratio of the knee joint are due to differences in neuromuscular recruitment during maximal voluntary actions, with women having a lower percentage recruitment than men during concentric actions but not eccentric actions (Westing and Seger 1989). However, there are limited data investigating sex differences in the FH/Q ratio in children.

In a pair-matched control study, Myer *et al.* (2009) determined the association of quadriceps and hamstrings isokinetic strength to ACL injury risk in female athletes. They measured the strength of the hamstring and quadriceps of 132 female and male collegiate soccer and basketball players, who were prospectively screened between 2002 and 2007. After that period of time, they reported 22 ACL ruptures (16 during soccer and 6 during basketball play). Those females who suffered an ACL injury had significantly decreased hamstrings strength, but similar, or not decreased, quadriceps strength, compared to matched control males. Those females who did not have an ACL injury however, had significantly decreased quadriceps strength, but not decreased hamstrings strength, compared to matched male athletes. These data can be seen in figure 2.4 below:

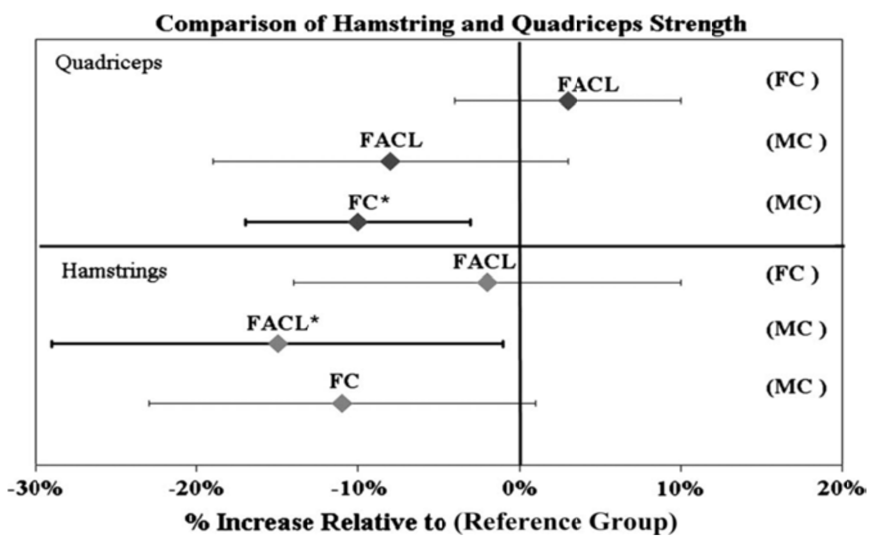


Figure 2.4: Percent increase in responses and 95% CI comparing study group to reference group. FACL, female ACL injured; FC, female control; MC, male control. *P , 0.05. FACL, female ACL injured; FC, female control; MC, male control (Myer *et al.*, 2009).

These finding indicates that decreased relative hamstrings strength and recruitment may be a potential contributing mechanism to ACL injury in high-risk female athletes. They concluded that female athletes who demonstrate the combination of decreased relative hamstrings and high relative quadriceps strength may be at increased risk for ACL injury.

Another factor that might explain these sex differences is the capacity to store and release elastic energy in the muscle-tendon unit during eccentric actions. There is evidence suggesting a superior ability of females compared to males in utilizing stored elastic energy in the muscle-tendon unit (Komi and Bosco 1978). Komi and Bosco (1978) discovered that female participants were able to utilize significantly more of the energy produced in the pre-stretching phase compared with males. However, the cross-sectional study of De Ste Croix *et al.* (2007) appears to be the only study that has examined sex differences in the FH/Q ratio in children. These authors reported no significant sex differences in FH/Q ratio at either test velocity in prepubertal, teenage or adults. These findings may in part be attributed to that fact that isokinetic concentric/eccentric tests do not allow a great deal of elastic energy storage and thus any potential benefits that females may have in utilizing stored elastic energy is not evident. More longitudinal data are required to reinforce whether or not there are sex differences in the FH/Q ratio throughout childhood. However, based on very limited data concerning sex differences in the FH/Q ratio we might speculate that sex differences in the relative risk of non-contact ACL injury may be attributed to factors other than those that are muscular in nature. These data would suggest that neuromuscular recruitment plays a crucial role in the sex difference related to relative risk of injury during childhood.

Interestingly, the same study reported significant sex differences for the FQ/H ratio at slow and fast movement velocities, irrespective of age, with males demonstrating significantly higher ratios than females (De Ste Croix *et al.* 2007). Females had similar quadriceps eccentric muscle torque compared to males and the lower ratio can be attributed to a lower capacity for hamstring concentric actions. It is generally accepted that females have a greater capacity for generating eccentric torque compared to males (Seger and Thorstensson 2000). However, females irrespective of age or movement velocity, appear to have a lower capacity for generating concentric hamstring torque than males. It is speculated that this trend is due to lower motor unit activation during concentric actions in females (Westing and Seger 1989). In an applied sense, these data suggest that stabilization of the knee joint during flexion movements, with high eccentric knee extension actions, is lower in females compared to males during childhood and into adulthood.

One of the biggest issues with the current literature examining the FH/Q ratio during childhood is that no studies appear to have determined the functional ratio at the point where knee injury is most likely to occur (0-30º of full knee extension). All of the studies reported in this Chapter have used a peak torque value for concentric quadriceps torque and divided by eccentric peak torque value for eccentric hamstring actions. This may not accurately represent the functional ratio as it is likely that the angle at which PT occurs for each of these actions and muscle groups is different. The recent work of Forbes *et al*. (2009) highlights this issue where angles of peak torque for concentric quadriceps ranged from 72 - 78º in 12 to 18 year-olds compared with eccentric hamstring angles which ranged from 31-38º. Therefore, within current literature, concentric and eccentric torques determined at different joint angles are used to represent the FH/Q ratio, rather than torque achieved at the same joint angle. This clearly does not help in elucidating the functional role that these muscle play in stabilizing the knee during childhood. Furthermore, the joint angle where non-contact ACL injury is mostly likely to occur is not at the point where peak torque is generated. Peak concentric and eccentric torque production is likely to occur in the mid-late range of the movement (around 30-80º of knee flexion), whereas it is well recognized that injury is likely to occur when the knee is closer to full extension (0-30º). Based on this knowledge, it would seem more appropriate to calculate the FH/Q ratio using angle specific torque values close to full extension. It is clear that more data are required on the FH/Q ratio during childhood, especially using angle specific data and in females. Whether this will change our understanding of the age and sex associated changes in dynamic knee stability and the susceptibility to knee injury during childhood remains to be established.

In their review of literature Hewett *et al.* (2008) wanted to determine if there were differences in CH/Q ratios between sexes and to evaluate the effects of increased isokinetic velocity on these measures. They found that at slower testing velocities, no gender differences in CH/Q ratio were observed. However, with increased knee flexion/extension angular velocities, approaching those that occur during sports activities, significantly greater CH/Q ratios were observed in male than female athletes. They concluded that females, contrary to males, do not increase the hamstring to quadriceps torque ratio at high velocities during seated open-chain isokinetic activity.

Even though Myer *et al.* (2009) and Ahmad *et al.* (2006) measured the CH/Q ratio, females that suffered ACL injury had a lower ratio values compared to non-injured females and matched control males. Low or lowered values for FH/Q ratios representative of knee extension (for example, as the result of increased concentric quadriceps strength) may indicate that more high-resistance strength training should be performed for the knee flexors, preferably including eccentric muscle contraction (Aagaard *et al.*, 1998).

2.8: Neuromuscular control and knee stability

The effectiveness of the active muscular control system in working synergistically with the passive joint helps to create dynamics knee stability. Any neuromuscular imbalances that limit this partnership may increase the risk for an ACL injury (Myer *et al.,* 2004). When the function of the ACL in controlling anterior tibial translation is considered, the hamstring muscle, in particular, becomes of interest (Huston and Wojtys, 1996). At low knee-flexion angles (0 to 30° of knee flexion), quadriceps contractions pull the tibia forward and increase stress on the ACL, especially without balanced knee-flexor (hamstring and gastrocnemius) co-contraction to decrease strain on the ligament (Myer *et al.,* 2004). Myer *et al.* (2009) stated that adequate co-contraction of the knee flexors may help balance active contraction of the quadriceps that can compress the joint and assist in the control of high knee abduction torques or valgus collapse. They also suggested that developing an appropriate neuromuscular control may prevent the critical loading necessary to rupture the ACL during manoeuvres that place the athlete at risk for an injury.

There is still some debate as to whether reflective muscular activation and joint stiffening can occur quickly enough to protect the joint once a large force is applied to the ligament, and data during childhood are particularly sparse. There are few studies that have examined the age and sex associated changes in knee muscle activation during landing or pivoting tasks. Russell *et al.* (2007) determined co-contraction ratios (CCR) of the hamstrings and quadriceps during a landing task in prepubertal children compared with those of adults. They reported significant age differences in relation to pre-landing CCR, indicating that adults pre-activated their hamstring muscles prior to landing to a greater extent than children. This suggests that this feed-forward mechanism is more mature in adults compared with children. However, there were no age- or sex-related differences in activation levels during the reflective or voluntary muscle activation phases. These data are supported by work of Lazaridis *et al.* (2010) who also demonstrated higher and longer pre-activation in adult males compared with prepubertal boys for the calf muscles. They provide an interesting insight into landing mechanisms during the prepubertal years. As the incidence rate of ACL injury is smaller in prepubertal children than in adults, they probably rely on different strategies to control the forces of landing. This may include relying on a proximal strategy (one that uses the large muscles in the hip and torso) as opposed to a knee and ankle strategy (Russell *et al.* 2007). As there appear to be no comparable data to support this view it remains an interesting but unproven hypothesis.

EMD has been implicated as a risk factor for knee injury in adults (Troy Blackburn *et al.* 2009). Figure 10.2 demonstrates one method of calculation of EMD assuming the onset of electrical activity as a ±15 μV deviation from baseline measurement to the onset of force production which is assumed as 9.6 Nm. Data from adults suggest that EMDs vary between 30 to 50 ms up to as much as a few hundred milliseconds depending on the muscle examined and movement velocity (Shultz and Perrin 1999).

Considering this time lapse and the need to develop sufficient muscle tension rapidly enough to provide dynamic knee stability, EMD should be considered when evaluating muscular responses to an imposed perturbation or injurious stress. It is important to note that it has been suggested that EMD is influenced by a number of factors including the transmission of muscle force through the series of elastic component (SEC), changes in the ability of the action potential to propogate and the properties of the excitation contraction coupling process (Howatson 2010). Recent adult data comparing sex differences in EMD of the hamstrings during eccentric muscle actions showed no significant sex difference (Troy Blackburn *et al.* 2009). However, conflicting data are available for other muscle groups (Bell and Jacobs 1986). Very few studies appear to have examined EMD during childhood (Cohen *et al.* 2010; Falk *et al.* 2009; Grosset *et al.* 2010; Zhou *et al.* 1995) and there appears to be only one longitudinal study.

The work of Gosset *et al.* (2010) focused on ankle stiffness and EMD of the triceps surae in normal and diseased children. They reported a greater EMD in children suffering with Legg-Calve_Perthes disease (a hip disorder) compared to healthy controls, albeit only in 6 children. Cohen *et al.* (2010) also found significantly greater EMD in young children (9-12 years) compared to adults (65ms vs 57ms) for knee and elbow extension and flexion during isometric actions. However, they did not show any significant difference between the endurance trained and untrained children suggesting that level of training status did not have an effect on EMD in 9-12 year olds.

Falk *et al.* (2009) reported a significantly longer EMD in prepubertal boys compared with adult males and Zhou *et al.* (1995) found significantly longer EMD values in 8-12 year-olds (61ms for boys; 58ms for girls) compared with 13-16 year-olds (44ms for boys; 47ms for girls) and adults (40ms for males; 46ms for females). This longer EMD in children may be as a result of differences in muscle composition. However, current limited evidence suggests that differences in muscle composition are not sufficient to account for the child-adult differences. Therefore differences in muscle activation, such as excitation-contraction coupling and muscle fibre conduction velocity have been implicated in this longer EMD. Therefore this lower rate of force development may reduce muscle-tendinous stiffness and increase the potential for injury in children. However, Grosset *et al.* (2007) reported greater electrically stimulated EMD for the triceps surae in 7 year-old compared with 11 year-old prepubertal children indicating a potential increase in muscle-tendinous stiffness with age independent of maturation. However, this hypothesis requires further investigation employing longitudinal studies throughout childhood and including female participants.

A number of adult studies have suggested that males demonstrate a shorter EMD compared to females and have attributed this to greater musculo-tendinous stiffness in males (Troy Blackburn *et al.* 2009; Zhou *et al.* 1995). Conflicting data from our laboratory on adults have indicated no sex difference in EMD of the hamstrings during eccentric muscle actions over a range of velocities (De Ste Croix *et al.* unpublished observations). Only one study appears to have explored sex differences in EMD of the knee extensors during isometric actions (Zhou *et al.* 1995) during childhood, and no sex differences were reported in either the 8-12 year-old or 13-16 year-old age groups. Whether

EMD accounts for the greater relative risk of non-contact ACL injury in girls is unclear as there are no studies that have examined sex differences in EMD in children during eccentric muscle actions. It is surprising that there is only one study on the EMD of young girls given that they are the most at risk group for non-contact ACL injury. It remains to be identified how EMD may change during childhood, particularly for the knee flexors and eccentric actions. Further study is needed to explore the age and sex related changes in EMD, linked to the relative risk of injury.

2.9 Effect of fatigue on dynamic knee stability

After a fatiguing exercise bout, biomechanical and neuromuscular factors such as muscle activation patterns, co-activation, kinematics and kinetics, and stiffness properties are altered (Padua *et al.* 2006). Adult studies have reported that submaximal fatigue not only increases anterior tibial translation but that this is accompanied by significantly longer latency of the hamstring muscles, subsequently decreasing joint stability (Melnyk and Gollhofer 2007). In a fatigued state, adults also use antagonist inhibition strategies by reducing hamstring activation (Padua *et al.* 2006). The work of Padua *et al.* (2006) demonstrated greater co-activation ratios in females compared to males in a fatigued state and also suggest that adults move to an ankle dominant strategy compared to knee strategy to protect the knee on landing. It is well recognized in the available literature that injury to the ACL appears to be more prevalent in the latter stages of sporting performance and most likely when muscle fatigue is present (Small *et al.* 2010). For example a recent study has indicated that the FH/Q ratio significantly decreases at the end of each half of a soccer match using a simulated soccer specific fatiguing task (Small *et al.* 2010). There appear to be no comparable data available during childhood but if fatigue has a similar effect on children then the ability to resist fatigue and maintain joint stability should form a major part of prevention programmes. Work by Kawakami *et al*. (1993) suggested that at least for the elbow flexors, concentric and eccentric torque production decreases at a similar rate with advancing muscular fatigue in 13 year-old boys. These limited data would suggest that the FH/Q ratio would remain similar in the fatigued and non-fatigued state in children. However, this suggestion requires further investigation, especially on the knee joint and using female participants across the age range.

Available data are reasonably consistent indicating that children fatigue at a slower rate than adults during either a single or repeated bout of high intensity exercise (De Ste Croix *et al.* 2009; Ratel *et al.* 2006). The ability of children to maintain force output during repeated bouts of high intensity exercise may be related to lower levels of fatigue, which in turn may be reflective of different muscle characteristics compared to adults. Proposed mechanisms for the greater fatigue resistance in children compared with adults include: a) children use oxidative pathways quicker than adults and therefore lead to a lower accumulation of by-products, b) children’s lower ability to activate their type

II muscle fibres and c) possible faster phosphocreatine resynthesis, improved acid base regulation and faster removal of metabolic by-products. Similar mechanisms have been proposed for a possible sex difference in fatigue resistance with females showing less fatigue than males during repeated high intensity tasks (Hicks *et al.* 2001). One of the few studies to examine this sex difference in young children and adults found no sex differences in either age group (De Ste Croix *et al.* 2009). However, most of these data are from limited sources that do not adequately cover the period of maturation and have used varying methods to induce fatigue. Importantly no available studies have examined the effect of eccentric fatigue on eccentric torque production during childhood or between the sexes.

Data on the effects of muscle action specific fatigue on either the FH/Q ratio or EMD are sparse. It is well recognized in adults that fatigue affects eccentric and concentric actions differently and that generally, eccentric actions are more fatigue resistant than concentric actions (Roig *et al.* 2009). Therefore, in a fatigued state the FH/Q ratio should increase and the knee should be more stable. However, no studies appear to have reported angle specific FH/Q ratio after fatiguing tasks. Interestingly a recent study on adults suggests that heavy intensity aerobic training reduces the FH/Q ratio but does not affect the FH/Q_{con} ratio which points to greater fatigue during eccentric muscle actions (Oliveira *et al.* 2009). Robineau et al. (2012) recently reported on neuromuscular fatigue induced by a simulated 90min football match in a very small sample (n=8) of amateur male players. They found significant reductions in both concentric (12.2%) and eccentric (25.4%) torque production of the quadriceps both at half time and at the end of the simulated match. Unfortunately they did not determine eccentric torque of the hamstrings but did also show a decline in the eccentric torque production of the quadriceps (13.3%). Interestingly they found a significant decline in quadriceps EMG activity only at the end of the match by no significant changes in the EMG activity of the hamstrings.

Howatson (2010) examined the chronic effects of eccentric fatigue and muscle damage on the biceps brachii in male adults and reported significantly greater EMD up to 96 hours following the exercise bout. This finding was despite an apparent return of muscle function and suggests that caution should be taken if a task requiring a fast reaction time or fast generation of high forces is needed following this type of exercise. Zhou *et al*. (1996) also demonstrated a significant increase in EMD following 4 bouts of 30s all out cycling exercise in adult males. All of the available adult studies seem to show a significant increase in EMD after fatiguing trails which would predispose the knee to greater injury risk. The mechanisms involved in the increase in EMD after fatigue could be due to the deterioration in muscle conductive, contractile or elastic properties and requires further study. Unfortunately, there are no current data available that permit us to explore the age and sex associated changes in EMD after fatiguing exercise in children. This data is urgently needed to elucidate the relative risk of non-contact knee injury during childhood when the child is in a fatigued state.

2.10 Conclusions

Despite evidence that the incidence rate of ACL injury per hours of exposure is most prevalent in 13-18 year old girls there is a surprising lack of empirical data on this age group. The mechanisms that are associated with this increased risk are poorly understood, even though muscular and neuromuscular mechanisms have been shown to be important in reducing the relative risk of injury. As ACL risk is multifactorial it is important to critically examine the underlying causes related to the relative risk in female youth athletes. Limited evidence suggesting that females are more quadriceps dominant than males and that traditional football training may predispose individuals further to quadriceps dominance then they is an obvious need to explore FH/Q ratios and neuromuscular functioning in this age group. This aligned with evidence surrounding incidence and fatigue means that exploring these mechanisms when fatigue is present as paramount. By utilising complex study designs, and examining a range of muscular and neuromuscular outcome variables in relation to fatigue, we can start to build a more comprehensive and accurate understanding of the most relevant risk factors that negatively affect weight bearing knee joint function. This should then enable us to identify potential risk factors and implement appropriate age and maturation based training strategies to reduce the relative risk of injury – Protect Her Knees.

Research Questions and Objectives

Research Questions

1. To what extent does football specific fatigue influence the functional hamstring to quadriceps ratio, leg stiffness and electromechanical delay in female youth football players?

2. To what extent do age and maturation related differences influence the functional hamstring to quadriceps ratio, leg stiffness and electromechanical delay after football specific fatigue in youth female football players?

3. To what extent are muscle specific compensatory mechanisms in place following football specific fatigue in youth female football players?

Research Objectives

1. Explore the effects of football specific fatiguing task on the muscular (H/Q_{FUNC}) and neuromuscular (EMD) components of dynamic knee stability that relate to the relative risk of knee/hamstring injury in young girls.

2. Investigate whether any age and maturation related differences in key outcome measures exist after football specific fatigue.

3. To explore whether there are muscle specific compensatory mechanisms that contribute to knee stability in a fatigued state.

4. To propose appropriate age/maturational related training to improve dynamic knee stability when fatigue is present in young female footballers

Methods

4.1 Participants

36 females aged 12-18 y from an FA Women's Super League Team professional youth academy were recruited to participate in this study and both player and parent written informed consent was obtained (see appendix). Verbal consent was also obtained from the club prior to approaching players. Participants were given an information sheet to explain the procedures involved as well as receiving verbal instructions (see appendix). Players in each squad were recruited from the three age groups U13's (n = 14), U15's (n = 9) and U17's (n = 13). Participants were instructed to avoid their regular training regimens throughout the experimental period and not to take part in any vigorous physical activity 48 hours proceeding each testing day. All participants completed a health questionnaire (see appendix) in accordance with the University of Gloucestershire Sport and Exercise Laboratory procedures. Acceptance to the study was approved if the participants satisfied the acceptance criterion as described in the health questionnaire flow chart. There were 2 exclusion criteria in this study: (1) histories of orthopedic problems, such as episodes of hamstrings injuries, fractures, surgery or pain in the spine or hamstring muscles over the past six months; (2) self reported presence of delayed onset muscle soreness (DOMS) at a testing session. Participants were also instructed not to: 1) Drink or eat anything other than water in the final 3 h before each visit; 2) Drink alcohol in the final 24 h before each visit; drink caffeine within the final 12 h before the test. None of the participants reported any form of musculoskeletal disorder at the time of testing.

4.1.1: Ethical Considerations

Ethical approval was obtained from the University of Gloucestershire's Research Ethics Committee (RESC) (see Appendix A). RESC approved laboratory procedures and University guidelines for working with children were followed at all times and all researchers involved in the study had obtained a Criminal Record Bureau (CRB) check before data collection began (Criminal Records Bureau approval is required in the UK before undertaking any work with children).

Participants were made aware that they could withdraw from the study at any point without affecting their relationship with the University, research team or the club. All the data collected was stored on a computer using an ID code and only accessible by the research team or club if prior consent was given (the players provided additional consent for the club to have access to their data). The participants name will not be used and any hard copies of data were stored in a lockable draw and only available to the principal investigator.

4.2 Study Design

Participants were required to visit the laboratory at the University on 2 separate occasions. An additional testing session took place during a training session and involved repeated hops on a portable contact mat to calculate leg stiffness (figure 4.1).

The purpose of the habituation session was to familiarise the participant with the testing protocol on the isokinetic dynamometer as well as an introduction to the SAFT90. During this session anthropometric data was also collected in order to determine maturational status defined as years from peak height velocity (Mirwald et al. 2002), as well as static 'Q' angle.

During session 3 participants performed baseline isokinetic/EMG tests in a pre-fatigued state followed by the football-specific fatigue task. Immediately post the football specific fatigue task repeat leg stiffness, isokinetic, and EMG tests were performed.

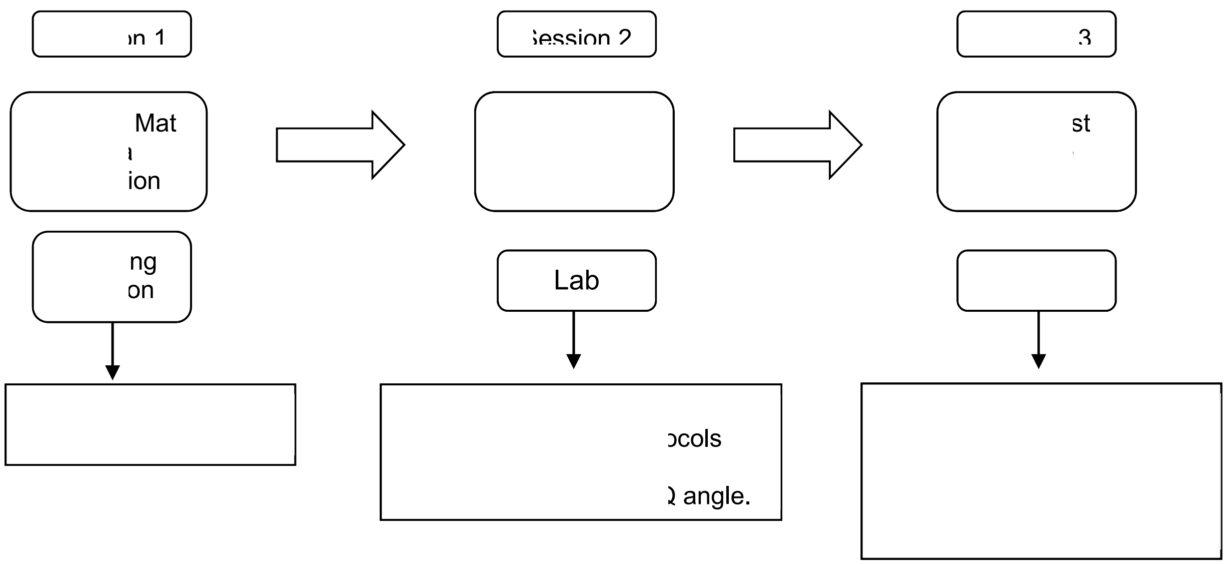


Figure 4.1: Research design schematic

4.3 Procedures

4.3.1 Anthropometry

Age was computed from date of birth and date of testing. Stature and body mass were measured on the first visit to the laboratory according to the procedures of Weiner and Lourie (1988) using a Stadiometer (Holtain Harpenden, Crymych, UK) and calibrated balance beam scales (Weylux Birmingham, UK). Sitting height was measured with a Holtain Stadiometer (Crymych, Dyfed, UK) with the posterior surface of the knee tight against the measuring box. Age from PHV was determined using the following equation of Mirwald et al. (2002):

For females: Maturity offset = − 9.376 + (0.0001882 x (leg length x sitting height)) + (− 0.0022 x (age x leg length)) + (0.005841 x (age x sitting height)) + (0.002658 age x weight) + (0.07693 x (mass by stature ratio))

4.3.2 Leg stiffness

Participant performed 4 sets of sub-maximal two-legged hopping with 3 min rest between sets at a frequency of 2.0 Hz. Participants were asked to hop two-legged on top of the contact mat for a period of 20 consecutive hops in each set, at each given frequency. Hopping frequency was maintained by an audio signal from a quartz metronome (SQ-44, Seiko, UK). Monitoring of hopping frequency using the digital metronome, as opposed to allowing participants to self select their preferred frequency, enabled greater control of movement coordination in the lower extremities whilst hopping. The choice of 2.0 Hz was within the boundaries previously highlighted as allowing the broadest possible range of hopping frequencies (1.5 Hz – 3.0 Hz; Hobara et al., 2008) and has been advocated in previous studies using paediatric populations (Lloyd et al., 2009). Frequencies below 1.5 Hz have led to an inability of maintaining true spring-mass model behaviour in the lower extremities (Farley et al., 1991), whilst frequencies above 3.0 Hz have prevented the successful maintenance of desired hopping pace (Hobara et al., 2008). Additionally, owing to the fact that those studies used adult populations, the choice of 2.0 Hz was deemed more attainable and sustainable for paediatric populations, who may arguably utilize less consistent movement patterns. All jumps were performed on a mobile contact mat (Smartjump, Fusion Sport, Australia), and data instantaneously collected via a hand-held PDA (iPAQ, Hewlett Packard, USA). Leg stiffness was calculated from the sub-maximal hopping test using the following equation:

$$K_N = \frac{M.\pi(T_f + T_c)}{T_c^2\left(\frac{T_f + T_c}{\pi} - \frac{T_c}{4}\right)} \qquad \text{(in N x m}^{-1}\text{)}$$

Where K_n is the leg stiffness, as calculated using a contact mat, M is total body mass, T_c the ground contact time and T_f the flight time (Dalleau *et al*., 2003).This method has been previously shown to be reliable in paediatric populations (Lloyd *et al.,* 2009). Relative leg stiffness will be determined by normalising absolute stiffness by body mass and leg length to account for group/individual differences and this is a dimensionless value.

4.3.3 Determining the 'Q' Angle

The Q angle was determined using whole body kinematics with the participant in a static standing position. Six high speed cameras (Proreflex, Qualysis, Gothenburg) using infrared light to detect 19mm and 30mm diameter spherical reflective markers were placed on anatomical landmarks on the participant. These landmarks were: lateral boarder of the acromion, sternum, sacrum, spina iliaca anterior superior, trochanter major, immediately superior and central to patella, mid-lateral knee joint line, tibial tubercle, lateral malleolus, between second and third metatarsal heads and on lateral on fifth metatarsal head (see plate 4.1). Exposure time was 2s and a sampling frequency of 240Hz was used. Marker identification was undertaken using the Qualysis Track Manager software and 3D analysis performed in visual 3D. A virtual marker for the centre of the patella will be used as the software model does not provide a position for this marker. The virtual marker is positioned 60mm above the tibial tubercle. The local co-ordinates for each segment were determined for analysis of the segmental rotations for the femur and tibia. The quadriceps angle was calculated for each participant using the Qualysis Track Manager software. The markers for the anterior superior iliac spine, the patella and the tibial tuberosity of the ipsilateral side were identified from the static postural analysis trial and entered as components of an angle analysis calculation.

Plate 4.1: Placement of reflective markers

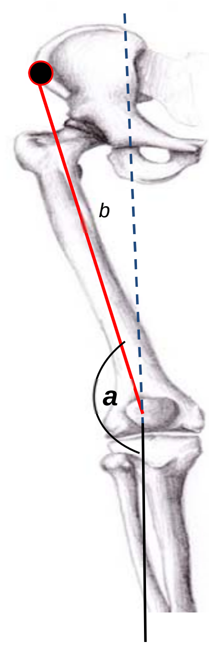


Figure 4.2: Calculation of Q Angle

Qualysis Track manager then provided a graph, plotted to demonstrate the trajectory of the three markers, over the 10s trial. The data was then exported to a text file in a numerical format which was then transferred onto an excel worksheet. The mean was then calculated from this data to provide angle *a.* This was then subtracted from 180º to determine the quadriceps angle (*b*) (see figure 4.2). The procedure was then repeated on the non dominant side.

4.3.4 Functional Hamstring/quadriceps ratio

Peak torque assessments were made on the dominant leg, determined by kicking preference. Tested range of motion was from 90° to 0° of knee flexion (0° = full voluntary extension), determined for each participant individually by placing mechanical stops at the beginning and end of their full active range of motion. The range stop control was set as soft so as to reduce the possibility of sudden resistance at the end of each range of motion. Gravity corrections for limb mass were performed before each isokinetic assessment in accordance with the manufacturer's instructions (Biodex Pro Manual, Applications/ Operations, Biodex Medical Systems, Inc., Shirley, NY). Therefore, at the start of each test session the participant relax their leg so that passive determination of the effects of gravity on the limb and lever arm could be accounted for.

All assessments are performed in a prone position for both concentric and eccentric testing (Figure 4.2). Testing occurred at a slow (60˚/s), intermediate (120˚/s) and fast angular velocity (180˚/s) for both concentric and eccentric actions with extension undertaken first. Testing always started with the slowest angular velocity (60˚/s) and continued with increasing angular velocity (120˚/s followed by 240˚/s) to reduce the risk of injury (Gaul, 1996). Concentric quadriceps muscle strength was determined during dual concentric-concentric actions and the passive eccentric mode was used to determine eccentric torque during ECC/ECC cycles. The passive eccentric mode was used as previous work with paediatric populations has indicated that children have difficulties in maintaining consistent torque resistance throughout the entire range of movement in the reactive eccentric mode. This in turn causes stalling of the lever arm and subsequently influences the calculation of average torque. In the concentric quadriceps measurement participants were instructed to push the lever arm up, and pull it down as hard and fast as possible. However, in the eccentric hamstring measurement participants were instructed to resist the lever arm as hard and as fast as possible.

Participants performed three maximal efforts at each angular velocity with a 30 s rest period between movements at different angular velocity. In particular it was stressed to participants the importance of either pushing, pulling or resist the lever arm as hard and as fast as possible throughout the entire range of motion. Standardised verbal encouragement was given during each maximal effort and visual feedback of the recorded torque provided. Peak torque values were recorded during concentric quadriceps and eccentric hamstrings action and later used to calculate the FH/Q ratio by expressing average eccentric hamstring torque to average concentric quadriceps torque over three knee angles (0-10°, 10-20° and 20-30°) and at three angular velocities (60˚/s, 120˚/s and 180˚/s).

Plate 4.2: Prone position adopted during isokinetic testing

4.3.5 Electromyography

Electromyography was quantified with an 8-channel DelSys EMG telemetry system (DelSys Myomonitor III, DelSys Inc., Boston, MA, USA) to investigate the activity of the hamstrings during the eccentric hamstring movement to determine the EMD. For maximum signal detection each bipolar surface electrode (DE- 2.3 MA; DelSys Inc., Boston, MA, USA) was positioned in the mid-line of the belly of the muscle perpendicular to the muscle fibres because in this location the electromyographic signal with the greatest amplitude is detected (Gleeson, 2001).

The Biodex square wave synchronization pulse is configurable via the Biodex ASA software allowing the triggering of the EMG software. The EMG system signal was interfaced to the Biodex via a trigger box. The EMG works software offers full triggering capabilities to control the start and stop of all data acquisition systems in a given experimental setup. The Biodex ASA software is used to activate the Biodex square wave signal output to trigger the start signal. The Trigger Module only accepts signals that are between 0 to 5 volts, and could be configured for either positive-edge signals or negative-edge signals. Positive-edge or "rising" was defined to start from 0V and rise to 5V. The transition points of these voltages were defined as the event. Once the 5V level is reached, the duration of the trigger pulse is kept in the high state for a minimum amount of time before returning back to the low state. The trigger box detects a change in the Biodex output square wave signal, so that when the appropriate voltage change had taken place, the trigger box triggered the EMG PC (laptop) software to start recording the EMG data. When the hold button (Biodex) is pressed by the researcher the Biodex signal (trigger) is switched on. Therefore, the data from the EMG and biodex are completely time aligned.

Three electrodes were placed on the dominant limb on the medial and lateral hamstring muscles represented by semitendinosus (ST), and biceps femoris (BF) and on the calf muscle represented by the Gastrocnemius (G), as recommended by Seniam (2007). The standard for the skin preparation is an electrical resistance between the three electrodes of less than 5kΩ. The skin was cleaned with an alcohol wipe to improve application of the electrodes and reduce the acceptable impedance to below 5kΩ. Participants were also asked not to use any type of moisturiser on their legs during testing. A permanent pen was used to mark the position of electrodes for repeat testing, although where possible the electrodes were kept on during the fatigue task ready for post fatigue testing (figure 4.3).

Plate 4.3: an illustration of the positioning of the electrodes on the hamstrings

Following the application of surface electrodes participants a baseline measure of EMG activity was recorded with the participant in a prone position with the leg fully extended and supported by the couch. Participants were instructed to relax and lay as still as possible whilst 3 measurements each lasting 10s were taken. If muscle activity was evident this procedure was repeated or the electrodes repositioned until the principal investigator was confident that a true baseline value was recorded. Participants were provided with 2 external triggers for the start of the movement of the lever arm: 1) the physical movement of the lever arm; 2) a light appeared on the trigger box when the movement was initiated and at a constant velocity (see plate 4.4). Participants were encouraged to relax the leg as much as possible before the movement to reduce the influence of pre-activation on the measurement. If the investigator could observe that pre-activation was taking place they would remind the participant to relax before starting the lever arm. The participants were reminded to exert maximal voluntary actions as quickly as possible when seeing the light and feeling the lever arm move (using the same isokinetic procedures previously highlighted).

Plate 4.4: Trigger port indicating the trigger light

Raw EMG data was collected at a sampling frequency of 1024 Hz and sent directly to the DelSys Acquisition software, set up on a Toshiba Laptop (L20, Toshiba Corp. Tokyo, Japan). The EMG unit includes a common mode rejection ratio of >80 dB and an amplifier gain of 1000. Raw EMG data was band pass filtered at 20 – 450 Hz using the DelSys Acquisition software. The EMG data for each of the three angular velocities (60˚/s, 120˚/s and 180˚/s) was normalised against the maximum EMG RMS amplitude recorded in the activity of the hamstrings muscles (semitendinosus ST, Gastrocnemius SM and biceps femoris BF).

The EMD was determined as the time interval between the onset of EMG activity and force development according to the procedure developed by Zhou et al (1995). Based on this procedure the onset of EMD was determined as a change from the EMG mean baseline level to +15 μV deviation and the offset of EMD was determined as the time taken (ms) to generate 9.6-Nm torque (see Figure 4.3). The maximal EMD value was determined as the shortest EMD from the three measurements for each angular velocity.

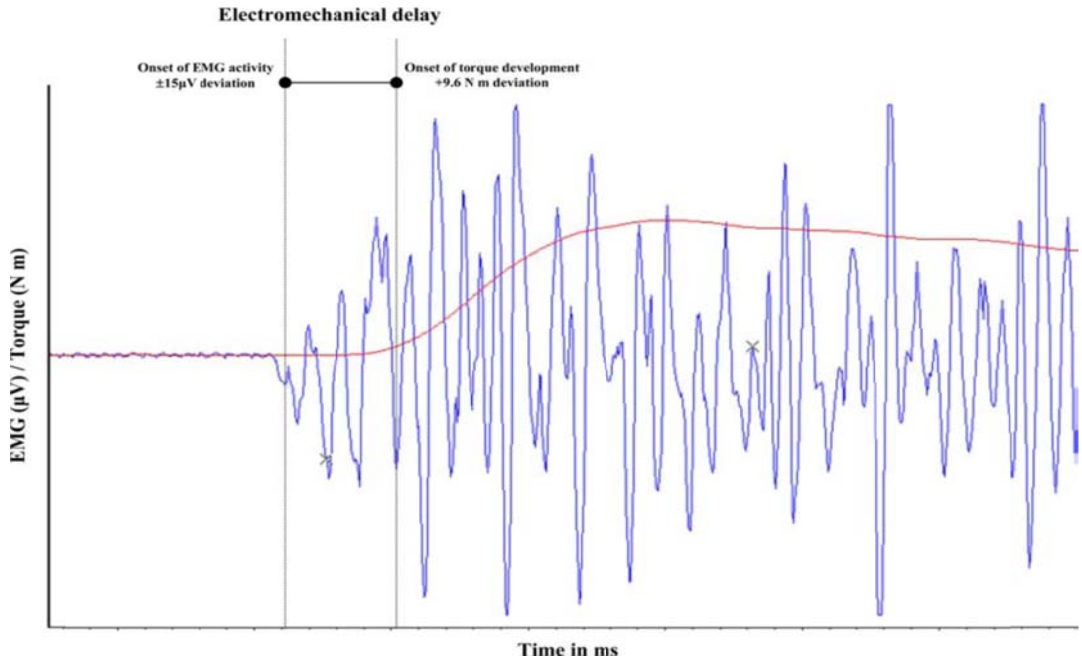


Figure 4.3: an illustration of the process used to determine the EMD (Zhou et al. 1995).

4.3.6 SAFT90 Protocol

The SAFT90 is based on contemporary time-motion analysis using data obtained from English Championship level match play (Prozone) and has been validated by Lovell et al (2008) to replicate the fatigue response of football match-play (Small, 2010). The free running protocol includes various changes in both direction and speed over the age appropriate time period inherent to match-play, with passive rest intervals equivalent to those experienced on a match day (eg 2 min rest between playing bouts for U13). The design of the course is based around a shuttle run over a 20m distance, with the incorporation of four positioned poles for the participants to navigate using utility movements (see figure 4.4).

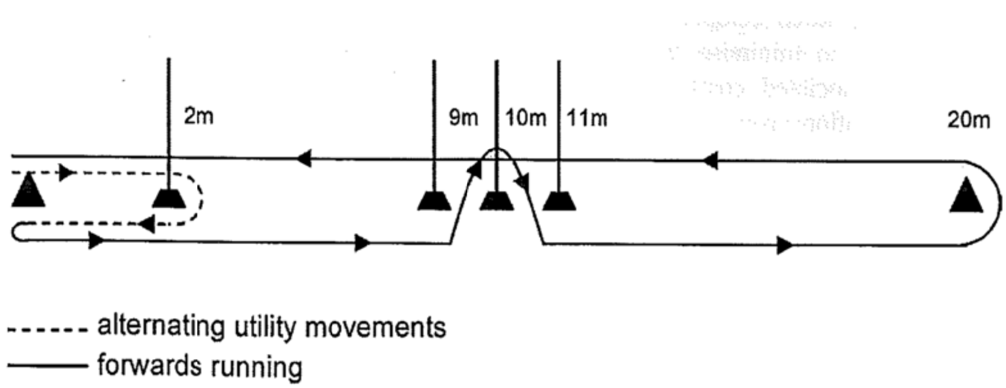


Figure 4.4: A diagrammatic representation of the SAFT90 course

The course was performed with the participant performing either backwards running or sidestepping around the first field pole, followed by forward running through the course, navigating the middle three field poles, although no contact actions such as kicking or tackling were performed. The movement intensity and activity performed by the participants whilst completing the SAFT90 course was maintained using verbal signals on an audio CD. The audio CD contains a 15 min activity protocol which was repeated randomly and intermittently in order to last for the duration of a game the participant usually competes in (Table 4.1). The coach and a member of the research team provided strong verbal encouragement throughout the protocol. Participants ran in groups of 3 or 4 staggered at 30min intervals.

Table 4.1: Academy game duration.

Age Group	Format	Total playing time
Under 13's	4 x 15 minutes (2min rest between quarters)	60 minutes
Unger 15's	2 x 40 minutes (10min rest between halves)	80 minutes
Under 17's	2 x 45 minutes (15min rest between halves)	90 minutes

Before any testing is completed, players performed a standard 10 minute warm up which is usually performed at their club before matches. This will include running and familiar dynamic movements to players. All procedures can be viewed on the CD attached to this report

4.4 Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, v. 19.0 for Windows; SPSS Inc, Chicago). Firstly the distribution of raw data sets was checked for homogeneity and skewness using the Kolmogorov-Smirnov test. Descriptive statistics including means and standard deviation were calculated for each measure. Pearson product moment correlation co-efficients were run to explore relationships between the key outcome variables. A 3 (group) x 2 (time) x 3 (angle) x 3 (velocity) repeated measures analysis of variance (RMANOVA) for the H/Q_{FUNC}, and a group (3) x time (2) RMANOVA for leg stiffness was used to explore interaction and main effects. A 3 (muscle) x 3 (group) x 2 (time) RMANOVA was used to explore interaction and main effects for EMD. Significant interaction or main effects were further examined using Bonferroni-corrected post hoc *t*-tests. The level of significance was set at $P \leq 0.05$ for all tests.

Results

5.1 Participant Characteristics

A total of 36 participants completed the study consisting of 14 U13 players, 9 U15 players and 13 U17 players. Age was determined from date of birth and test date. Four of the participants determined the left leg as their dominant leg (based on kicking preference) and 32 participants were right footed. Only one participant in the U13 age group self reported that they had started menstruating and was in the follicular phase during testing. 5 participants in the U15 and 11 participants in the U17 age group self reported that they were menstruating (two U17s were taking the contraceptive pill) and were in the following phases during testing: Luteal phase (n = 10) Follicular phase (n = 7). Participant characteristics can be seen in table 5.1. There were significant group differences in stature, body mass, leg length and offset from PHV. There were no significant group differences in Q angle. Distance covered during the SAFT90 was 6320 ± 69 m (U13), 10525 ± 592 m (U15) and 10590 ± 662 m (U17) for each age group respectively.

Table 5.1: Participant characteristics by age group

	Under 13	Under 15	Under 17
Age (y)	12.1 ± 0.5	13.9 ± 0.6	15.8 ± 0.5
Stature (m)	1.46 ± 0.06*	1.59 ± 0.08	1.66 ± 0.06
Body mass (kg)	40.8 ± 6.7*	51.9 ± 8.8	61.9 ± 8.2
Leg length (cm)	68.6 ± 3.4*	73.4 ± 3.8	79.8 ± 3.8
Offset from PHV (y)	-0.28 ± 0.55*	1.11 ± 0.55	2.93 ± 0.58
Q angle (°)	14.52 ± 0.46	16.96 ± 0.30	15.22 ± 0.31

* significant difference between groups

5.2: FH/Q ratio

Mean (SD) data for FH/Q ratio by angle, velocity and age group, pre and post fatigue can be found in table 5.2. Significant interaction effects for angle x velocity ($p = 0.00$) and time x angle ($p = 0.033$) were found for the FH/Q ratio. The time x angle interaction (figure 5.1) demonstrated that the ratio decreased from pre to post fatigue at 0-10° (1.56 ± 0.94 vs 1.29 ± 1.07 [absolute change -0.27]), remained similar at 10-20° (1.76 ± 0.74 vs 1.82 ± 1.12 [absolute change +0.06]) and increased at

20-30° (1.45 ± 0.42 vs 1.68 ± 1.02 [absolute change +0.23]). The difference in the absolute change in the FH/Q ratio from pre to post fatigue between 0-10° and 20-30° was 0.50. The angle x velocity interaction (figure 5.2) demonstrated a reduction in the FH/Q ratio with increasing velocity at 0-10° of flexion compared with an increase in the FH/Q ratio with increasing velocity at 10-20° and 20-30° of flexion. No other significant interaction effects were observed. A significant main effect for angle ($p = 0.019$) was found with the ratio significantly higher between 10-20° than 0-10° and 20-30° (figure 5.1). Importantly there were no main effects for time (pre fatigue 1.59 ± 0.70 vs post fatigue 1.60 ± 1.07). Although the time x group interaction effect did not reach statistical significance ($p = 0.07$) there were age related differences in the response to the fatigue task. The FH/Q ratio remained unchanged in the U13s (absolute change = +0.02), decreased in the U15s (absolute change = -0.39) and increased in the U17s (absolute change = +0.38). The difference in the absolute change between the U15 and U17 age groups was 0.77.

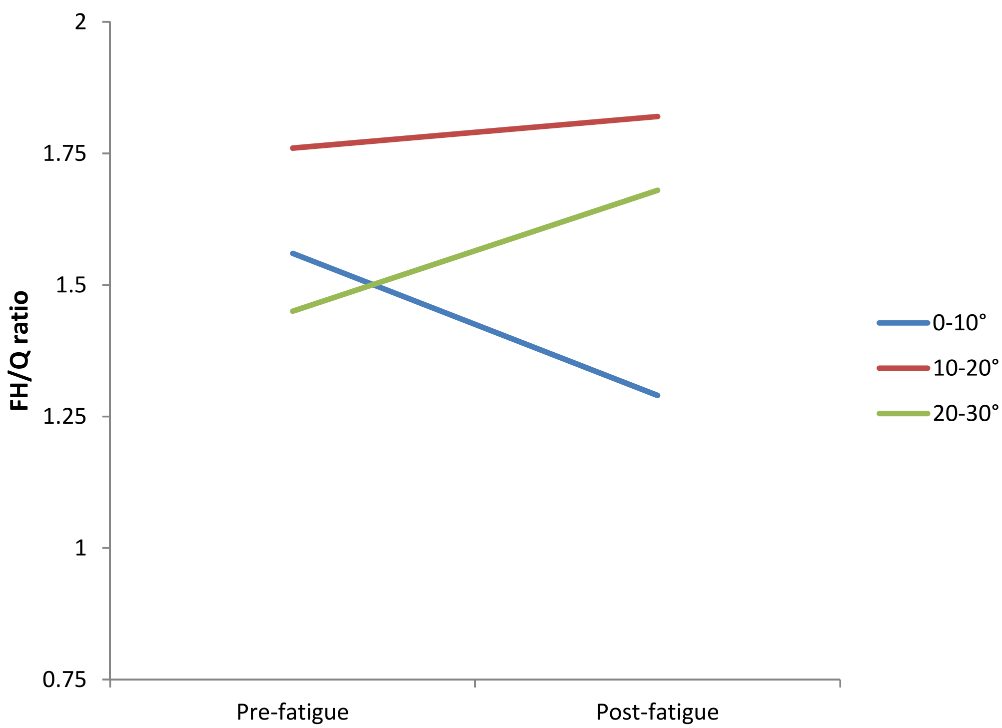


Figure 5.1: FH/Q ratio pre and post fatigue by joint angle

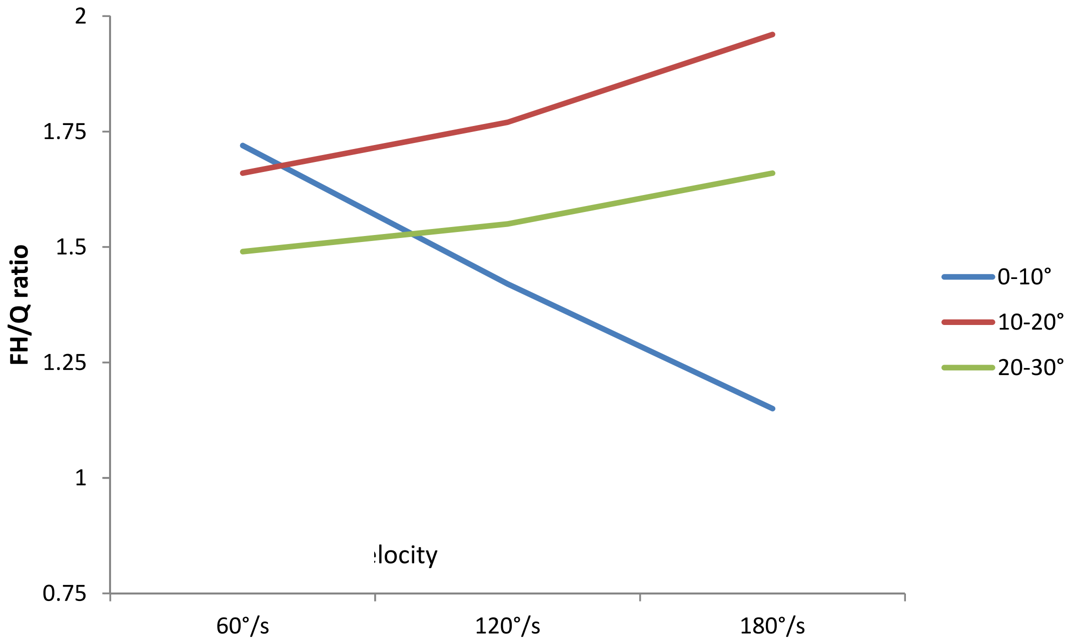


Figure 5.2: FH/Q ratio by joint angle and movement velocity

Table 5.2 FH/Q ratio pre and post fatigue by age group, movement velocity and joint angle

Velocity/Angle	FH/Q ratio Pre Fatigue			FH/Q ratio Post Fatigue		
0-10°	U13	U15	U17	U13	U15	U17
60°/s	1.40 ± 1.13	1.79 ± 1.01	1.42 ± 0.70*	1.59 ± 1.61	0.95 ± 1.18	1.31 ± 0.68
120°/s	2.01 ± 1.09	1.43 ± 0.92	1.49 ± 0.79	1.71 ± 1.38	1.27 ± 1.32	1.04 ± 1.0
180°/s	1.62 ± 1.15	0.75 ± 0.75	1.08 ± 0.57	1.45 ± 0.92	0.89 ± 0.49	1.10 ± 1.1
10-20°						
60°/s	1.50 ± 0.63	1.75 ± 0.86	1.30 ± 0.57*	1.69 ± 1.45	1.34 ± 1.24	2.17 ± 1.0
120°/s	1.63 ± 0.59	1.93 ± 0.88	1.90 ± 0.75	1.71 ± 1.35	1.43 ± 0.62	1.58 ± 1.0
180°/s	1.76 ± 0.65	2.09 ± 1.23	1.70 ± 0.57	1.76 ± 0.88	1.60 ± 0.42	1.99 ± 1.3
20-30°						
60°/s	1.27 ± 0.40	1.35 ± 0.54	1.18 ± 0.26*	1.78 ± 1.40	1.03 ± 0.50	2.30 ± 1.0
120°/s	1.38 ± 0.45	1.46 ± 0.48	1.46 ± 0.34	1.36 ± 1.05	1.23 ± 0.37	1.88 ± 0.9
180°/s	1.54 ± 0.40	1.52 ± 0.69	1.49 ± 0.30	1.50 ± 0.93	1.33 ± 0.30	2.07 ± 1.

* Significant angle x velocity interaction effect
† Significant time x angle interaction effect

5.3: Relative Leg Stiffness

Mean (SD) data for relative leg stiffness by age group, pre and post fatigue can be found in table 5.3. RMANOVA revealed a significant group x time interaction effect ($p = 0.022$) for relative leg stiffness but no significant main effects of group or time. This interaction effect can be attributed to a slight decrease in stiffness in the U13 age group compared to an increase in stiffness in the U17 group post fatigue (figure 5.3). There was an individualised response to fatigue in the U15 age group with 4 girls increasing stiffness and 5 girls decreasing stiffness. Most girls stiffness decreased in the U13 age group (n = 10) but a few did increase their stiffness (n = 4). All girls in the U17 age group increased stiffness post fatigue.

Table 5.3: Relative leg stiffness (dimensionless) by group and time

Group	Pre Fatigue	Post Fatigue
U13	44.6 ± 5.5*	42.4 ± 7.7
U15	46.4 ± 8.8	47.1 ± 6.2
U17	36.5 ± 6.6	41.0 ± 6.1

* Significant group by time interaction effect

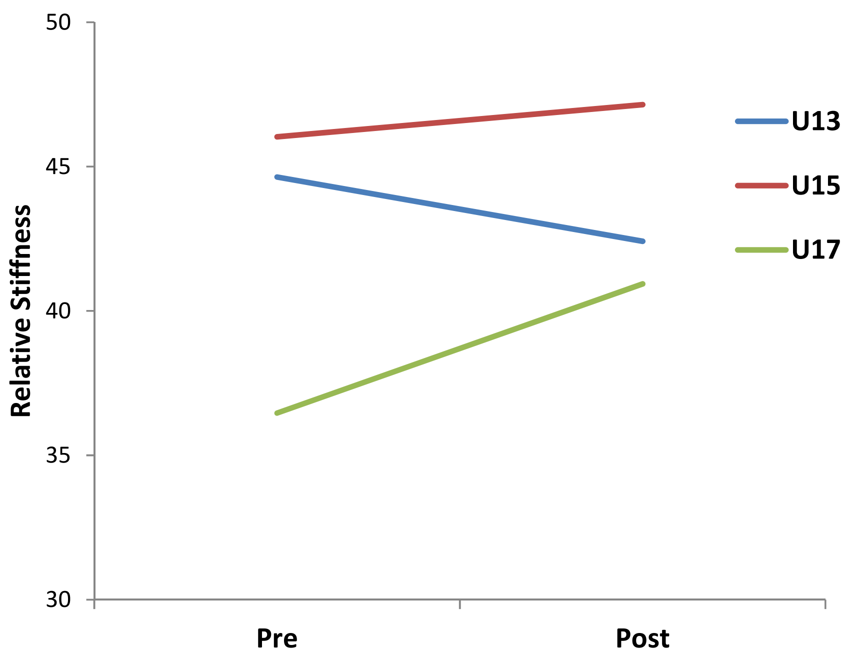


Figure 5.3: Relative leg stiffness pre and post fatigue by group

5.4 EMD

Mean (SD) data for EMD by age group, pre and post fatigue can be found in table 5.4. Data is presented for each muscle group and split by movement velocity. RMANOVA revealed a significant time by group interaction effect ($p = 0.046$) but no other interaction effects were observed. Post hoc analysis revealed that EMD was significantly longer in the U13 age group compared with the U15 and U17 groups and this difference was greater post fatigue (absolute increase in EMD from pre to post fatigue was 78ms [U13], 40ms [U15] and 50ms [U17] respectively). These data can be seen in figures 5.4, 5.5 and 5.6. A significant main effect for time ($p = 0.000$) and group ($p = 0.005$) were also observed. Irrespective of group, muscle or movement velocity EMD was significantly longer post fatigue compared with pre fatigue [160 ± 68 ms Vs 101 ± 43 ms] (see table 5.4). Likewise, irrespective of time, muscle or movement velocity EMD was significantly longer in the U13 group compared with the U15 (158 ± 66ms vs 113 ± 39ms) and U17 (158 ± 66ms vs 120 ± 40ms) age groups (see figures 5.4, 5.5 and 5.6). There were no significant differences in EMD between the U15 and U17 age groups (113 ± 39ms vs 120 ± 40ms). No significant ($p>0.05$) main effects for muscle (132 ± 51ms [BF], 133 ± 52ms [ST], 127 ± 63ms [G]) or movement velocity (139 ± 69ms [60°/s], 122 ± 46ms [120°/s], 131 ± 51ms [180°/s]) were found.

Table 5.4: EMD pre and post fatigue by age group, muscle and movement velocity

Muscle/Velocity	EMD Pre Fatigue (ms)				EMD post fatigue (ms)			
Biceps Femoris	U13	U15	U17	Combined	U13	U15	U17	Combined
60°/s	136 ± 62	99 ± 36	96 ± 35	**113 ± 51**	220 ± 111	143 ± 44	157 ± 52*	**179 ± 85**
120°/s	103 ± 32	86 ± 28	95 ± 36	**96 ± 32**	178 ± 57	127 ± 42	146 ± 40*	**154 ± 51**
180°/s	117 ± 38	93 ± 27	85 ± 33	**100 ± 36**	197 ± 60	145 ± 46	148 ± 32*	**167 ± 53**
Semitendonosis								
60°/s	144 ± 54	97 ± 34	97 ± 40	**116 ± 49**	223 ± 106	141 ±46	153 ± 48	**178 ± 84**
120°/s	106 ± 31	96 ± 37	95 ± 35	**100 ± 33**	179 ± 45	125 ± 42	141 ± 36	**152 ± 46**
180°/s	124 ± 46	101 ± 41	84 ± 28	**104 ± 42**	200 ± 62	150 ± 56	141 ± 39	**167 ± 59**
Gastrocnemis								
60°/s	124 ± 61	79 ± 17	102 ± 61	**105 ± 55**	212 ± 122	117 ± 42	165 ± 45	**171 ± 91**
120°/s	97 ± 48	93 ± 24	92 ± 35	**94 ± 38**	185 ± 102	111 ± 42	137 ± 46	**150 ± 78**
180°/s	123 ± 68	92 ± 43	81 ± 31	**101 ± 53**	177 ± 80	145 ± 53	144 ± 42	**157 ± 63**
All Combined	**119 ± 49**	**93 ± 32**	**92 ± 37 ‡**	**101 ± 43 †**	**197 ± 83**	**133 ± 46**	**148 ± 42**	**160 ± 67**

* Significant group x time interaction effect
† Significant main effect for time
‡ Significant main effect for group

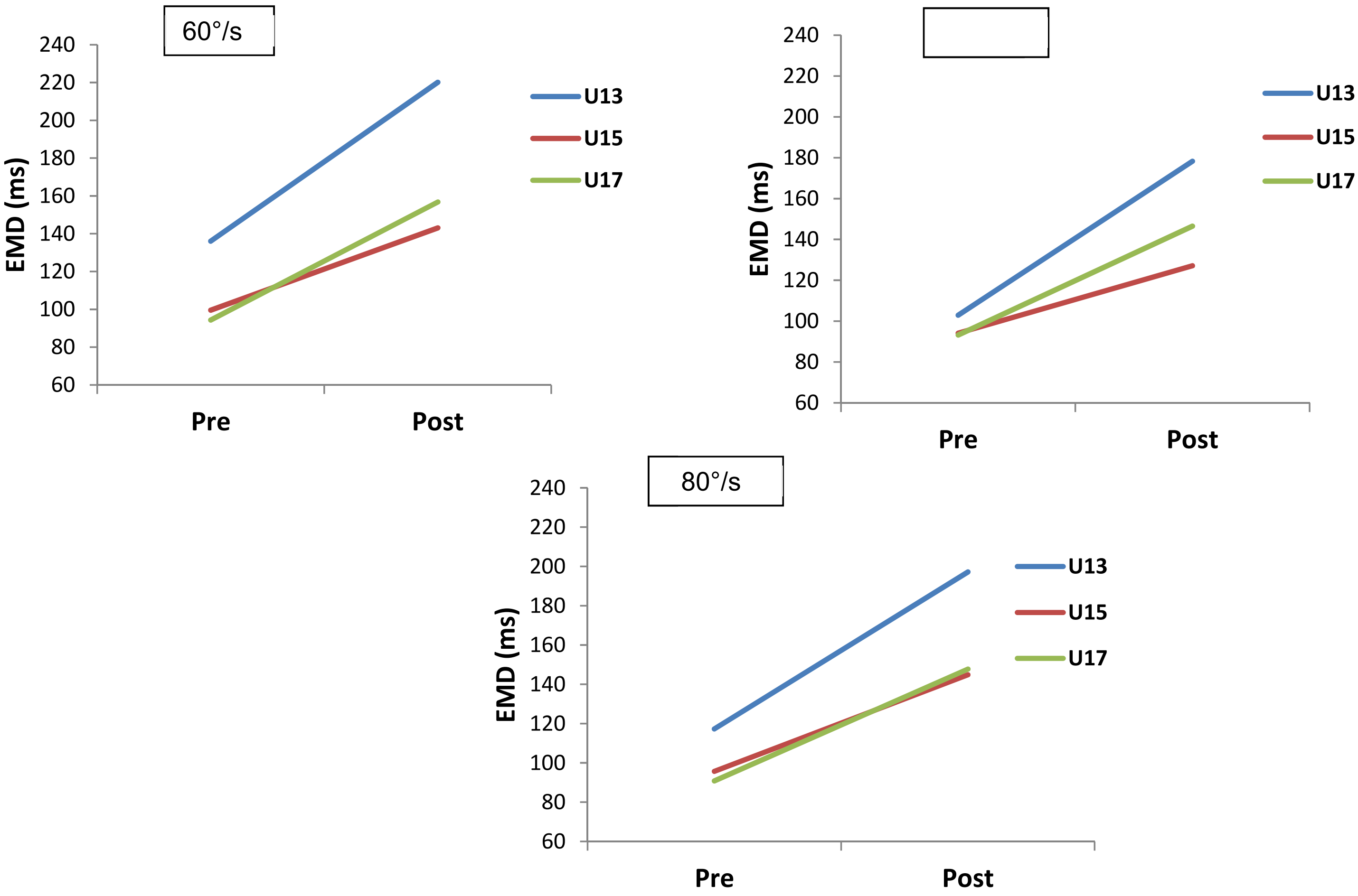


Figure 5.4: EMD by group pre and post fatigue for the biceps femoris

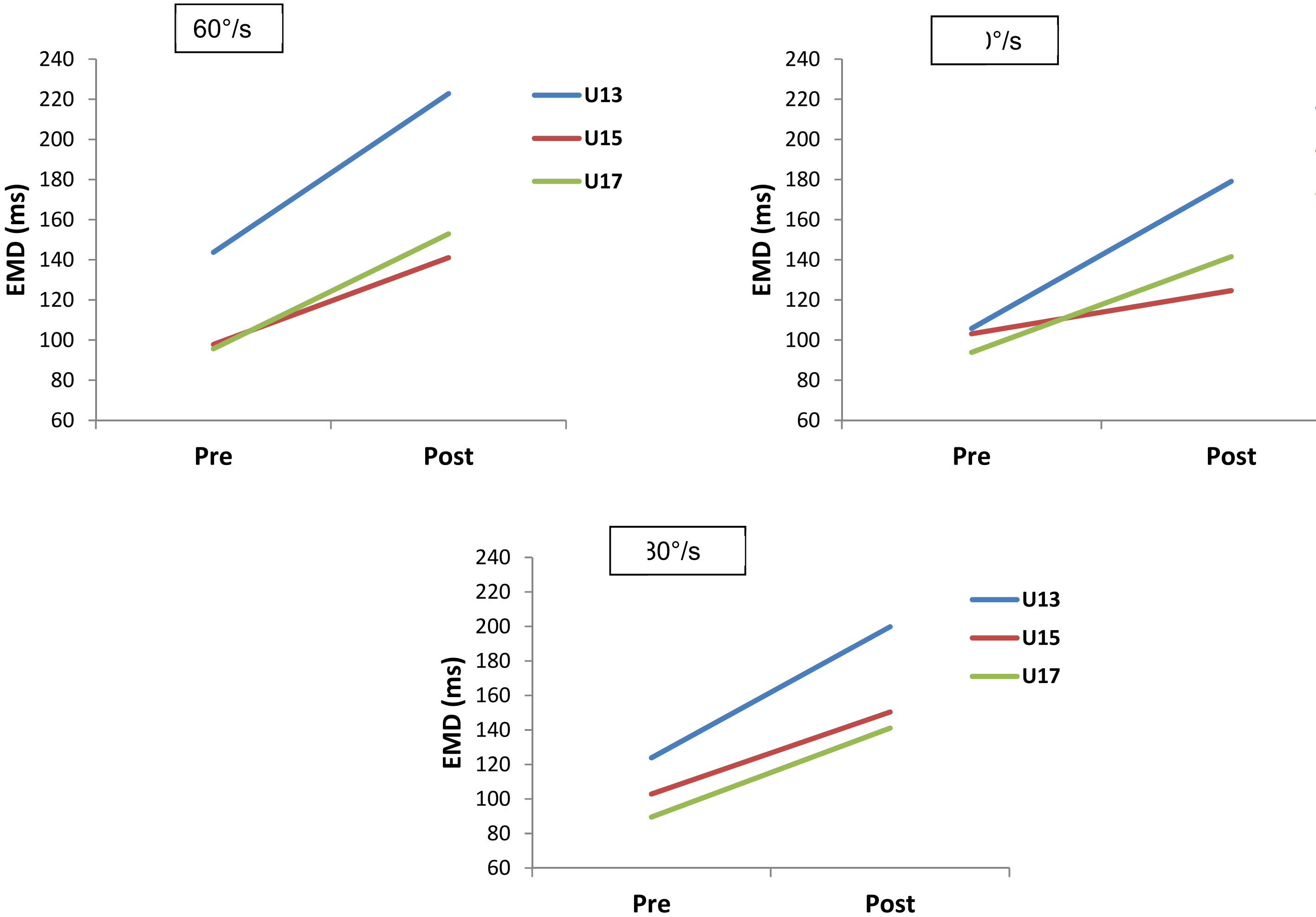


Figure 5.5: EMD by group pre and post fatigue for the semitendonosis

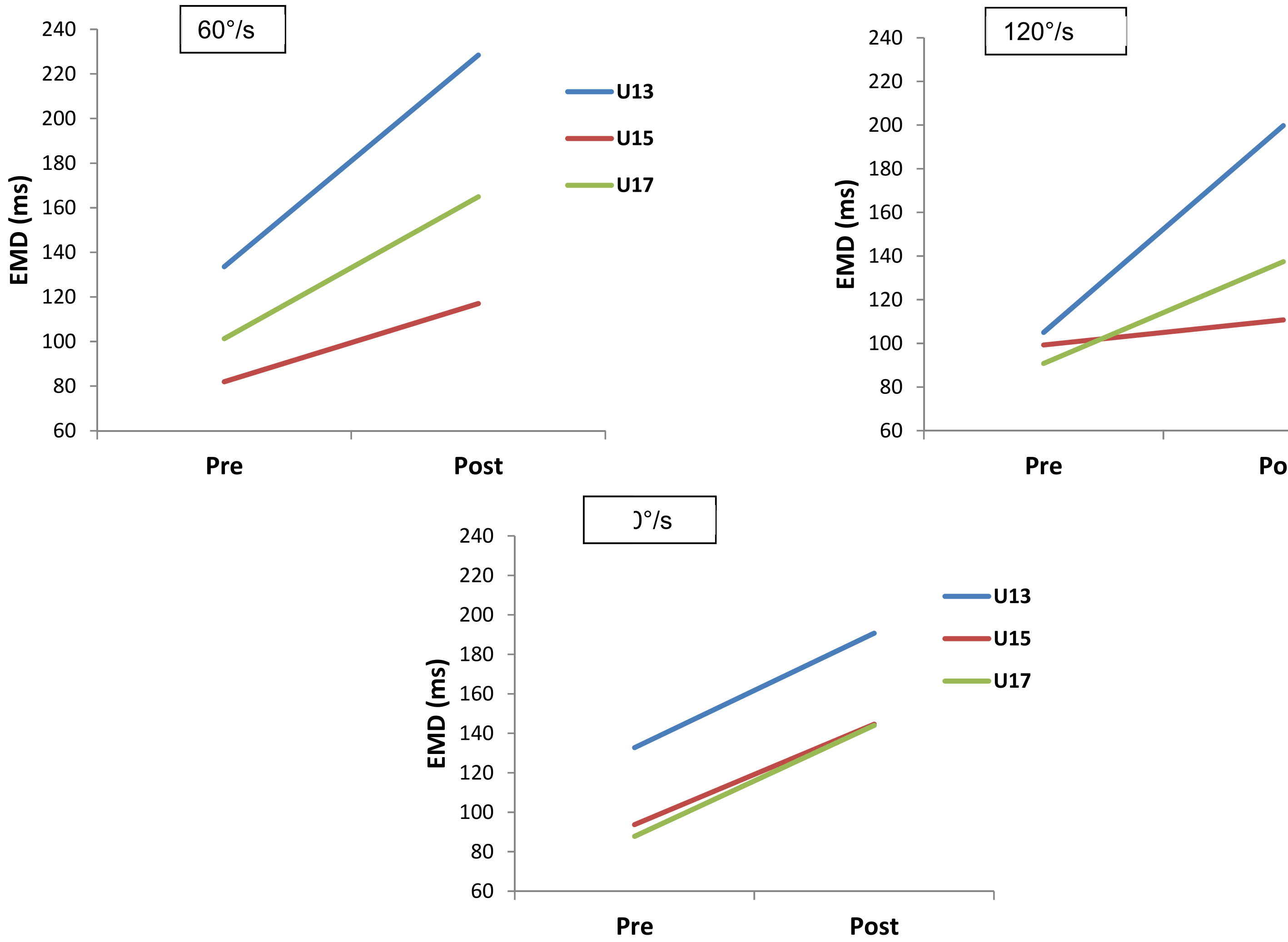


Figure 5.6: EMD by group pre and post fatigue for the Gastrocnemus

5.5: Summary of Results

1. A time x angle interaction effect demonstrated a significant decrease in the FH/Q ratio post fatigue, irrespective of age, between 0-10° of knee flexion, compared with an increase at more flexed angles.

2. A significant angle x velocity interaction effect indicated that irrespective of age as velocity of movement increases the FH/Q ratio decreases only at the most extended knee position

3. EMD was significantly longer post fatigue in all age groups but the change from pre to post fatigue was greatest in the U13 age group

4. A significant time x age interaction effect for relative leg stiffness indicated a slight decrease in the U13 age group, no change in the U15s and a significant increase in the U17 post fatigue

Discussion

6.1: Over-view of the main findings

This study has reinforced the importance of using angle specific data to explore the FH/Q ratio as the negative effects of fatigue on female youth footballers was evident closer to full knee extension but this detrimental effect disappeared as the knee flexed. Additionally this study has shown that in young girls when the knee is closer to full extension the FH/Q ratio decreases with increasing angular velocity. This has important implications for the determination of muscular dynamic knee stability in young female footballers as the ability of the muscular system to stabilise the knee (determined as the FH/Q) is impaired when fatigue is present only near full knee extension, and at faster movement velocities. These findings have specific implications for females who tend to land with the knee in more extended positions (Lephart et al., 2002) and especially postpubertal females who land in more extended positions with more mediolateral joint forces compared to prepubertal children (Hass et al., 2005). Also, our findings suggest that previous studies that have used peak torque values to describe the FH/Q ratio (which generally occur in the mid range of the movement), and/or only assessed the ratio at slow to medium angular velocities should be viewed with a degree of caution as they do not represent what is occurring closer to full knee extension and during faster velocity movements. Our findings question the functional relevance of most previous literature as determination of the FH/Q ratio is not in the position that the knee is in when it is likely to sustain an injury. Our statistical findings suggest that when using the current field based test to examine football specific fatigue on dynamic knee stability there are no detrimental effects on muscular stability as determined by the FH/Q ratio, irrespective of age or maturation. This can be mainly attributed to the relative fatigue resistance of eccentric hamstring torque compared with a relatively greater decrease in concentric quadriceps torque after fatiguing exercise. However, although our age related data did not reach statistical significance ($p = 0.07$) there were clear age related differences in the fatigue related response to muscular stability. From pre to post fatigue the U13 groups showed no change in FH/Q ratio, the U15 age groups FH/Q ratio declined and the U17 age groups FH/Q ratio increased. However, the non-significant findings would lead us to suggest that other mechanisms, rather than muscular stability, may contribute to a greater extent to the increased relative risk of knee injury in female youth players when fatigue is present. This assumption is supported by the neuromuscular findings in the current study. A significant increase in the EMD post fatigue demonstrates that neuromuscular feedback is impaired in youth female players when fatigue is induced, irrespective of age or maturation. Importantly this effect is greater in younger, less mature girls and reduces with age, maturation and training status. Interestingly there is an age effect in the response of feed-forward, pre-activation mechanisms when fatigue is present. The leg stiffness results suggest that feed-forward mechanisms are compromised with fatigue in younger aged players (U13), remain similar in pubertal aged players (U15) and are improved in older players (U17). The relative leg stiffness data obtained in the current study may demonstrate the effectiveness of football specific training, with enhanced pre-activation evident in the older age groups post fatigue, and this may be a potential compensatory mechanism for the reduced ability to utilise neuromuscular feedback mechanisms. However, this hypothesis requires further investigation using non-trained control groups, as this may simply reflect changes with normal growth and maturation. The age related variability in our findings suggest that the response of muscular stability mechanisms and neuromuscular feed-forward and feedback mechanisms to cope with fatigue may be related to either a growth/maturational effect and/or a training effect. As no significant difference in EMD of the three muscles examined was found in the current study, we would tentatively suggest that youth female footballers do not move to an ankle dominant strategy when fatigue is present.

This research is the first of its kind to demonstrate that football specific fatigue has a detrimental effect on neuromuscular functioning of the muscles that support the knee in youth female footballers and therefore may be a potential factor in the increased relative risk of injury in this population. The reduced neuromuscular functioning in both feed-forward and feed-back mechanisms in the youngest age group (U13) may be a protective mechanism for their relatively immature musculoskeletal systems. The U15 age group were the only group to show reduced muscular (FH/Q ratio) and neuromuscular (EMD) capability post fatigue and may be considered a high injury risk group. This status may be due to their pubertal status and the interacting effects of hormones, changes in limb proportions as well as shifts in metabolic specialisation. The oldest age group (U17) appear to show signs of compensatory mechanisms that may help to protect the joint despite significant impairment to feed-back mechanisms. This age group increased both muscular stability and neuromuscular feed-forward mechanisms when fatigue was present. Our findings would reinforce the suggestion from previous research that training to improve neuromuscular functioning is essential in female youth footballers to reduce the relative risk of injury (Hewett 2000; Myer et al. 2006, 2009) however this improvement should be age/maturation specific and importantly related to fatigue resistance, which has not previously been prescribed.

6.2: Influence of football specific fatigue on FH/Q ratio

The manifestations of functional changes occurring with fatigue are multiple and depend on the joint angle, angular velocity, and action type (Green, 1997). One of the aims of the present investigation was to examine the effects of football specific fatigue on the FH/Q ratio at different joint angles and angular velocities. A significant time by joint angle interaction effect was found in the current study, indicating that irrespective of age the FH/Q ratio at the most extended joint position (0-10°) was lower post-fatigue compared to pre-fatigue. As the knee moves into a more flexed position the influence of fatigue disappears and by 20-30° of knee flexion the FH/Q ratio increased post compared with pre fatigue. These findings are important as near full knee extension, static stability is reduced and functional stability relies mainly on dynamic stability to protect the knee structures (Griffin et al., 2006). The data from the current study highlights that the influence of fatigue on muscular dynamic knee stability is joint angle specific and reinforces the inappropriate use of using peak torque to calculate the FH/Q ratio. Epidemiological data suggests that injury is more frequent in the later stages of football matches when fatigue is present and after numerous repetitons of the same movement (Hawkins et al., 2001, Olsen et al., 2004). The data from the present study suggest that muscular stability is reduced when fatigue is present, but only as the knee approaches the last 10° of knee extension. These findings support the view that the stability systems are affected by changes in the joint angle in youth female players. By observing the change in the angle-specific FH/Q ratio following football specific fatigue, we have shown that in youth female footballers the eccentric hamstring muscle action is less effective in extended knee positions. By exploring the concentric and eccentric torque data in the current study it is evident that when in a fatigued state as the knee approaches extension that the ability of the hamstrings to act eccentrically to counteract the quadriceps concentric torque production is diminished and the ratio decreases. This suggests that in female youth players the ability of the dynamic muscular system to stabilise the knee close to full knee extension when fatigue is present is compromised. These findings has particular implications for footballers as common movement patterns within the game situation place the knee in extended positions (eg pivoting, twisting, kicking, landing). Landing mechanics data have indicated that females tend to land with the knee in more extended positions (Hass et al., 2005) and therefore the findings of the current study would propose that landing from a jump in the later stages of a football match may be a cause for increased relative risk of injury in youth female footballers.

It is difficult to compare our findings to previous literature as this is the first study to have examined angle specific FH/Q ratios in youth female footballers post fatigue. There is also limited adult data with which to compare our findings. A number of adult studies have demonstrated a significant reduction in the FH/Q ratio calculated in the mid range of the movement (using peak torque values) after fatiguing exercise (Small et al., 2010; Rahnama et al., 2003). These studies have used a range of laboratory based fatigue protocols including repeated isokinetic actions and downhill running, and have determined torque with the hip in a flexed position which is not functionally relevant. There appear to be no studies that have explored the influence of fatigue on angle specific FH/Q ratios, however unpublished data from our laboratory, in a large sample of adult participants (n = 110), has indicated that the angle specific FH/Q ratio, determined with the hip extended, increases closer to full knee extension in adults when fatigue is present (El Nagar, 2012: unpublished PhD). This compensatory mechanism of increasing the FH/Q when fatigue is present was in response to reduced neuromuscular functioning (longer EMD post fatigue) and proposed as a muscular response to protect the joint. The data from the current study may suggest that young girl's immature and developing muscular system is not developed enough to compensate for the compromised neuromuscular effects of fatigue (see EMD section). In particular the ability of the hamstrings to act eccentrically close to full knee extension when fatigue is present. We also found no significant main effect for joint angle indicating that the FH/Q ratio in youth female footballers does not change with increasing knee extension when fatigue is not present. This is in contrast with the adult literature that suggests that there is a protective mechanism of an increased FH/Q ratio (due to a larger decrease in the quadriceps concentric torque than in eccentric hamstrings torque) as the knee approaches full extension (Aagaard et al., 1998, Coombs and Garbutt, 2002; El Nagar, 2012). For example Coombs and Garbutt (2002) used a small sample (n = 15) of adult recreational athletes to calculate joint angle-specific FH/Q ratio values throughout a 90° range of movement and found increasing FH/Q ratio values as the knee extended, and the highest values were at 10° of knee flexion. Our data suggest that even when fatigue is not present that female youth footballers may be at risk during movements when the knee is almost fully extended due to the lack of a protective mechanism of increasing muscular stability. The mechanisms behind this adult/child angle specific difference are difficult to prescribe but could be related to either a) maturational effects of eccentric torque production; b) quadriceps dominance in females; c) football specific training effects and/or d) a combination of these factors. In the current study it is unlikely that this first hypothesis is true for the U17 age group given their post pubertal status. However, whether there are protective mechamism acting on the younger age groups (U13 and U15) to reduce muscle lengthening (and subsequently limit eccentric torque production) at the extremes of the range of movement, remains to be investigated. Its is also unlikely that the second hypothesis has crediance given the angle related effects in adults is not different between males and females. However, whether a quadriceps dominance is evident in the female youth footballers in the current study, and can account for the lack of protective effect at more extended knee positions is an interesting hypothesis. It may be that our findings of a non-significant effect of joint angle on the FH/Q ratio may be related to traditional football training effects. Recent work has confirmed that loading patterns experienced during football training in youth male footballers asymmetrically strengthen the muscles about the knee, altering the balance towards quadriceps dominance (Iga *et al.* 2009). Iga *et al.* (2009) demonstrated a training effect in 15 year-old football players on FH/Q ratio with lower ratios found in conventionally trained footballers compared with resistance trained footballers and controls.

However, if traditional football training in female youth players produces quadriceps dominant individuals we would have expected to see significant age effects in the current study as the older age groups would have had more exposure to traditional training. The fact that we found no significant age differences in the FH/Q ratio is in contrast to a recent cross-sectional study on a large group of male youth footballers aged 12-18 years (Forbes et al., 2009). Forbes et al (2009) reported significantly lower FH/Q ratios, determined in a seated position using peak toque values, in 18 year olds (0.84) compared with 12 year olds (1.01) (Forbes *et al.* 2009). This reduction in FH/Q ratio with age was attributed to a relatively greater increase in concentric quadriceps torque compared with the relative increase in eccentric hamstring torque, reinforcing a potential for the development of quadriceps dominance in traditional football training in male players. Conflictingly one study using non-trained children reported age associated effects between prepubertal children, teenagers and adults with 9-10 year-olds producing a significantly lower FH/Q ratio (0.97) than teenagers (1.23) and adults (1.19) (De Ste Croix et al., 2007). There is one study on trained male youth basketball players (12-17 year-old) who have reported similar findings to the current study of a non-significant age effect on FH/Q ratios (Gerodimos *et al.* 2003). It should be noted that all of the previous studies cited here used peak torque to determine the FH/Q ratio and from a seated position with the hip flexed. This is particularly important given that torque appears to be significantly greater for knee flexion in a sitting position when compared to the more ecologically valid supine or prone position (Black et al., 1993). Therefore, direct comparison of torque obtained from seated versus supine or prone positions should be viewed with a degree of caution, as the length –tension relationship is altered. An advantage of the assessment of torque in the supine position, as used in the present study, is that it provides a closer approximation of the length tension relationship of the hamstring and quadriceps muscles during many functional and sporting activities, and is therefore functionally relevant (Worrell et al., 1990).

The data from the current study shows no interaction or main effects of fatigue and age on the FH/Q ratio. One of the reasons why we have found no statistically significant fatigue related effects may potentially be ascribed to the football specific protocol. The movements in the SAFT90 contain periods of rapid deceleration, however the protocol has limited cutting and sideways movements, which may reduce the amount of eccentric work done during the protocol. A football specific procedure that involves more extreme changes in direction and also includes jumping, landing and kicking motions may place greater stress on the eccentric proportion of the muscle. It would be useful, if not more logistically difficult, to explore changes in the FH/Q ratio post competitive match play. The lack of any statistically significant age effects in the current study must be viewed with a degree of caution, as although the exploration of age effects did not reach statistical significance ($P = 0.07$) there appears to be age group associated differences in the FH/Q ratio from pre to post fatigue. We found no significant absolute change in the FH/Q ratio in the U13 age group (0.02), but a significant decrease in the ratio in the U15's (-0.39) and a significant increase in the U17's (+0.38). This equates to an absolute difference in the FH/Q ratio between the U15 and U17 age group, from pre to post fatigue of 0.77. This difference in the fatigue related response to dynamic muscular stability clearly has some practical significance and may have reached statistical significance with a larger sample size. It is difficult to prescribe these findings to physiological and biomechanical or biological mechanisms as well as relate them to previous studies as this is the first study to have explored the influence of football specific fatigue on the FH/Q ratio in female youth footballers using angle specific data. In support of the U17 findings of increased FH/Q ratio when fatigue is present the study of Wright et al., (2009) reported a significant increase in the FH/Q ratio (0.88 vs. 1.08) following a fatiguing protocol consisting of 50 maximal concentric knee flexion/extension repetitions. EMG analysis indicated that the co-activation of the hamstrings during concentric quadriceps muscle actions significantly increased post fatigue (Wright et al., 2009) and this may be a mechanism that could be attributed to the greater FH/Q ratio found in the U17 group in the current study. Comparable data from youth players is limited but we have previously explored football specific fatigue using the SAFT90 protocol on angle specific FH/Q ratio in U18 male professional footballers and found no statistically significant change in the FH/Q post fatigue, irrespective of joint angle or movement velocity (Davenport, 2011 ; Unpublished MSc thesis). These comparative data from male footballers to the U17 female group in the current study suggests that FH/Q ratio is not compromised after football specific fatigue. Indeed, our data suggest that post puberty muscular stability is increased in a fatigued state in female footballers. It is difficult to prescribe mechanisms to this increase in the FH/Q ratio post fatigue but it is related to a greater reduction in concentric quadriceps torque production post fatigue compared with the decline in eccentric hamstring torque. There are data that have shown that eccentric actions are more resistant to fatigue and that these effects are more pronounced in females compared to males (Hicks et al. 2001). This may account for the differences between the findings of the current study and the significant decrease in the FH/Q ratio reported in previous football fatigue related studies that have used males as participants. Horstmann et al., (2001) also suggested that usually eccentric exercise leads to less acute fatigue and lower lactate and ammonia reaction than concentric exercise at comparable work levels. Our findings tentatively suggest that there may be development of fatigue resistance following repeated eccentric muscle actions with maturation in young girls towards the adult state. Another suggestion based on the comparative findings from our U18 male professional players is that there may also be an additional protective effect that is developed through football training and total hours of exposure. This hypothesis would require verification by exploring whether the fatigue related affects observed in the current study differ in non-trained young girls. At this point we would tentatively suggest that the increase in the FH/Q ratio post fatigue in the U17 age group may be related to both maturational status and training status to protect the joint due to diminished neuromuscular stability (to be discussed in the EMD section).

Other studies have reported conflicting findings that would support the U15 data showing a decrease in the FH/Q ratio when fatigue is present (Delextrat et al. 2010; Rahnama et al. 2003;

Small et al., 2010). The contradictory findings may be attributed to the nature of the varying fatiguing protocols (including the mode and intensity of exercise, the nature of loading during muscle activation and the environment) and proportion of concentric vs. eccentric work performed, which would influence the mechanisms of fatigue (Maluf and Enoka, 2005). However, there are a number of studies that have explored changes in the FH/Q ratio in male adults after a bout of football simulated exercise. The findings of these studies are relatively consistent, demonstrating a significant decrease in the FH/Q ratio when fatigue is present (Delextrat et al. 2010; Rahnama et al. 2003; Small et al. 2010). All of these studies reported that the eccentric hamstring torque decreased to a significantly greater extent than the concentric quadriceps torque when fatigue was present. As previously stated these data should be viewed with a degree of caution as they all used PT to determine the FH/Q ratio and testing occurred with the hip in a flexed position.

It is well recognized in adults that fatigue affects eccentric and concentric actions differently and that generally, eccentric actions are more fatigue resistant than concentric actions (Roig *et al.* 2009). Therefore, in a fatigued state the FH/Q ratio should increase and the knee should be more stable, as found in the U17 age group in the current study. However, we found a reduction in the FH/Q ratio in the U15 age group and these findings are comparable to the previously cited adult findings (Delextrat et al. 2010; Rahnama et al. 2003; Small et al. 2010; Oliveira *et al.* 2009), but the mechanisms associated with this decrease at this age are more difficult to prescribe. The decrease in eccentric hamstring torque following fatigue in the present study would appear to suggest that the stability of the joint is compromised during forceful knee extension movement in female footballers during the period of puberty. In adults this has been prescribed to a relatively greater decrease in eccentric compared to concentric torque production and has been more apparent in studies that have focused on eccentrically fatiguing the muscles. The current fatigue task was a football specific task that included eccentric actions but did not specifically focus on eccentrically fatiguing the hamstring muscles. Tentative speculation on the reason why we observed a shift in the fatigue related response in the FH/Q ratio from no change in the U13s, to a decrease in the U15s, to an increase in the U17s may be due to developmental changes in metabolic specialisation. Previous studies have suggested that young children are metabolic non-specialists, which may be related to their faster oxygen uptake kinetics compared to adults (Fawkner and Armstrong, 2007). It is possible that due to the pubertal nature of the U15 age group that they are in the transition from being non-specialists to becoming metabolic specialists. This hypothesis would suggest that the muscular fatigue response in the U13 age group was negligible due to their non-specialists nature and greater contribution from aerobic sources. The lack of a noticeable eccentric fatigue effect in the U17 age group is more difficult to describe but potentially through normal growth and maturation, combined with extensive training, these players may have adapted to the negative effects that come with using predominantly anaerobic metabolism during the football related exercise. However, the

U15 group may be in the transition from relying on aerobic sources to using predominantly anaerobic sources and have not yet developed metabolic strategies to cope with the negative effects that come with anaerobic metabolism. These assumptions are supported by evidence that younger children are more fatigue resistance compared with adults due to: a) children use oxidative pathways quicker than adults and therefore lead to a lower accumulation of by-products, b) children's lower ability to activate their type II muscle fibres and c) possible faster phosphocreatine resynthesis, improved acid base regulation and faster removal of metabolic by-products (De Ste Croix and Deighan, 2011). Another explanation of the U15 data may be related to the rapid change in body weight (peak weight velocity [PWV]) during this pubertal stage and the increased likelihood of injury due to increased loading from increased body mass. Disproportional increases in body fat as a percentage of body weight that occur during PWV may mean that there is increased ground contact loading in this age group without the concomitant increases in muscle strength from muscle growth. Additionally there may be a protective/inhibitory mechanism in the U15 age group that does not allow them to develop large eccentric force in fatigue conditions. This speculation is somewhat intriguing in that in very young children an inhibitory feedback control in the muscle during eccentric actions is seen as a protective mechanism during muscle lengthening to avoid muscle damage. However, in this case the inhibitory effect is unwanted and may potentially increase anatomical injury risk. These proposed reasons for the findings of the current study are provided tentatively as they require further investigation.

A further explanation for why we found a negative fatigue related effect in the U15 group compared with to the U17 age group may be attributed to the nature of the football specific fatigue task. As the SAFT90 protocol is self regulated and workload is not matched it may be that on average the U15 group completed more work than the U17 group, therefore inducing a greater fatigue response. This hypothesis is partly supported by the data we collected on the distance travelled during the SAFT90 protocol. The mean difference between the U15 and U17 age groups was only 65m (10,525m Vs 10,590m) despite the fact that the U15 performed for 10min less (80 V 90mins). Finally, although there was not a significant age effect for the Q angles the U15 age group had larger angles compared with the U13 and U17 respectively (17° Vs 14.5° and 15.2° respectively). It may be that this greater Q angle could be a contributing factor to the negative fatigue related effects found on the FH/Q in the current study. However, this hypothesis requires further investigation. Nevertheless, it would appear that this reduced muscular stability, combined with a reduced neuromuscular stability (see EMD section) when fatigue is present, places the pubertal female footballer at the greatest risk of knee injury. Our findings may start to explain the mechanisms behind the significantly greater risk of injury in the pubertal child, as recently demonstrated by Rumpf and Cronin (2012). Rumpf and Cronin (2012) reported a significant increase in injury incidence of 8.0 injuries/1000h in 9-12y-olds compared to 65.8 injuries/1000h in 13-16y-olds. They tentatively speculate that this dramatic increase seems to coincide, especially in boys with peak height velocity (PHV). The girls in the U15 age group in the current study were, on average 1y post PHV, and should have benefited from peak strength gains that occur around 0.5y after PHV in girls (De Ste Croix and Deighan, 2011). It may potentially be a case that this group is experiencing large gains in strength, without concomitant increases in neuromuscular functioning. Therefore they may not have had time to 'train' this larger muscle mass to work effectively from a neuromuscular perspective. This hypothesis seems plausible, given our limited knowledge on development of muscular and neuromuscular functioning around PHV in girls, but requires further investigation. Also although we did not set out to collect incidence data this hypothesis resonates with data obtained from the club used in the current study as the reason why the sample is the smallest in the U15 age group is that 4 players had knee injuries during the testing period.

6.3: Influence of football specific fatigue on leg stiffness

The importance of feed-forward mechanisms in preparing the joint for contact with the ground (either from a jump or during pivoting/twisting actions) are crucial for dynamic stability of the knee. It is difficult to compare the findings from the current study to the extant literature as this is the first study to have explored changes in leg stiffness following football specific fatigue in youth female players. The significant time by group interaction effect observed in the current study was due to the slight decrease in stiffness in the U13s compared to relatively no change in the U15s and an increase in the U17s from pre to post fatigue. These data would suggest that football specific fatigue induces an inhibitory response in younger players and an excitatory response in older players. The slight reduction in stiffness in the U13 group indicates longer ground contact times and a likely shift in the neural control to a lower contribution from pre-activation and short latency stretch reflexes. This in turn would likely lead to more of a yielding action with an increase in centre of mass displacement during ground contact and subsequently increase the relative risk of injury, especially during landing.

Adult studies have provided conflicting results with some studies showing an increase in stiffness after fatigue (Morin et al., 2011), some showing a decrease in stiffness (Dutto and Smith 2002; Comys et al. 2006) and some showing no change in stiffness (Girard et al. 2011; Morin et al. 2006). It is difficult to attribute these findings to mechanistic structures but they could be influenced by the nature of the fatigue protocol, and the methods used to calculate leg stiffness. Currently unpublished data from our research group has shown an individualised response of leg stiffness to 45min of football specific exercise in 16-year-old boys, with strong linear relationships between changes in both feed-forward muscle activity and eccentric muscle activity with leg stiffness ($r = 0.94$, $r = 0.87$ respectively) (Oliver, 2008). Data has also shown that differences in stiffness between children and adults are related to the speed of the movement (Oliver and Smith, 2010) and therefore this needs to be taken into consideration when comparing data from different studies. Care must also be taken when examining data from studies that have reported relative stiffness as most studies have simply taken body mass into account but this is not appropriate in children where proportional changes in limb length during growth and maturation play a key role in biomechanical and muscular functioning. Thus it is important that when exploring age related differences in leg stiffness that both body mass and limb length are taken into account, as has been done in the current study. Therefore differences in the findings of previous studies may be attributed to the varying method used to determine relative stiffness. However, the conflicting findings may of course simply describe the individualised response of fatigue to neuromuscular functioning in terms of feed-forward mechanisms.

The stiffness values reported in the current study for the U13 and U15 age groups are similar to those previously found using the same protocol in young boys (Lloyd et al., 2012; Oliver and Smith, 2010). Lloyd et al (2012) reported comparative relative leg stiffness data for 12 year olds (44 vs 45) and 15 year olds (49 vs 46) to our pre-fatigue data for U13 and U15s respectively. Both Lloyd et al (2012) and Oliver and Smith (2010) have reported significant age related effects in the neural control of leg stiffness in boys with stiffness increasing with age. We also found age related differences but the stiffness was lower pre fatigue in the U17 compared with U15 and U13 age groups. These data are difficult to explain as others have proposed that in boys there is a clear maturational effect in leg stiffness that is related to growth and the development of motor control. Lloyd et al (2012a) suggest that as children mature they become more reliant on supra-spinal feed-forward input and short latency stretch reflexes to regulate greater levels of leg stiffness. We have found age related changes that may support this mechanism in relation to maturation in youth female footballers, but they are only evident when fatigue is present. It is likely therefore that the age related effects found in the current study are more likely due to the effect of fatigue rather than from normal growth and maturation. There is supporting data that has also demonstrated no significant maturation effects in leg stiffness (Korff et al., 2010, Lloyd et al., 2012).

The U13 data showing a small decrease in leg stiffness from pre to post fatigue indicates a detrimental fatiguing effect on neuromuscular feed-forward mechanisms in this age group. Importantly there were no individualised effects of fatigue on leg stiffness and all participants relative stiffness was reduced post fatigue. This reduction in relative leg stiffness indicates longer ground contact times, shorter flight times, and a likely shift in the neural control to a lower contribution from pre-activation and short latency stretch reflexes. The reduction in angular displacement may also suggest a transition from fast stretch-shortening cycle activity to slow stretch-shortening type activity, but this hypothesis requires investigation using contact time data. What drives this neural response in this age group is unclear but could be related to a protective mechanism to prevent excessive rapid overload of the relatively immature musculotendon system upon ground contact. It is well recognised that the Golgi complex is considerably diminished in size and number in adults compared to children (Oliver and Smith, 2010) and this in part may account for the increased protective mechanism. It has also been proposed that intrafusal fibre development throughout childhood may also dampen the excitatory response to a stretch stimulus in children. How this development is influenced by fatigue in children remains to be investigated. As it is likely to be pre-programmed commands that play a decisive role in stiffness regulation together with a barrage of proprioceptive feedback, our findings suggest that young female footballers (U13) ability to recall pre-programmed commands may not be optimal when fatigue is present.

The U15 data found in the current study are in agreement with previous unpublished observations that have shown no significant change in relative leg stiffness in U16 year old male footballers after 45min of football specific exercise (Oliver, 2008). However, the Oliver (2008) data demonstrated an individualised response to the fatigue protocol with some individuals increasing leg stiffness post fatigue whilst others demonstrated a decrease in leg stiffness. Adult data following a 1h exhaustive run have also demonstrated an individualised response to a fatigue related task, with some participants increasing stiffness, some showing no change and some showing a decrease (Hunter and Smith, 2007). Our data also demonstrated some individualised response to the change in stiffness pre-post fatigue in the U15 age group, indicating that pre-activation was enhanced in some individuals but reduced in others post fatigue. However, the increase or decrease in stiffness was relatively small and in line with our findings for the FH/Q ratio this variability may be due to the pubertal status of this group with individuals at differing stages of maturation (range in offset from PHV was +0.41 – 1.95y). These findings may also reflect differing amounts of fatigue experienced by individuals after the football specific fatigue task as the workload was not set and was in some ways self regulated. These data suggest that female players who are progressing through puberty do not exhibit significant detrimental effects of fatigue on feed-forward neuromuscular mechanisms but also do not use feed-forward mechanisms in a compensatory manner, as shown in the U17 age group.

The U17 data was somewhat unexpected and suggests enhanced feed-forward mechanisms in post pubertal female footballers when fatigue is present. The proposed reason for this is difficult to explain but as with the enhanced FH/Q ratio post fatigue we tentatively suggest that this may be some form of compensatory mechanism to counteract the reduced feedback system (EMD) when fatigue is present. This finding may also support some form of quadriceps-dominant strategy, which has been shown in previous studies examining fatigue effects in females (Padua et al., 2006). It has been reported that females use an enhanced feed-forward strategy of the quadriceps when fatigued by activating the quadriceps 45% more than male counterparts in preparation for ground contact (Padua et al., 2006). Our findings tentatively suggest that via either normal growth and maturation and/or football specific training that post pubertal female players compensate for longer latency times (longer EMD) by moving to a quadriceps dominant strategy and increasing muscular stability (FH/Q ratio) and feed-forward mechanisms (stiffness) to protect the joint. Oliver and Smith (2010) demonstrated that men were able to increase their feed-forward mechanisms by demonstrating greater relative stiffness compared to boys at faster hopping velocities. EMG also indicated a greater extensor activity and ability to use reflex muscle activity (Oliver and Smith, 2010). Lloyd et al. (2012) reported similar findings in 9, 12 and 15year old boys, demonstrating a significantly greater absolute and relative leg stiffness in 15 year olds compared to 12 and 9 year olds. These findings may support our data indicating that there may be an age related effect in enhanced leg stiffness in female youth footballers, but only when fatigue is present. Whether this effect is related simply to age and maturation, or is further enhanced through football specific training in female youth footballers requires further investigation using non-trained controls. It could be that greater exposure to football training may develop the ability, when fatigue is present, to increase the reflex response and musculoarticular stiffness (Grosset et al., 2007), increase and produce earlier muscle pre-activation (Lazaridis et al., 2010), improve spindle sensitivity (Grosset et al., 2007) and decrease co-contraction ratios. There may also potentially be some form of training/learning effect and skill mastery associated with the ability to increase stiffness when fatigue is present. The work of Laffaye et al (2005) indicated that those individual classified as experts were able to demonstrate increased stiffness to maximise jump height compared to novices. It may be that the U17 age group in the current study have 'learned' to increase stiffness when fatigue is present to maintain performance and this mastery may also help to reduce the relative risk of injury.

Therefore, our data may potentially demonstrate some form of training effect that may be over and above changes that we would expect to see through normal growth and development. This is of course speculation as we do not have any control group data to compare our findings against and therefore this should be an avenue for further study. In adults certain forms of resistance and flexibility training have been shown to lead to both increases or decrease in leg stiffness (Wilson et al., 1992). There is very little data on children however, one recent study has indicated that just 4 weeks of plyometric training improved leg stiffness in 12 and 15-year old boys (Lloyd et al., 2012). Although Lloyd et al (2012) did not find any training related effects in 9-year old children a recent study exploring the effects of 10 weeks of resistance training in prepubertal children demonstrated an increase in Achilles tendon stiffness (Waugh et al., 2011; cited in Blazvich et al., 2011). These findings suggest that training may be able to influence both relative leg stiffness and tendon properties in children through physical training and may in part explain the findings of the current study. Potentially the change from an inhibitory effect of fatigue in the U13 group to an excitatory response in the U17 group could be attributed to some form of training effect where the ability to compensate for a reduction in other stability mechanisms with fatigue (eg longer EMD) is developed through training. It should be noted that none of the groups in the current study have undergone systematic plyometric, resistance or eccentric conditioning programmes, so this hypothesis would be related to football specific training effects.

6.4: Influence of football specific fatigue on EMD

The EMD, which is a component of the reflex time, is important for sports performance as it affects muscle response to sudden movements. Clinically, alterations in the EMD of the hamstring muscle-tendon unit compromise knee integrity and/or impair performance by modifying the transfer time of muscle tension to the tibia. Previous studies have highlighted the importance of changes in EMD during physical activities. Vos et al., (1991) observed that changes in EMD play an important role in the organization of the movement and probably result in impairment of neuromuscular control, through its relationship with the reflex time.

The significant group by time interaction effect and main effect for time and group for EMD indicated that football specific fatigue significantly increases EMD post fatigue for all age groups. These data demonstrate that irrespective of age neuromuscular feedback mechanisms are significantly compromised with football performance in youth female footballers. Importantly the ability of the neuromuscular system to contribute to the stabilisation of the knee when fatigue is present was affected most in younger age groups. The significant main effect for time, indicating that the EMD for all muscles assessed was longer post-fatigue compared to pre-fatigue can be attributable to a number of mechanisms such as metabolic inhibition of the contractile process; excitation-contraction coupling failure as well as structural changes that have been suggested to explain peripheral fatigue (Pasquet et al., 2000, Gibala et al., 1995, Baker et al., 1993). The present findings, together with corroborating findings from other studies (e.g., Gleeson et al. 1998; Zhou et al. 1996), suggest a reduced capability of the dynamic stabilisers to provide forceful corrective responses to mechanical loading of the knee from a neuromuscular perspective (feedback mechanisms) when fatigue is present. Such fatigue-related changes in neuromuscular performance may be interpreted to represent an increased risk of injury (Chan et al., 2001; Gleeson et al., 1998; Mercer et al., 1998), which may be amplified particularly at knee angles where key ligamentous structures are already under greatest mechanical strain (e.g., near full knee extension) (Beynnon and Johnson, 1996).

It is difficult to compare our data to the work of others as there are no studies that have examined changes in EMD before and after fatigue in young girls, and during eccentric actions of the hamstrings. There are a number of adult studies that have explored fatigue effect on EMD but mainly during isometric muscle actions. There is an acknowledgement in the literature that EMD is influenced by the type of muscle action (Cavanagh and Komi, 1979), joint angle (Grabiner, 1986), the level of effort (Grabiner, 1986; Vos et al., 1991), fatigue (Nilsson et al., 1977, Kroll, 1974) and the age and sex of the participants (Clarkson and Kroll, 1978, Bell and Jacobs, 1986). Despite this the majority of adult studies have shown a significant increase in EMD following fatigue (El Nagar, 2012; Nilsson et al., 1977, Horita and Ishiko, 1987; Howatson 2010; Zhou et al., 1996). For example Nilsson et al. (1977) reported an increase in EMD of the vastus lateralis muscle (VL) during concentric muscle actions from 95ms at rest to 121ms after 100 maximal isokinetic knee extensions. The study of Howatson (2010) indicated that after fatiguing exercise with a large eccentric component that EMD remains compromised 96h after the fatiguing task even though force production returns to pre fatigue levels. These data from adult participants have important implications for injury risk as neuromuscular feedback mechanisms appear to remain compromised even after muscular components have recovered. We did not explore the chronic effects of the bout of football specific exercise on EMD, however given the large increase in EMD found in the current study it may be that youth female footballers neuromuscular feedback mechanisms remain compromised until their next training session or game which could increase the relative risk of injury. This hypothesis requires further investigation plotting the chronic effects of football specific fatigue on both muscular and neuromuscular components in youth female footballers. This may provide coaches with important information regarding neuromuscular readiness to re-perform in this population.

To our knowledge no studies have examined the effect of fatigue on changes in EMD during eccentric muscle actions of the hamstrings. However, unpublished observations from our laboratory have shown a significant increase in EMD during eccentric hamstring exercise in both male and female adults, with the effects significantly greater in females (El Nagar, 2012). These data agree with the findings of the current study indicating that EMD during eccentric hamstring actions are compromised when fatigue is present. The absolute change in EMD in the current study is greater than that observed for adults, indicating that football specific fatigue effects appear to negatively influence neuromuscular stability to a greater extent in females during childhood compared to adulthood.

There are no paediatric studies that have explored the influence of fatigue on EMD in either males or females. The limited EMD data in children have been exclusively on male participants, during isometric actions and in a non-fatigued state (Cohen *et al.* 2010; Falk *et al.* 2009; Grosset *et al.* 2010; Zhou *et al.* 1995). The work of Gosset *et al.* (2010) focused on ankle stiffness and EMD of the triceps surae in normal and diseased children. They reported a greater EMD in children suffering with Legg-Calve_Perthes disease (a hip disorder) compared to healthy controls, albeit only in 6 children. Cohen *et al.* (2010), Falk et al (2009) and Zhou et al (1995) have all found significantly longer EMD in young children (8-12 years) compared to adults. This longer EMD in children may be as a result of differences in muscle composition. However, current limited evidence suggests that differences in muscle composition are not sufficient to account for the child-adult differences. Therefore differences in muscle activation, such as excitation-contraction coupling and muscle fibre conduction velocity have been implicated in this longer EMD. Therefore this lower rate of force development may reduce muscle-tendinous stiffness and increase the potential for injury in children. Our data support this age related difference in EMD of the hamstring during eccentric muscle actions. The U13 had significantly longer EMD for all muscles assessed (average 119ms) compared with the U15 (93ms) and U17 (92ms) age groups. The pre-fatigue EMD values in the current study are longer than those previously reported in the literature for isometric actions (range 44-65ms) (Cohen *et al.* 2010; Falk *et al.* 2009; Grosset *et al.* 2010; Zhou *et al.* 1995) but this may be related to the muscle group, muscle action and movement velocity used. It has been previously recognised that comparison of EMD data between studies is problematic as EMD values in adults have been shown to vary between 30 to 50ms up to a few hundred ms depending on the muscle examined, muscle action, joint angle and movement velocity (Shultz and Perrin, 1999). For example a recent study by Minshull et al. (2011) demonstrated that volitional EMD of the quadriceps femoris at joint angles proximal to full knee extension were shortened by increased knee flexion. This joint angle specific change in EMD is likely to reflect the influence of the degree of myofilament overlap (McComas, 1996), the discharge properties of the motoneurons and the capability for neural activation (Komi et al., 2000) and the compliance characteristics of the musculo-tendinous complex (Muraoka et al., 2004).

Additionally the current study is the first to demonstrate age related effects in EMD when fatigue is present. The significantly longer EMD both pre and post fatigue in the U13 age group may indicate a reduced ability of younger children to quickly activate their muscles until the first mechanical response or that they have a less stiff musculo-tendinous system. It is possible that the younger girls have a more compliant muscle–tendon system when fatigue is present compared with older girls and this requires more time to produce a mechanical response, given the same stimulus. The observed age difference in the present study in relations to fatigue could also represent differences in fibre type distribution (Viitasalo and Komi, 1981, Woledge et al., 1985), with type II fibres having shorter force-developing times than type I fibres. It may be that there is an increase of type II fibres with maturation although the evidence to support this view is limited. Interestingly, Cohen et al (2010) did not show any significant difference between endurance trained and untrained children suggesting that level of training status did not have an effect on EMD in 9-12 year olds. Our findings may challenge this conclusion and suggest that number of total hours of athlete exposure may influence EMD by reducing the detrimental effects of fatigue, over and above the effects of normal growth and maturation (as shown in the U17 compared with the U13 age groups). However, this hypothesis requires further investigation investigating fatigue effects on EMD in non trained participants at each age group.

The main proposed mechanism for the lengthening of EMD with fatigue in adults is from the excitation-contraction coupling contractile mechanisms and the stretching of the series elastic components (SEC), resulting in prolonged EMD (Shi, 1996). After stimulation, the rate of calcium ions (Ca^{2+}) release from the sarcoplasmic reticulum (SR) has been shown to require 2-3 ms to reach its peak level (Zhou et al., 1996), which would account for approximately 5%-7.5% of an EMD time of 40ms. Impaired membrane conductivity with fatigue could reduce the sarcoplasmic reticulum (SR) Ca^{2+} release and therefore, contribute to the reduced rate of force generation and prolonged EMD during fatigue (Westerblad et al., 1991). Thus, it has been suggested that acidosis induced by intense muscle actions may reduce SR Ca^{2+} release (consequently increasing the time to reach its peak), Ca^{2+} sensitivity and force production, and prolong EMD (Westerblad et al., 1991, Horita and Ishiko, 1987b, Maclaren et al., 1989). It would appear therefore that the prolongation of the EMD during fatigue may be largely attributed to the failure of the muscle contractile process. Given this mechanism we would not have expected to observe greater fatigue related effects in EMD in the U13 age group. Current data on children suggest that younger children experience less metabolic fatigue (eg acidosis or accumulation of Pi) due to faster oxygen uptake kinetics (Fawkner and Arnstrong, 1997). We can only speculate that possibly even in the youngest age group that football specific training, which includes large elements of anaerobic work, may have increased the use of anaerobic metabolism in this age group but they are not trained enough to effectively remove detrimental by-products of anaerobic metabolism. It has been suggested that the time required for the contractile component to stretch the SEC probably accounts for the major portion of EMD (Zhou et al., 1996, Viitasalo and Komi, 1980, Winter and Brookes, 1991). If the major influencing factor of EMD is the time for the contractile components to stretch the series elastic component (SEC), increases in the percentage of type II fibres, contractile force and rate of force development may alter the EMD time. The current study clearly demonstrated an elongation of EMD with fatigue. It is likely that this reflects impaired contractile mechanisms, increased compliance of the SEC, with a reduction in muscle fibre conduction velocity. But how the elastic properties of the muscle affect EMD during fatigue is not clear. The mechanisms involved in the increase in EMD after fatigue in the current study could be due to the deterioration in muscle conductive, contractile or elastic properties and requires further study.

In response to muscular fatigue, Rozzi et al., (1999) indicated that there was an overall decrease in the ability to detect joint motion moving into the direction of extension, an increase in the onset time of contraction for the medial hamstring and lateral gastrocnemius muscles in response to a landing task. Muscular fatigue is considered an important factor in impaired neuromuscular mechanisms, as research has demonstrated its deleterious effects on knee joint laxity as well as both the afferent and efferent neuromuscular pathways (Ribeiro et al., 2007). A importance aspect of altered joint proprioception due to fatigue is a decrease in neuromuscular control (Rizzu et al., 2000). As a consequence of the increased latency periods during the fatigued state muscles are not able to respond quickly enough to protect a joint from injury especially in females. Alterations in the afferent input to the alpha motor neurons can potentially affect reactive muscular function and decrease the protection of the joints (Rizzu et al., 2000). Skinner et al. (1986) reported that during fatigue conditions participants had significantly decreased proprioceptive abilities. They hypothesized that this was due to either altered afferent impulses from the muscles themselves or from abnormal stresses in the joint capsule as a result of the muscle fatigue (Skinner et al., 1986). Altered joint proprioception due to fatigue may impact on neuromuscular control (Rizzu et al., 2000). Our findings support the view that the influence of fatigue on the EMD on hamstring muscles in female youth footballers potentially influences dynamic muscular control of knee joint alignment, and that specifically differences in muscle recruitment and EMD may be partly responsible for the risk of ACL injury. Our findings of increased EMD with fatigue tentatively suggest that proprioception and joint control are altered when fatigue is present, due to decreased neuromuscular control. However, more studies are needed to explore the influence of fatigue on proprioception and knee joint position sense, which may alter landing mechanics, when individuals youth female footballers are fatigued.

We found no significant muscle specific differences in the EMD either pre or post fatigue in the current study. These findings suggest that in youth female footballers, and during eccentric actions, that there are no differences in the response rate of feedback mechanisms between lateral and medial hamstrings or the calf muscles. Previous studies have suggested that adult females move away from a knee dominant strategy to an ankle dominant strategy when fatigue is present (Padua et al., 2006). Using peak EMG determined during the preparatory phase Padua et al., (2006) demonstrated that females shift towards greater reliance of the ankle musculature to help stabilise the joint when fatigue is present as these muscles tend to be less fatigued. To our knowledge the current study is the first study to have examined changed in EMD pre-post fatigue to explore if an ankle dominant strategy is evident is any age group/sex, let alone in youth female footballers. Our findings suggest that at least for female youth footballers that after football specific fatigue there are no differences in the feedback response rate between the muscles of the hamstrings or the calf. This would suggest that female youth footballers do not move towards an ankle dominant strategy when fatigue is present, irrespective of age, maturational status or training status. As with Padua et al. (2006) we acknowledge that we did not explore the role of the gluteal muscles and it may be possible that the football specific fatigue protocol used in the current study may have altered EMD of the gluteals in order to compensate for fatigue. Ethically it may be difficult to explore the role of the gluteals in youth female footballers but future studies need to investigate the effects of fatigue on the neuromuscular response of both the hip musculature and core muscles.

Applied Implications and Recommendations

The findings from the current study suggest that muscular functioning may not be dramatically impaired when football specific fatigue is present for all age groups. However, there may be implications for youth female footballers of diminished muscular stability when fatigue is present at near full knee extension and during faster velocity movements. It would appear that football specific fatigue specifically reduces neuromuscular feedback mechanisms in female youth footballers and training studies need to be designed that specifically target neuromuscular functioning rather than simply improving absolute torque production during eccentric actions of the hamstrings. As female footballers mature they may use compensatory mechanism to maintain stability when fatigue is present by increasing feed-forward mechanisms and muscular stability. How football specific training may contribute to this compensatory mechanisms remains to be identified.

Our findings suggest that the following need to be considered in the further development of neuromuscular conditioning programmes for elite female youth footballers:

1. That a neuromuscular conditioning programme is focused on being related to fatigue resistance and is undertaken in the middle or end of training sessions rather than solely during the warm-up when fatigue is not present
2. That muscular conditioning includes and focuses on the portion of the movement that is towards full knee extension (in particular the first 15° of knee flexion) – this should include training aimed at improving landing performance
3. That training includes fast velocity movements as well as more controlled slower velocity movements
4. That training programmes are especially effective in enhancing neuromuscular functioning as a primary goal as well as improving torque production as a secondary goal
5. That training is both age group and maturational stage specific. Specific neuromuscular training should be recommended, implemented and developed as early as possible
6. That training in younger age groups focuses on the development and enhancement of neuromuscular feed-forward mechanisms in response to fatigue. This training should also include fundamental movement skill development
7. That training during puberty is enhanced and individualised to focus on both muscular and neuromuscular qualities

7.1 Current evidence of effectiveness of neuromuscular training during childhood

Evidence from adult studies have indicated that individuals who routinely perform sport activities under stressful conditions can protect against the rotational stresses at the knee joint by increasing eccentric hamstring actions, as the limb is striking the ground (Bonci 1999). The proposed components of prevention programmes have been subject to much debate and surround developing strength ratios versus improving landing mechanics. However, most programmes currently aim to improve both movement strategies and neuromuscular/muscular performance and routinely include: strengthening, stretching, plyometric and balance components. It is beyond the scope of this study to explore these different neuromuscular training programmes. However, most importantly, they all contain some eccentric conditioning of the hamstring muscles, which emphasizes the importance of paying particular attention to eccentric hamstring training with the goal of injury prevention. For further information regarding training programmes the reader is directed towards the text of Hewett, Shultz and Griffins (2007) on understanding and preventing noncontact ACL injuries.

Our research group have recently demonstrated that a relatively modest load of eccentric hamstring conditioning in professional adult male footballers can significantly increase eccentric torque production in just 4 weeks (Iga et al. 2012). This study also demonstrated that slow eccentric training can improve fast eccentric torque production and therefore controlled Nordic Hamstring Exercise can have positive effects for fast velocity movements. A systematic review by Roig *et al.* (2009) has also pointed to effective improvements in eccentric strength following eccentric training at high intensities in adults. However, there appear to be no available studies that have directly determined the effectiveness of an eccentric training programme in female youth footballers and eccentric torque production, leg stiffness or EMD. Most training studies have focused on changes in either maximal muscle activation determined using EMG or biomechanical landing mechanics.

For example, an injury prevention programme by Lim *et al.* (2009) on young girls indicated positive changes in biomechanical factors associated with non-contact ACL injury (e.g. knee internal rotation angle, knee valgus moment, knee flexion angle). Studies focused on improving landing performance have been shown to increase knee flexion angles and reduce ground contact forces in both prepubertal and postpubertal participants (Hewett et al., 1996; Prapavessis et al., 2003). Data from children are limited and the large majority of studies have focused on high school girls, probably due to the available incidence data that suggests that this is the most ‘at risk’ group for non-contact ACL injury (Hewett *et al.* 1999; McLeod *et al.* 2009; Myer *et al.* 2006a, 2006b, 2008).These studies are relatively consistent demonstrating a reduction in the relative risk of injury in young girls irrespective of the type of neuromuscular training employed.

Due to the individual timing and tempo of both muscle growth and neuromuscular development during childhood it has been proposed that unbalanced development of these systems may result in neuromuscular imbalances that make the individual more susceptible to injury (McLeod *et al.* 2009). Previous studies have suggested that neuromuscular training should be targeted to a period when rapid musculoskeletal growth is occurring and/or when limb lengths change decreasing balance and co-ordination. All of the available neuromuscular training studies during childhood have focused on older children (14-17 year-old) except for the cluster-randomized controlled trial of Emery and Meeuwisse (2010), which looked at under 13 to under 18 year-old age groups. This current limitation with the extant literature makes the description of the effectiveness of neuromuscular training during childhood difficult. Our findings clearly indicate that neuromuscular training should be advocated as early as possible due to the detrimental effects fatigue has on neuromuscular functioning.

Despite this limitation, most of the available data demonstrate the effectiveness of a range of neuromuscular training programmes (plyometrics, balance training, dynamic stabilization training, eccentric training, trunk and hip training, static stretching) on a range of biomechanical, muscular, neuromuscular and performance outcomes (e.g. hip adduction angle, maximum ankle eversion angle, isokinetic torque production, centre of pressure, vertical ground reaction force, vertical jump height). Current data have provided us with a sense of which mechanisms may change after training and reduce the relative risk of injury during childhood. However, it is perhaps more important to explore if such programmes reduce injury rates. A systematic review by Hewett *et al.* (2005) indicated that all but one study that met the inclusion criteria indicated significant reductions in injury rates following intervention programmes to reduce injury rate in females ranging from 36-87%. Although these studies clearly indicate a role of neuromuscular training to reduce injury risk it must be noted that these studies pertain to females and generally to the 14-16 year-old age group. Whether such programmes have the same protective effects in younger age group and how this develops with age and maturation remain to be identified.

There appear to be no studies that have investigated changes in the FH/Q ratio in children after a training programme. However one recent study has indicated that 6 weeks of neuromuscular training significantly increased balance and proprioceptive capabilities of 15-16 year-old female basketball players (McLeod *et al.* 2009). Myer *et al.* (2008) also examined the effectiveness of a trunk and hip focused neuromuscular training programme on knee and hip isokinetic strength in 15 year-old girls. After a 10 week training programme the experimental group improved their hip strength by 16%, whereas the control group reported no significant increases in strength. The authors however, do acknowledge the limitations of their findings as the strength measure was determined from concentric rather than eccentric actions and during open rather than closed chain actions. Further work by this group have also explored differences in the effects of plyometric versus dynamic stabilization and balance training on lower extremity biomechanics, power and balance (Meyer *et al.* 2006a, 2006b). In the biomechanical 3D motion analysis they reported that both types of training reduced lower extremity valgus measurements with plyometric exercise predominately influencing sagittal plane kinematics during a drop vertical jump and balance training during single legged drop landing. Nelson and Brandy (2004) also reported improved hamstring flexibility in 16 year old high school boys after a 6 week eccentric training programme, having linked the proposed benefits of enhanced flexibility to reduction of injury. Emery and Meeuwisse (2010) recently reported that a soccer-specific neuromuscular training programme for 13-18 year-old males and females, was protective of all injuries and reduced the injury rate to 2.08 per 1000 player hours in the training group compared with 3.35 per 1000 player hours in the control group. Therefore, the majority of available data clearly indicates that neuromuscular training appears to reduce the relative risk of injury and enhance physical performance in late adolescence in females. It is well recognized that selection into elite sport is biased towards early maturing children and it may be pertinent to explore these effects in early maturers and in younger age groups. Well designed longitudinal studies are required to further elucidate answers to the effectiveness of such programmes throughout childhood.

Our research group have just completed an unpublished full systematic review exploring the effectiveness of intervention programmes (either muscular or neuromuscular) on the FH/Q ratio in young females. A total of 4582 journal articles were found in the initial database search. After the inclusion and exclusion criteria were employed using a PICOS study design (Population, Intervention, Control) a total of 13 articles met the inclusion criteria, and there were a total of 317 females (age range 14.5 - 23 y) who were investigated regarding their knee dynamic stability. On average, each intervention lasted for 15 weeks, ranging from 6- to 20 weeks, with half of all studies using an intervention period between 6 to 8 weeks. Only 2 studies measured specifically the H/Q ratio in their sample. Only one of the studies that found a positive effect in the intervention applied was a randomised controlled trial. The main finding of the systematic review suggests that intervention programs with an emphasis on strength and plyometric exercises are effective in improving H/Q ratio and strength imbalances in young females. However, our extensive search highlights the paucity of data in this area and on the population of interest (e.g. youth female footballers).

Future Research Directions

This study has provided some unique findings into the effects of football specific fatigue on dynamic knee stability in female youth footballers. We are currently collecting 3D landing mechanics data on these participants to additionally explore the role of landing mechanics in relation to growth and maturation. However, all of this data is cross sectional in nature, which although providing essential information regarding differences with age and maturation, longitudinal studies are needed to explore the effects of growth and maturation on dynamic knee stability. Future studies should continue to examine a range of potential risk factors (including hormonal influences), and the findings of the current study have highlighted the need to examine both neuromuscular feed-forward, as well as feedback mechanisms as their response to fatigue may be age and maturation dependent. Due to the large effects of fatigue on EMD demonstrated in the current study, and some evidence that EMD remains compromised in adults up to 92h after fatigue (Howatson 2010), future studies should explore the chronic effects of football specific exercise on both muscular and neuromuscular functioning in youth female players. This neuromuscular readiness to re-perform would appear not only to have implications for performance but may be associated with the increased relative risk of injury within a competitive season. Tracking these carry-over affects alongside metabolic markers such as creatine kianase and cortisol/testosterone ratios may provide coaches and sports scientists with knowledge regarding individuals readiness to re-perform (whether at training or in match play). Future studies in youth female players may benefit from exploring fatigue effects after competitive match play, but this may restrict outcome measures to those than can be achieved using portable equipment (such as leg stiffness, portable force plates, EMG). There is a need to further explore whether compensatory mechanisms are evident in female youth footballers by exploring the effects of football specific fatigue on a wider range of muscles that contribute to stabilising the knee (eg the hip and core muscles). This may identify, as with adults, whether female youth footballers move to a muscular strategy of using muscles that are less fatigued during the fatigue task.

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APPENDICIES

Appendix A: Letter for participants

Protect her knees - Exploring the role of football specific fatigue on dynamic knee stability in female youth football players

Dear Team member,

Thank you for your interest in taking part in this study. This sheet will tell you a bit more about the study and what we would like you to do. Please read this carefully. If you decide not to take part it will not change your relationship with the research team, the University or Club.

What is the project about?

We are interested in looking at the effect of football fatigue on the lower leg, particularly on the knee in female youth players. This is a unique study investigating how well the muscles work when they are fatigued, so that we can design training to try to reduce the risk of injury.

About the study

This study has been funded by UEFA and will be run by a research team from the University of Gloucestershire. All researchers have experience working with athletes/children and have completed a full CRB check. The study has also been approved by the University Research Ethics Sub-Committee (RESC).

Who is taking part in the study?

Females aged 12-17 years who are participating in professional women's football in the UK (players linked to Bristol Academy Ladies).

You will visit the University of Gloucestershire twice and we will come to one of your club training sessions to do some quick tests.

On your first visit to the University all tests will be explained to you and you will have a go on a machine called an isokinetic dynamometer to get used to the movements you will be performing, as well as practice the running test. Your posture and landing technique will also be examined using a camera system. Other things like height and weight will be measured on this visit to the University.

On your second visit to the University you will perform the actual tests. Initially, you will perform a standard ten-minute warm-up that is usually performed at your club before matches. This will include running and movements familiar to you. You will then perform a baseline test involving a repeated kicking action with your preferred leg on the isokinetic dynamometer. There will be 30-seconds rest between each effort. Measurements of muscular activity will be taken using pads placed on different muscles in the leg.

Following baseline testing players will undertake the $SAFT^{90}$ exercise protocol. This protocol involves football specific movement around a sports hall for the same duration as your matches with a half-time interval, also the same as on a match day. 5-minutes after finishing, you will repeat the test on the isokinetic dynamometer a second time.

Water will be available to fill up drinks bottles when you visit the University in order to keep you hydrated throughout the testing session.

When we come to one of your club trainings, you will perform a variety of hopping and jumping tasks on a mobile contact mat. This will include jumping on both feet continuously for about 10 seconds. You will also complete a Functional Movement Screen (FMS) which examines how you move when you perform a squat, a hurdle step, a lunge, an assessment of shoulder mobility, a straight leg raise, a push up and a core stability exercise.

When will I do it?

Whenever you and the laboratory are available we will arrange a time for you to come to the laboratory. If you wish you may come as a group. All tests at the University of Gloucestershire will follow our RESC approved laboratory guidelines. Parents/guardians will be informed of the specific arrangements via written documentation.

Can I change my mind?

You can stop being a part of the study at any time, including anytime throughout data collection. All you have to do is let us know that you no longer want to take part. This will not affect your relationship with the research team, the University or Club.

What will you do with the information?

All the information collected will be stored on a computer using ID codes and the results will only be seen by the research team. Your name will never be used. The data will be stored on password protected computers and in lockable filing cabinets for 10 years and will then be destroyed.

The tests we perform are nothing to do with performance and your coaches will not use the results to pick the team. The results could be used to design a specific individual training program by your coaches in order to reduce the risk of future injury. If you want your coaches to have access to your individual results in order to make changes to your training, you should fill in the Data consent form.

What if I have any questions?

If you have any questions then please feel free to ask either of the people below at any time.

What do I do next?

If you have read and understood everything that we want you to do and are happy to take part please sign the consent form that is attached to this sheet.

Miss Abigail Priestley

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Appendix B: Participant consent form

Protect her knees - Exploring the role of football specific fatigue on dynamic knee stability in female youth football players

Sport & Exercise Laboratories

Participant informed consent form

I have had full details of the tests I am about to complete explained to me. I understand the risks and benefits involved, and that I am free to withdraw from the tests at any point. I confirm that I have completed a health questionnaire and I am in a fit condition to undertake the required exercise.

Participant

Name:………………………………………

Signed:……………………………………................. Date:………………...

Miss Abigail Priestley

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Appendix C: Participant data release form

Protect her knees - Exploring the role of football specific fatigue on dynamic knee stability in female youth football players

(Data Consent Form)

Dear Participant,

The data gathered in this study will not be passed on to any other party and will remain anonymous if you so wish. However, the data may be beneficial for you if passed on to the relevant members of your club.

The data collected does not relate to actual performance, therefore your coaches will not use the data for team selection. It is purely to highlight any specific areas that could be improved through an individual training program in order to reduce your risk to injury.

If you wish for your data to be made available for your team management please sign below.

Participant

Name:…………………………………………..

Signed…………………………………………. Date:………………………………

Thank you again for your cooperation.

Miss Abigail Priestley

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Appendix D: Parent/guardian consent form

Protect her knees - Exploring the role of football specific fatigue on dynamic knee stability in female youth football players

Sport & Exercise Laboratories

Parent/ guardian informed consent form

I have had full details of the tests my child is about to complete explained to me. I understand the risks and benefits involved, and that she is free to withdraw from the tests at any point. I confirm my child has completed a health questionnaire and is in a fit condition to undertake the required exercise.

Parent/ guardian

Name:……………………………………

Signed:……………………………………............... Date:………………...

Miss Abigail Priestley

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Appendix E: Letter of ethical approval for the study

Research Ethics Sub-Committee Approval

Protect her knees: Exploring the role of football specific fatigue on dynamic knee stability in female youth football players.

After attending the Research Ethics Sub-Committee meeting to discuss the project and making the suggested amendments, the project has again been considered and we are pleased to inform you that it has now been approved.

Thank you for taking the time to attend the RESC meeting and good luck with your project.

Best wishes

Dr Malcolm MacLean
Chair - Research Ethics Sub-Committee

Associate Dean, Academic Frameworks
University of Gloucestershire
The Park
Cheltenham GL50 2RH

Appendix F: Pre-testing health questionnaire

SPORT & EXERCISE LABORATORIES

Health Questionnaire

About this questionnaire:

The purpose of this questionnaire is to gather information about your health and lifestyle. We will use this information to decide whether you are eligible to take part in the testing for which you have volunteered. It is important that you answer the questions truthfully. The information you give will be treated in confidence. Your completed form will be stored securely for 5 years and then destroyed.

Section 1, which has been completed by the tester, provides basic information about the testing for which you have volunteered. Sections 2 to 7 are for you to complete: please circle the appropriate response or write your answer in the space provided. Please also complete section 8. Sections 9 and 10 will be completed by the tester, after you have completed sections 2 to 8.

1.1 Section 1: The testing (completed by tester)

To complete the testing for which you have volunteered you will be required to undertake:

Moderate exercise (i.e., exercise that makes you breathe more heavily than you do at rest but not so heavily that you are unable to maintain a conversation) ☐

Vigorous exercise (i.e., exercise that makes you breath so heavily that you are unable to maintain a conversation) ☐

The testing involves:

Walking	☐	Generating or absorbing high forces through your arms	☐
Running	☐	Generating or absorbing high forces through your shoulders	☐
Cycling	☐	Generating or absorbing high forces through your trunk	☐
Rowing	☐	Generating or absorbing high forces through your hips	☐
Swimming	☐	Generating or absorbing high forces through your legs	☐
Jumping	☐		

1.2 Section 2: General information

Name: .... Sex: M F Age:

Height (approx.): .... Weight (approx.):

1.2.1 Section 3: Initial considerations

1. Do any of the following apply to you? No Yes
   - a) I have HIV, Hepatitis A, Hepatitis B or Hepatitis C
   - b) I am pregnant
   - c) I have a muscle or joint problem that could be aggravated by the testing described in section 1
   - d) I am feeling unwell today
   - e) I have had a fever in the last 7 days

(If you have answered "Yes" to question 1, go straight to section 8)

1.3

1.4

1.5 Section 4: Habitual physical activity

2a. Do you typically perform moderate exercise (as defined in section 1) No Yes

for 20 minutes or longer at least twice a week?

2b. Have you performed this type of exercise within the last 10 days? No Yes

3a. Do you typically perform vigorous exercise (as defined in section 1) No Yes

at least once a week?

3b. Have you performed this type of exercise within the last 10 days? No Yes

1.6

1.7 Section 5: Known medical conditions

4. Do **any** of the following apply to you? No Yes
   - a) I have had insulin-dependent diabetes for more than 15 years
   - b) I have insulin-dependent diabetes and am over 30 years old
   - c) I have non-insulin-dependent diabetes and am over 35 years old

5. Have you ever had a stroke? No Yes

6. Has your doctor ever said you have heart trouble? No Yes

7. Do **both** of the following apply to you? No Yes
   - a) I take asthma medication
   - b) I have experienced shortness of breath or difficulty with breathing in the last 4 weeks?

8. Do you have any of the following: cancer, COPD, cystic fibrosis, No Yes

other lung disease, liver disease, kidney disease, mental illness, osteoporosis, severe arthritis, a thyroid problem?

(If you have answered "Yes" to any questions in section 5, go straight to section 8.)

1.8 Section 6: Signs and symptoms

9. Do you often have pains in your heart, chest, or the surrounding areas? No Yes

10. Do you experience shortness of breath, either at rest or with mild exertion? No Yes

11. Do you often feel faint or have spells of severe dizziness? No Yes

12. Have you, in the last 12 months, experienced difficulty with breathing when lying down or been awakened at night by shortness of breath? No Yes

13. Do you experience swelling or a build up of fluid in or around your ankles? No Yes

14. Do you often get the feeling that your heart is racing or skipping beats, either at rest or during exercise? No Yes

15. Do you regularly get pains in your calves and lower legs during exercise that are not due to soreness or stiffness? No Yes

16. Has your doctor ever told you that you have a heart murmur? No Yes

17. Do you experience unusual fatigue or shortness of breath during everyday activities? No Yes

(If you have answered "Yes" to any questions in section 6, go straight to section 8.)

1.9

1.10 Section 7: Risk factors

18. Does **either** of the following apply to you? No Yes
    a) I smoke cigarettes on a daily basis
    b) I stopped smoking cigarettes on a daily basis less than 6 months ago

19. Has your doctor ever told you that you have high blood pressure? No Yes

20. Has your doctor ever told you that you have high cholesterol? No Yes

21. Has your father or any of your brothers had a heart attack, heart surgery, or a stroke before the age of 55? No Yes

22. Has your mother or any of your sisters had a heart attack, heart surgery, or a stroke before the age of 65? No Yes

23. Do **any** of the following apply to you? No Yes
    a) I have had insulin-dependent diabetes for less than 15 years
    b) I have insulin-dependent diabetes and am 30 or younger
    c) I have non-insulin-dependent diabetes and am 35 or younger

1.11 Section 8: Signatures

Participant: ………………………………………………………….. Date: ....……….……........

Guardian*: ....……………………......... Date: ....……….……........
(*Required only if the participant is under 18 years of age.)

Section 9: Additional risk factors (to be completed by the tester if relevant)

24. Is the participant's body mass index >30 kg/m^2? No Yes

25. Has the participant answered no to questions 2a **and** 3a? No Yes

Section 10: Eligibility (to be completed by the tester)

26. Is the participant eligible for the testing? No Yes

Name (of tester): ....………...........……………………

Signature: ……………………………………………………… Date: …………....….……….....

Appendix F: Concentric and Eccentric torque values pre and post fatigue

Table 1: U13 Eccentric and Concentric torque pre and post fatigue by joint angle

	Pre			Post		
	0-10°	***10-20°***	***20-30°***	***0-10°***	***10-20°***	***20-30°***
Concentric Q at 60°/s	21 ± 7	32 ± 11	42 ± 11	14 ± 8	27 ± 12	36 ± 12
Concentric Q at 120°/s	18 ± 7	30 ± 8	37 ± 9	16 ± 5	23 ± 9	31 ± 11
Concentric Q at 180°/s	19 ± 6	28 ± 8	35 ± 9	18 ± 9	24 ± 9	30 ± 10
Eccentric H at 60°/s	33 ± 22	44 ± 20	52 ± 18	34 ± 18	47 ± 23	50 ± 25
Eccentric H at 120°/s	33 ± 21	46 ± 19	50 ± 17	24 ± 16	50 ± 23	54 ± 25
Eccentric H at 180°/s	31 ± 20	46 ± 19	51 ± 17	48 ±18	24 ±16	52 ± 21

Q = Quadriceps H = Hamstrings

Table 2: U15 Eccentric and Concentric torque pre and post fatigue by joint angle

	Pre			Post		
	0-10°	***10-20°***	***20-30°***	***0-10°***	***10-20°***	***20-30°***
Concentric Q at 60°/s	17 ± 9	33 ± 8	46 ± 10	18 ± 6	34 ± 12	46 ±10
Concentric Q at 120°/s	17 ± 6	33 ± 8	44 ± 10	19 ± 7	33 ± 8	42 ± 9
Concentric Q at 180°/s	23 ± 7	31 ± 8	40 ± 9	22 ± 6	31 ± 9	40 ± 8
Eccentric H at 60°/s	26 ± 9	51 ± 13	54 ± 13	23 ± 17	39 ± 21	45 ± 18
Eccentric H at 120°/s	30 ± 14	55 ± 10	57 ± 12	22 ± 11	44 ± 13	50 ± 12
Eccentric H at 180°/s	24 ± 15	50 ± 14	52 ± 13	22 ± 7	49 ± 14	52 ± 12

Q = Quadriceps H = Hamstrings

Table 3: U17 Eccentric and Concentric torque pre and post fatigue by joint angle

	Pre			**Post**		
	0-10°	***10-20°***	***20-30°***	***0-10°***	***10-20°***	***20-30°***
Concentric Q at 60°/s	26 ± 10	48 ± 15	62 ±17	24 ± 6	39 ± 9	42 ± 12
Concentric Q at 120°/s	24 ± 10	41 ± 13	54 ± 15	23 ± 7	33 ± 8	39 ± 9
Concentric Q at 180°/s	26 ± 9	39 ± 13	51 ± 15	25 ± 6	27 ± 10	33 ± 9
Eccentric H at 60°/s	35 ± 13	67 ± 21	71 ± 23	31 ± 19	85 ± 48	93 ± 43
Eccentric H at 120°/s	34 ± 15	75 ± 24	80 ± 20	25 ± 22	49 ± 34	66 ± 28
Eccentric H at 180°/s	30 ± 13	67 ± 27	77 ± 23	28 ± 28	65 ± 39	75 ± 28

Q = Quadriceps **H = Hamstrings**