MATURATION, PLAYING POSITION AND GENETIC VARIATION: INJURY RISK FACTORS IN HIGH-LEVEL YOUTH SOCCER

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ABSTRACT

The negative impact of injury on the development of youth soccer players (YSP) means that efforts must be made to prevent and control their occurrence. Although a range of risk factors have been investigated in YSP, they remain poorly understood due to limitations with previous studies (as discussed in Chapter Two). Greater understanding of the factors affecting injury risk could help identify and improve management of 'at risk' players, thus improving the likelihood of them reaching their full potential of a professional contract. Accordingly, the overriding aim of this thesis was to investigate the association of potentially key risk factors with injury risk in a large cohort of high-level YSP from academies within eight professional clubs from four different countries, across two continents.

For all four experimental chapters in this thesis, injuries were prospectively recorded over the course of a single season in high-level YSP to determine (i) whether the rate of these injuries varied in YSP between different nations; (ii) if maturation status, playing position and/or genetic variation were associated with injury risk in YSP. The injury audit (Chapter Three) revealed that non-contact injuries to skeletal muscle were amongst those frequently recorded, with most injuries located in the lower limb. The Under-14 (U14) and U16 age groups suffered relatively more severe injuries, with minimal differences in injury rate between nations. Thus, Chapter Three indicated that more severe injuries occurred around the timing of biological maturation, suggesting an association of maturation status with injury in YSP. When this hypothesis was tested directly in Chapter Four, maturation status (pre-, mid- and post- peak height velocity [PHV], estimated by maturity offset)

was indeed associated with injury risk, where soft-tissue, ligament/tendon and thigh injuries were more prevalent in post-PHV than pre- and mid-PHV, and muscle injuries were more prevalent in post- than pre-PHV. With all injuries combined, post-PHV missed more days in the season than pre-PHV. Thus, the results from this chapter strongly suggest that post-PHV are at greatest risk of injury, and miss more of the season due to injury than pre- and mid-PHV. Having identified a maturation-dependent link with injury risk in YSP, Chapter Five investigated whether there was an association between playing positon and injuries solely in post-PHV YSP (due to the greater prevalence and severity of injuries in post- *vs.* pre-PHV, and the fact that playing position is more firmly defined at post-PHV). Prevalence and severity did not differ between outfield positions, but relatively fewer goalkeepers suffered thigh injuries than lateral and forward players combined. These data indicate injury prevention strategies should be similar for outfield players in post-PHV, but should focus on preventing thigh injuries particularly for lateral and forward players. Finally, the results from Chapter Six showed that three single nucleotide polymorphisms (SNPs), i.e. *COL5A1* rs12722, *EMILIN1* rs2289360 and *VEGFA* rs2010963, were associated with injury prevalence in pre- and post-PHV combined, and that more SNPs were individually associated with injury risk in post- *vs.* pre-PHV. When combining those SNPs that were individually associated with injury risk, injured YSP demonstrated a worse polygenic profile (in terms of more ‘at risk’ genotypes) than uninjured YSP regarding non-contact and non-contact soft-tissue injuries, while pre- and post-PHV YSP had similar polygenic profiles. These data indicate a maturation-dependent influence of individual SNPs on injury, i.e. the

environment likely interacts with genetic predisposition for injury more so in post-PHV YSP, when the intensity of training/match-play is greater than it is for pre-PHV YSP.

The results from this thesis clearly identify post-PHV as being at higher risk of injury than their biologically younger counterparts, and that injury risk is similar between countries in this under-researched and important population. The results also show that playing position in physically mature YSP has minimal influence on injury risk but that genetic variation appears to be linked with the likelihood of suffering certain injuries, as well as the severity of those injuries. These novel findings could be used to help identify and manage ‘at risk’ YSP in order to reduce the burden of injury, thus increasing the chance of talented YSP reaching their full potential of a professional playing career.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ACTN3; α-actinin-3

CCL2; Chemokine (C-C motif) ligand-2

CENT; Centrally positioned player

COL1A1; Collagen, type I, α1

COL5A1; Collagen, type V, α1

DNA; Deoxyribonucleic acid

EMILIN1; Elastin microfibril interfacer-1

EPPP; Elite Player Performance Plan

FWD; Forward positioned player

GK; Goalkeeper

IL6; Interleukin-6

LAT; Laterally positioned player

MAF; Minor allele frequency

MMP3; Matrix metalloproteinase-3

MYLK; Myosin light chain kinase

NCST; Non-contact soft-tissue

PCR; Polymerase chain reaction

PHV; Peak height velocity

SNP; Single nucleotide polymorphism

TGS; Total genotype score

U9; Under-9 age group

U10; Under-10 age group

U11; Under-11 age group

U12; Under-12 age group

U13; Under-13 age group

U14; Under-14 age group

U15; Under-15 age group

U16; Under-16 age group

U17; Under-18 age group

U18; Under-18 age group

U19; Under-19 age group

U20; Under-20 age group

U23; Under-23 age group

VEGFA; Vascular endothelial growth factor-A

YSP; Youth soccer player

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

GENERAL INTRODUCTION

1.0 INTRODUCTION

Soccer is a popular team sport played by men, women and children. Professional soccer is the highest level of the sport, with elite players representing the world's most successful soccer clubs and nations. The competitive and financial benefits of having the best players (Reilly, Bangsbo and Franks, 2000; Vaeyens et al., 2008) means the identification of talented youth soccer players (YSP) is important to soccer clubs. The process of talent identification is defined as the recognition of players with potential to reach the elite level (Williams and Reilly, 2000), whilst talent development refers to the design and provision of the optimal learning environment for players to achieve their potential (Reilly, Bangsbo and Franks, 2000). Consequently, early enrolment into a professional soccer academy is considered a significant advantage in maximising the long-term development of YSP (le Gall et al., 2010). It follows that any factor interrupting the development process may threaten the prospect of YSP reaching the elite level.

The rate of injury in soccer is relatively high, even when compared to high-risk industrial occupations (Hawkins and Fuller, 1999). Importantly, injuries are the primary factor influencing player availability in professional players and are consequently associated with team success (Arnason et al., 2004a; Parry and Drust, 2006). For YSP, reduced availability limits the capacity to develop recognised potential, meaning that injuries suffered by YSP can significantly affect the likelihood of long-term success. Accordingly, injury prevention is a fundamental consideration for soccer academies attempting to optimise player development (Price et al., 2004) as well as for the health and wellbeing of YSP.

The initial step in the sequence of injury prevention is to audit and describe the extent of the problem created by injuries, followed by the identification of factors and mechanisms involved in their occurrence. This permits the design and implementation of measures designed to reduce injuries, the efficacy of which are then evaluated by repeating the audit step. In some injury prevention models this involves simply re-observing the same population following the implementation of a proposed intervention (Van Mechelen, Hlobil and Kemper, 1992; Bahr and Krosshaug, 2005), whilst in others, such as the Translating Research into Injury Prevention Practice (TRIPP) framework, the efficacy of an intervention is first tested within a controlled environment before deciding on potential “real-world” application (Finch, 2006). Despite a considerable body of literature investigating injuries in professional players (Ekstrand et al., 1983; Hawkins et al., 2001; Ekstrand, Hägglund and Waldén, 2009) and YSP (Price et al., 2004; Le Gall et al., 2006), recent evidence demonstrates that injuries remain a considerable burden to YSP (Renshaw and Goodwin, 2016; Read et al., 2018b). This is in spite of the fact that there are several recognised risk factors for injury in YSP, such as previous injury, chronological age, match/training intensity and physical stress (Brink et al., 2010). Accordingly, the continual prevalence of injury in YSP suggests that further research is required and that additional factors, which remain poorly understood, warrant consideration. Furthermore, there are no studies comparing the rate of injury between YSP from different nations. It is therefore unknown whether the country in which YSP are developed has any effect on the frequency or type of injury in this population. It is possible that coaching styles, match play and training schedules differ between nations,

which could influence the risk of injury. Consequently, research is required to compare the rate of injury in YSP from different nations.

The risk factors contributing to the occurrence of sports injuries are either intrinsic (relating to the individual athlete) or extrinsic (relating to the surrounding environment). In soccer, intrinsic factors include chronological age and previous injury, whilst extrinsic factors include external load, weather conditions and opposition behaviour (Brink et al., 2010). Injury occurrence involves the interaction of intrinsic and extrinsic factors, such as injury-inciting events, where it is improbable that a single factor in isolation causes injury. Despite the attention of scientific research, the understanding of soccer injury aetiology is limited; meaning the ability to limit or predict injury is a significant challenge. Importantly, there may be different risk factors for injury in YSP than professional adult players due to increased competition in adult soccer and consequent differences in the intensity of match play (Arnason et al., 2004a; Wong and Hong, 2005).

An important topic in YSP is biological maturation, where the point of greatest linear growth is known as peak height velocity (PHV) (Malina et al., 2005a). Accordingly, some suggest that substantial acute growth may increase the risk of injury, as might the fact that the rate and timing of PHV varies between individuals (Malina et al., 2000; Malina, Bouchard and Bar-Or, 2004). With YSP typically grouped by chronological age, this can lead to substantial differences in the stature and physical capacity of YSP within the same team and/or competing against one another (Figueiredo et al., 2010). These differences, combined with the fact that the development of talented YSP involves deliberate increments in training volume and intensity (Read et

al., 2018b), underpin suggestions that adolescent growth transiently increases injury risk (Le Gall, Carling and Reilly, 2007; Van der Sluis et al., 2015). Nevertheless, few studies have investigated these hypotheses using adequate samples sizes with which to offer convincing conclusions.

Soccer match play is intermittent in nature and involves various aerobic and anaerobic actions (Ekblom, 1986; Stølen et al., 2005). The type, frequency and intensity of these actions depends on the position a player occupies (Bloomfield, Polman and O'Donoghue, 2007), with each position representing a tactical role. Variability in playing actions is likely to affect the internal loads experienced by players and has direct implications for energy demands, neuromuscular fatigue and exercise-induced muscle damage (Byrne, Twist and Eston, 2004; Nedelec et al., 2014). Despite being considered by some as a risk factor for over a decade, there remains a lack of agreement regarding which positions are at greatest risk, the type or locations of injuries that are most likely for each position, and whether differences are due to variance in playing actions between positions. Evidence suggests that maturation and physical stature might influence the allocation of YSP to specific positions in reflection of tactical requirements (Towlson et al., 2017), which also poses the question as to whether the attributes associated with playing positions might also reflect the physical capacity required to withstand the physical demands of each position. This suggests that players' physicality is likely to influence the positional role they excel in and are selected to occupy, potentially affecting their risk of injury. However, YSP may not decide on one playing position until reaching older age categories, and there is no consensus

regarding the method used to separate playing positions, each highlighting the challenge of investigating playing position and injury in YSP.

Recently, inter-individual variation in soccer injuries has led to suggestions that genetic variation between individual players might influence susceptibility to soccer injury. Indeed, genetic variation is associated with traits such as muscle strength (Hubal et al., 2010; Erskine et al., 2014) and elite athlete status (Druzhevskaya et al., 2008; Eynon et al., 2012; Heffernan et al., 2016), the latter suggesting that some heritable factors are advantageous to sporting performance. In soccer, the attainment of elite status may also be partly determined by physical resilience to training loads and the ability to recover during and between bouts of activity (Durand-Bush, 2000). Indeed, the capacity to remain injury-free or to minimise injury frequency and injury-induced absence may enhance YSP chances of success by reducing the proportion of development time lost to of injury.

Despite a growing number of studies exploring the influence of genetic variation on soccer injuries, the literature has focussed on adult players (Ficek et al., 2013; Pruna et al., 2013; Massidda et al., 2015a; Massidda et al., 2015c; Artells et al., 2016; Pruna et al., 2016; Myosotis et al., 2017a; Larruskain et al., 2018). Research is therefore required to determine the influence of genetic variation on injury in YSP.

1.1 AIMS AND OBJECTIVES OF THE THESIS

The overarching aim of this PhD thesis is to investigate the epidemiology of injuries in high-level YSP from different countries, with particular reference to maturation status, playing position and genetic variation.

The PhD project has the following objectives:

1. To identify the most common types and locations of injuries sustained in a cohort of high-level YSP from multiple nations and to determine if any differences in injury rate exist between countries. This is addressed in the work described in Chapter Three.
2. To ascertain whether the maturation status of high-level YSP affects the type, frequency and severity of injuries suffered. This is addressed in the work described in Chapter Four.
3. To determine whether playing position affects the type, frequency and severity of injuries suffered in physically mature, high-level YSP. This is addressed in the work described in Chapter Five.
4. To identify specific gene polymorphisms that are associated with the type, prevalence and/or severity of common injuries in high-level YSP. This is addressed in the work described in Chapter Six.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Soccer is an intermittent team sport, in which the relative contributions of aerobic and anaerobic activities are influenced by playing position (Stølen et al., 2005; Bloomfield, Polman and O'Donoghue, 2007). Such differences are likely to elicit variances in fatigue, which is a risk factor for injury (Buchheit et al., 2010), which in turn is the primary factor influencing player availability in soccer (Parry and Drust, 2006). Reliable evidence and understanding of injury aetiology is therefore important to maintain performance and assist team success (Arnason et al., 2004a), and could enhance the chances of a professional career in youth soccer players (YSP).

Talent identification strategies in soccer are tasked with finding and developing promising YSP (Vaeyens et al., 2006). However, the negative influence injury has on player availability and development means injury prevention is also a priority for soccer academies (Price et al., 2004). Multiple factors influence injury risk in soccer, which can be distinguished as intrinsic (e.g. previous injury, chronological age, biological age) and extrinsic (e.g. match/training intensity, physical stress) (Brink et al., 2010) (Fig. 2.1). The presence of repeated high-intensity actions and physical contact means that players risk skeletal muscle, ligament, tendon and bone injuries and occasionally suffer concussions (Delaney et al., 2002; Price et al., 2004). Moreover, players occupy different playing positions for tactical reasons (Stølen et al., 2005), leading to differences in running distance and the number of high-intensity actions (Bloomfield, Polman and O'Donoghue, 2007). Consequently, playing position might influence injury risk in professional (Carling, Orhant and LeGall, 2010) and YSP (Price et al., 2004). Further, as

YSP develop during adolescence, some suggest that maturation status affects their risk of injury (Le Gall, Carling and Reilly, 2007). In addition to playing position and maturation status, the influence of genetic variation on injury risk has gained recent attention in professional players (Pruna et al., 2013; Massidda et al., 2015a; Massidda et al., 2015c; Massidda et al., 2016; Pruna et al., 2016; Larruskain et al., 2018), and there is evidence that specific gene variants influence injury to skeletal muscle (Collins and Raleigh, 2009), ligament (Ficek et al., 2013) and tendon (September, Schwellnus and Collins, 2007). That is to say that differences in DNA sequence between individuals can lead to variation in the type, rate and/or amount of protein produced, thus affecting the tissue material and/or mechanical properties, and potentially influencing injury risk (September, Schwellnus and Collins, 2007). It is not yet known how genetic variation influences injury risk in YSP.

The aims of this review are to evaluate the existing literature describing the incidence and severity of soft-tissue (e.g. muscle, tendon, ligament) injuries in YSP, with particular reference to the potential effects of maturation status and player position. Additionally, this review aims to evaluate the existing literature regarding genetic variation and injury in professional soccer players, which has potential relevance to YSP.

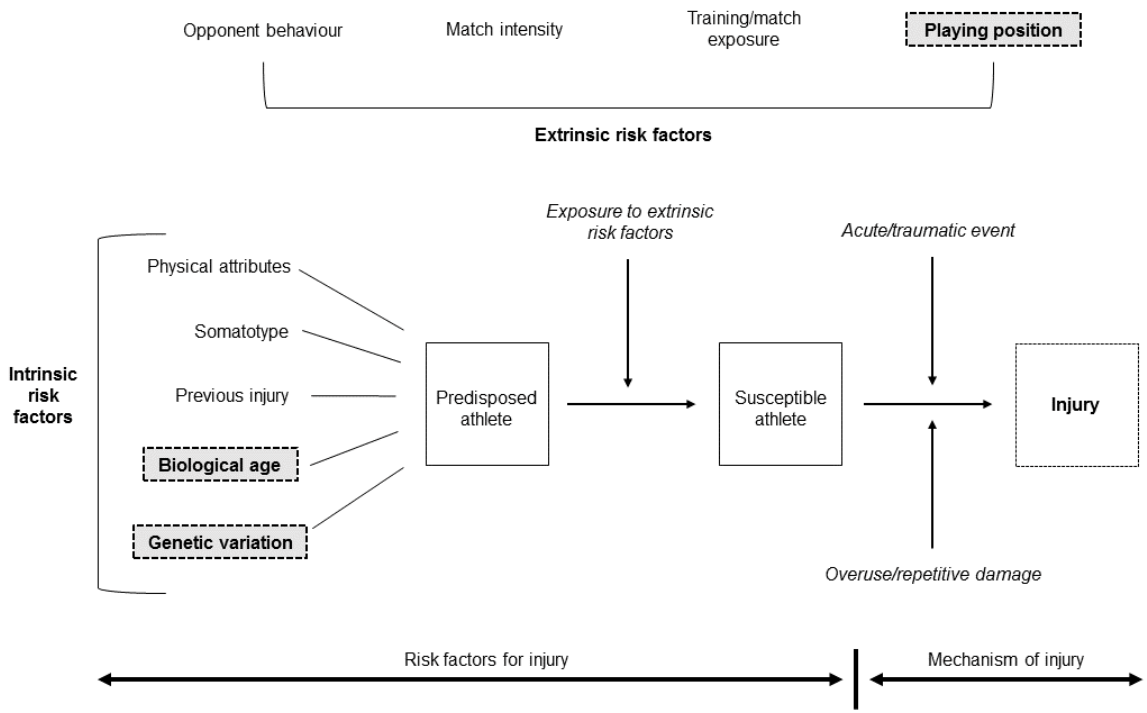

Figure 2.1. Interaction between intrinsic and extrinsic risk factors in the predisposition to injury. Physical attributes including (but not limited to) musculoskeletal properties (including muscle size and strength, tendon stiffness and bone mineral density), cardiovascular fitness and flexibility.

2.2 PHYSIOLOGICAL DEMANDS AND INJURY IN YOUTH SOCCER

Injury avoidance is important for soccer clubs and players because injury can lead to functional impairment (Hawkins and Fuller, 1999). The linear relationship between exposure to training and/or matches and injury risk (Nédélec et al., 2013) is an important consideration for YSP, whose exposure increases during athletic development (Le Gall et al., 2006). Players exposed to more soccer activity will run greater cumulative distances and experience larger volumes of high-intensity running and sprinting (Harley et al., 2010), which are risk factors for muscular injury (Ekstrand, Hägglund and Waldén, 2011). This questions whether the increase in injury incidence observed with chronological age (Price et al., 2004; Le Gall et al., 2006) is due to match factors, such as more intense play and increased ground reaction forces (Keller, Noyes and Buncher, 1988; Wong and Hong, 2005), or a product of accumulated exposure as players progress. The likelihood is that it involves a combination of these factors.

With the development of YSP intended to produce professional careers (Vaeyens et al., 2006), the preparation of physical qualities should reflect the demands of professional matches. From the available literature, the distance covered per match appears to increase with age in English players (Harley et al., 2010), though it is important to note that younger players usually play shorter matches with fewer players and smaller pitches (Carling et al., 2009). Elite YSP spend only 1% of matches sprinting (Harley et al., 2010) and, although soccer relies largely on aerobic metabolism (Stølen et al., 2005), initial and leading accelerations are key anaerobic actions in elite youth soccer (Murtagh et al., 2019) and contribute significantly to match outcomes (Wragg,

Maxwell and Doust, 2000). However, the number of these actions may depend on playing position (Reilly, 2003; Bloomfield, Polman and O'Donoghue, 2007). Some literature describing soccer match-play is becoming outdated, with the intensity of professional matches increasing in recent years (Andersen et al., 2004). For example, high-intensity running distances increased ~30% between 2006 and 2014 in the English Premier League (Barnes et al., 2014). Whilst running distance and running speeds influence fatigue, which is recognised as a risk factor for soccer injury (Buchheit et al., 2010), other playing actions such as landing and directional change are commonly involved when injuries occur (Rahnama, Reilly and Lees, 2002; Wong and Hong, 2005). In spite of this, much of the existing literature in professional and YSP focuses on running-based activity, with a lack of data concerning the quantity and intensity of other actions. Furthermore, the accuracy and validity of methods used to quantify running actions, such as Global Positioning System (GPS) and/or accelerometry in reflecting the internal loads experienced by players has been questioned (Rampinini et al., 2015; Dalen et al., 2018), suggesting there are limitations to the current knowledge of YSP activity. Another consideration is that the match demands described in studies of YSP are only representative of the age group described, with the broad spectrum of age groups contained within the youth soccer population making it unlikely that the observations described in one age group are wholly applicable to another. For example, differences in skeletal maturity (Van der Sluis et al., 2015) and the speed of matches (Arnason et al., 2004a) between younger and older YSP would suggest that considering match actions as injury risk factors is only valid in the specific age group in which they are assessed. Whilst different age

groups have been studied independently, there are no studies investigating the relationship between match demands and injury across multiple age groups of YSP. Consequently, it is not explicitly clear how the demands of youth soccer differ from the professional game, and how these demands influence injury risk in YSP.

The interaction of intrinsic and extrinsic factors is key to the aetiology of soft-tissue injuries, where the characteristics of one soft-tissue may influence the risk of injury to another. For example, muscular weakness may reduce the stability around a joint, thus increasing ligament injury risk (Solomonow, 2009), and a stiffer tendon could increase the risk of strain injury to the adjoining muscle during eccentric actions (Hawkins and Bey, 1997). As most soft-tissue injuries are non-contact (Price et al., 2004; Read et al., 2018b), understanding their intrinsic (e.g. genetic) mechanisms could aid their prevention. As many as 60% of injuries in YSP are to skeletal muscle (Ergün et al., 2013) and involve the interaction of risk factors including muscle architecture, fatigue and activity type (Maughan et al., 2010; Fuller, Junge and Dvorak, 2012). The most common muscle injuries in professional and YSP are traumatic injuries of the thigh and calf (Ekstrand and Gillquist, 1982; Price et al., 2004; Ekstrand, Hägglund and Waldén, 2009). With superior muscular strength considered protective against musculoskeletal injury (Lauersen, Bertelsen and Andersen, 2014), it follows that factors predisposing enhanced muscular strength are advantageous. Ligament injuries are the disruption of collagenous fibres and have a high risk of recurrence because post-injury healing cannot fully restore a ligament's structural and functional characteristics (Woo et al., 1980; Yeung et al., 1994). Most non-contact

ligament injuries in soccer occur during cutting and landing actions, with most contact ligament injuries suffered during tackling (Ekstrand et al., 1983; Kofotolis, Kellis and Vlachopoulos, 2007). Ankle ligament injuries constitute nearly one fifth of injuries in YSP (Price et al., 2004; Le Gall et al., 2006) and players with a history of ankle sprain exhibit greater risk of further injury (Ekstrand and Tropp, 1990). Most knee ligament injuries in YSP are to the MCL (Ekstrand and Gillquist, 1982; Moore et al., 2011) and whilst ACL injuries constitute fewer injuries (Moore et al., 2011), they incur extensive absences (Alentorn-Geli et al., 2009). Low muscle strength is proposed as a risk factor for ligament injury (Ekstrand and Gillquist, 1982). Indeed, poor hip extensor strength increases the risk of ankle sprains in YSP (De Ridder et al., 2016), suggesting that general muscular weakness affects ligament injury risk. Tendon injuries account for up to 13% in YSP (Price et al., 2004; Le Gall et al., 2006; Renshaw and Goodwin, 2016) and can be career-changing injuries (Fredberg, Bolvig and Andersen, 2008). The Achilles and the patellar tendons are involved in regular, repetitive movements during running, jumping and kicking, and are the most commonly affected tendons in soccer (Fredberg and Bolvig, 2002; Dvorak, 2007; Gajhede-Knudsen et al., 2013). In professional players, 27% of Achilles tendinopathies are recurrent, with inadequate rest a key contributor (Gajhede-Knudsen et al., 2013), whilst patellar tendinopathy describes degeneration of the patellar tendon and is also common in other sports (Lian, Engebretsen and Bahr, 2005).

With YSP progressing through chronological age groups as they grow older, puberty and physiological development are important considerations, where the process of biological maturation affects physical capacity,

anthropometry and the performance of soccer-specific qualities (Le Gall, Carling and Reilly, 2007). When experienced alongside increments in training volume and match exposure, the maturation process may also influence injury risk (Van der Sluis et al., 2014).

2.3 THE EFFECT OF MATURATION ON INJURY INCIDENCE IN YOUTH SOCCER

2.3.1 Skeletal maturation in youth soccer players

Youth soccer players compete according to chronological age. However, player selection can be biased by biological age rather than chronological age (Reilly, Bangsbo and Franks, 2000), because of the favourable physical attributes achieved through biological maturity (Le Gall, Carling and Reilly, 2007). Individuals who mature at earlier chronological ages are often taller and heavier than those who reach skeletal maturation “on-time” (i.e. skeletal age within one year of chronological age (Johnson, Doherty and Freemont, 2009)) or later (Bayer and Bayley, 1959). This variability can be substantial around peak height velocity (PHV) (Mirwald et al., 2002), referring to the point of greatest linear growth, typically observed between the ages of 13 and 15 in males (Figueiredo et al., 2010; Van der Sluis et al., 2015) (Fig. 2.2). During this period, male YSP in the same chronological age group can have height and mass differences of up to 15 cm and 21 kg, respectively (Figueiredo et al., 2010). In the months surrounding PHV, serum testosterone in boys is significantly greater than pre-pubertal levels (Albin and Norjavaara, 2013), contributing to muscle growth, fat mass reduction and a net increase in body mass (Tanner, 1965). However, the timing of these changes differs between

individuals (Rogol, Roemmich and Clark, 2002) and in players maturing at later ages, delayed physiological growth may affect performance. It is important to note that PHV is an indirect indication of pubertal status, and that some studies have used more direct measures such as radiological examination (Le Gall, Carling and Reilly, 2007; Johnson, Doherty and Freemont, 2009). This may limit comparisons between studies quantifying maturation in YSP.

Anthropometric differences affect the execution of strength, speed and endurance tasks (Malina, Bouchard and Bar-Or, 2004) and may create "competitive inequality", where early-maturing players perform best (Malina et al., 2000; Philippaerts et al., 2006). Accordingly, preferential selection of skeletally mature players (Malina et al., 2000; Figueiredo et al., 2010) suggests that late-maturing players are less likely to succeed in soccer. By age 18 years, the influence of skeletal maturation rate is effectively obsolete, yet many talented players are overlooked because of delayed maturation (Johnson, Farooq and Whiteley, 2017).

2.3.1.1 Methods of determining maturation status

A prominent consideration when seeking to account for differences in maturation status between youth athletes is the method used to determine maturation status. There are a number of available methods with varying levels of precision, and with different challenges in relation to research ethics and the logistics of the sampling process. Historically, the use of radiological examination has been utilised to determine the skeletal age of youth populations (Rotch and Smith, 1910). Specifically, x-rays of the wrist and hand have long been considered as the optimal choice, with the Greulich-Pyle

(Greulich and Pyle, 1959), Fels (Roche, Thissen and Chumlea, 1988) and Tanner-Whitehouse (Tanner, 1965) methods utilising similar approaches with different classification criteria. The use of x-rays are advantageous due to their reliability and precision, and modern technologies now mean exposure to radiation from these methods is relatively low compared to their original use (Malina, 2011). However, performing radiological examination requires specialist training and equipment, making the analysis of large sample sizes challenging and potentially expensive. Another method of determining skeletal age is the assessment of secondary sex characteristics, though the use of this method brings concerns surrounding participant privacy and cultural issues when assessed in non-clinical environments (Malina, Bouchard and Bar-Or, 2004). Whilst self-assessment has been proposed as an alternative, this relies on the interpretation of the individual participant as opposed to a trained researcher or physician, and could increase measurement error. Many recent investigations involving youth athletes, and particularly YSP, determine maturity using regression equations incorporating anthropometric measurements. The equations developed by Mirwald and colleagues (Mirwald et al., 2002) to predict maturity-offset (years to/from PHV) utilise non-invasive measures that are relatively quick and easy to perform, meaning a large number of individuals can be tested within a short time frame, and in field-based settings. This makes them favourable for use with populations of youth athletes, with minimal requirement for specialist training or equipment, which also eliminates any financial costs. In many cases, measures of stature and body mass are routinely collected by sporting organisations, meaning the use of regression equations are viewed favourably by governing bodies and

researchers. Despite a reasonable commonality between skeletal age measured by radiological examination and maturity offset using these equations (correlation coefficient of 0.83), the standard error of the equations developed by Mirwald and colleagues is approximately six months (Mirwald et al., 2002), meaning a small number of individuals may be incorrectly categorised when using this method. An additional method used to indicate maturity status is the Khamis-Roche method (Khamis and Roche, 1994), which predicts adult stature based on a child's stature, mass, date of birth and stature of each of the child's parents. In adolescent males, this method can predict adult height in boys ± 5.35 cm 95% of the time (Sherar et al., 2005). Whilst this method is relatively simple and does not require measurement of skeletal age, knowledge of the stature of each of the child's parents may not be available in all circumstances. Nevertheless, the advantages of using this method or that by Mirwald and colleagues (Mirwald et al., 2002) relate to low cost and ease of (non-invasive) measurement. This means they are commonly used amongst sporting organisations and researchers alike, particularly in comparison to more invasive and expensive methods, such as radiological examination.

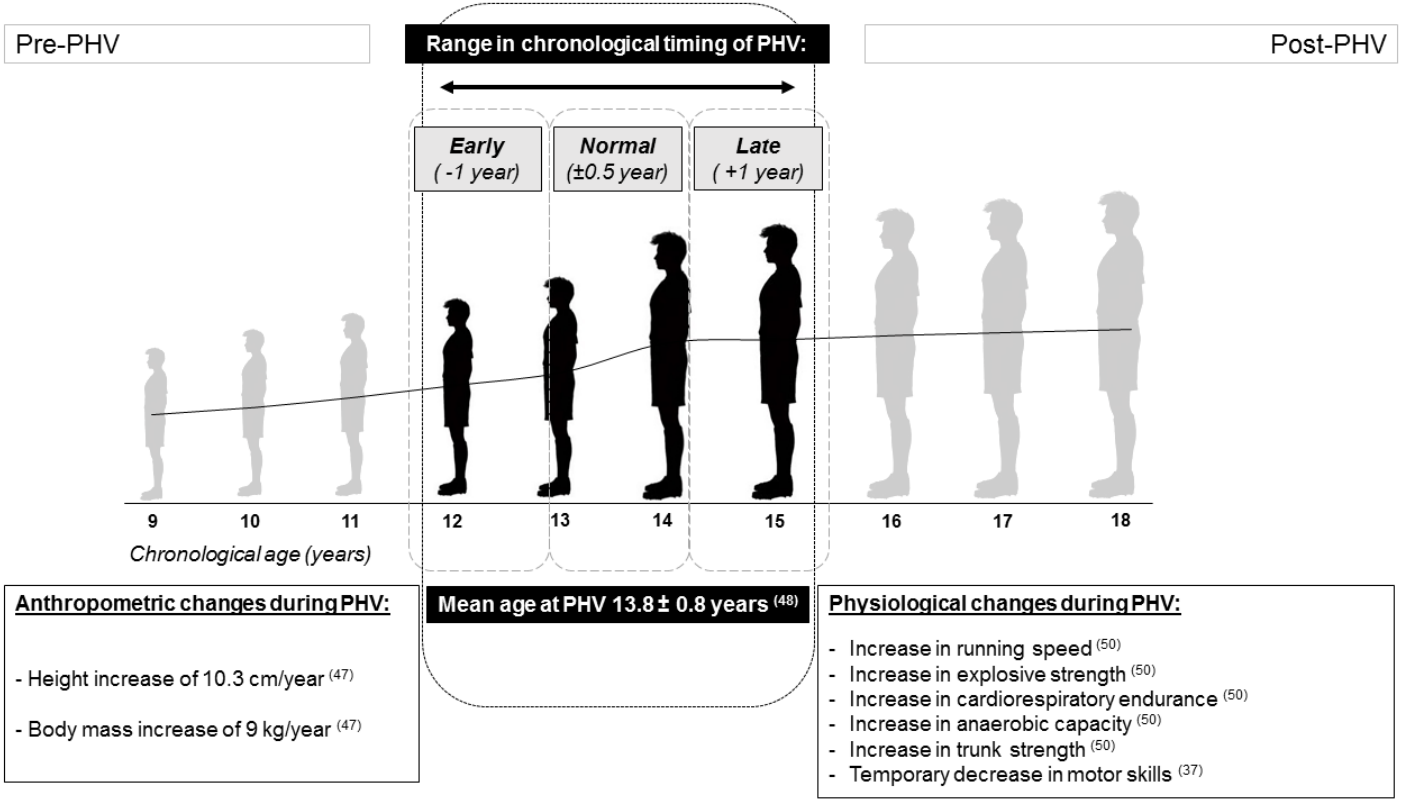

Figure 2.2. Anthropometric and physiological changes associated with peak height velocity (PHV) in adolescent males

2.3.2 The contribution of biological age to injury incidence

The possibility that biological maturation affects the risk of injury is a topic of interest in YSP (Malina et al., 2000; Malina et al., 2005a; Le Gall, Carling and Reilly, 2007; Figueiredo et al., 2010; Van der Sluis et al., 2014; Van der Sluis et al., 2015). There are concerns regarding a peak in injury incidence between the ages of 12 and 14 (Le Gall et al., 2006; Moore et al., 2011; Cloke et al., 2012; Renshaw and Goodwin, 2016) and that growing players concomitantly experience increments in training and match exposure (DiFiori, 2010). There is evidence that different stages of maturation affect landing mechanics (Read et al., 2018c) and lower limb asymmetries (Read et al., 2018d) in YSP, but fatigue related responses to match-play are not affected by maturation status (De Ste Croix et al., 2019). Whilst having the potential to affect injury risk, injuries were not recorded in these studies, meaning the influence of these factors on injury occurrence remains speculative.

Previous literature suggests that YSP suffer more injuries in the year surrounding PHV, which are more severe than injuries sustained in the periods before and after PHV (Van der Sluis et al., 2014). Indeed, injury risk in players who gain at least 0.6 cm in height or 0.3 kg in mass within one month is significantly greater than players with no change in height and mass (Kemper et al., 2015). Furthermore, Dutch YSP younger than 13.9 chronological years at the time of PHV suffered fewer injuries than players older than 13.9 at the time of PHV (Van der Sluis et al., 2015). Interestingly, more overuse injuries were recorded in the year preceding PHV amongst “late maturers”, suggesting these players experience relatively greater internal loads than their peers (Van der Sluis et al., 2015). Whilst no association was found between biological age

and injury incidence in French YSP, late maturers suffered more severe injuries (Le Gall, Carling and Reilly, 2007). Evidence suggests that the period around PHV is associated with increased injury risk, and that susceptibility to injury, particularly severe injury, is greater in players who mature later. This could be due to late maturers overreaching to keep up with their peers, or due to changes in limb length, muscle architecture and motor control. Despite these notable findings, the available literature has limitations. Two studies investigated the same cohort of 26 players (Van der Sluis et al., 2014; Van der Sluis et al., 2015) whilst another included players from 10 consecutive U14 age groups (Le Gall, Carling and Reilly, 2007). Such methodologies cannot compare injuries suffered around the timing of maturation with injuries suffered by players in younger or older chronological age groups. Only one study has included the influence of maturation across several age categories, concluding that later maturing players from the U13-U14 age groups experience greater risk of injury (Read et al., 2018a). Whilst the proposal that periods of adolescent growth increase the risk of injury is logical, supporting evidence is limited to a single study with a low number of players, with evidence that late maturing YSP experience greater injury risk also limited to a small number of investigations. Furthermore, the few studies to associate maturation with injury risk in YSP use different methods to quantify and/or define maturation status, restricting the collective interpretation of their findings. Whilst it would appear that late-maturing boys may be less physically robust to deal with the demands of soccer, and may therefore be at increased risk of injury until surpassing PHV, further research is required to substantiate this theory. Nevertheless, the biological maturity of players might also influence the nature of injury suffered.

More tendinopathies are reported in chronologically older players (Volpi, Pozzoni and Galli, 2003) and in those who mature either early or on-time (Le Gall, Carling and Reilly, 2007). Older, physically mature players typically suffer more muscle strains, which may reflect more intensive match-play styles (Arnason et al., 2004a). Crucially, recovery between training and matches may be increasingly important during periods of growth, where insufficient rest and recovery may increase the risk of overuse injury (Emery, 2003; Cassas and Cassettari-Wayhs, 2006). Under these circumstances, additional monitoring and modified training could assist in reducing the time missed through injury and could enhance player availability (Arnason et al., 2004a) and development (Price et al., 2004).

In summary, YSP may be susceptible to injury close to PHV, and those who mature later may suffer more severe injuries (Le Gall, Carling and Reilly, 2007; Van der Sluis et al., 2015) due to deficiencies in stature and physical capability. Existing literature suggests the risk of traumatic injury is elevated during PHV (Van der Sluis et al., 2014) and the risk of overuse injury is greater in late maturing young players (Van der Sluis et al., 2015). However, the limitations of these studies means there is still uncertainty concerning the influence of maturation status on injury risk in YSP. Though some suggest that reduced training load around peak growth could prove beneficial toward injury reduction, definitive evidence is required to demonstrate if players are at increased risk of injury whilst undergoing biological maturation.

2.4 THE EFFECT OF PLAYING POSITION ON INJURY INCIDENCE IN YOUTH SOCCER

2.4.1 Physiological demands of different playing positions in youth soccer

The anthropometry and physiological capacity of YSP often determines positional allocation and performance in specific tactical roles (Towlson et al., 2017). Furthermore, the different demands of playing positions (Bloomfield, Polman and O'Donoghue, 2007; Carling, Orhant and LeGall, 2010) may influence the type, incidence and severity of injury (Carling, Orhant and LeGall, 2010; Mallo and Dellal, 2012) (Fig. 2.3).

Playing position influences the type and quantity of playing actions in professional and YSP (Bloomfield, Polman and O'Donoghue, 2007; Harley et al., 2010). Literature regarding YSP suggests that central defenders cover the least total distance, with wide midfielders and strikers covering greater distance at high speeds (Buchheit et al., 2010; Mendez-Villanueva et al., 2013; Saward et al., 2015), whilst laterally positioned professional players (full-backs and wide midfielders) and strikers sprint most often (Mohr, Krustrup and Bangsbo, 2003). Clearly, in-game running is influenced by playing position, and players performing more high-speed running will experience greater muscular loads (Al Haddad et al., 2015), which increases fatigue and injury risk (Woods et al., 2004; Small et al., 2009; Guex and Millet, 2013). In particular, more sprinting increases the risk of muscular strain (Ekstrand, Hägglund and Waldén, 2011), whilst jumping and landing increase the risk of ligament injury (Ekstrand et al., 1983; Alentorn-Geli et al., 2009). The influence of playing position on the rate, type and severity of injury is of interest to coaches, players and practitioners and could support position-specific training

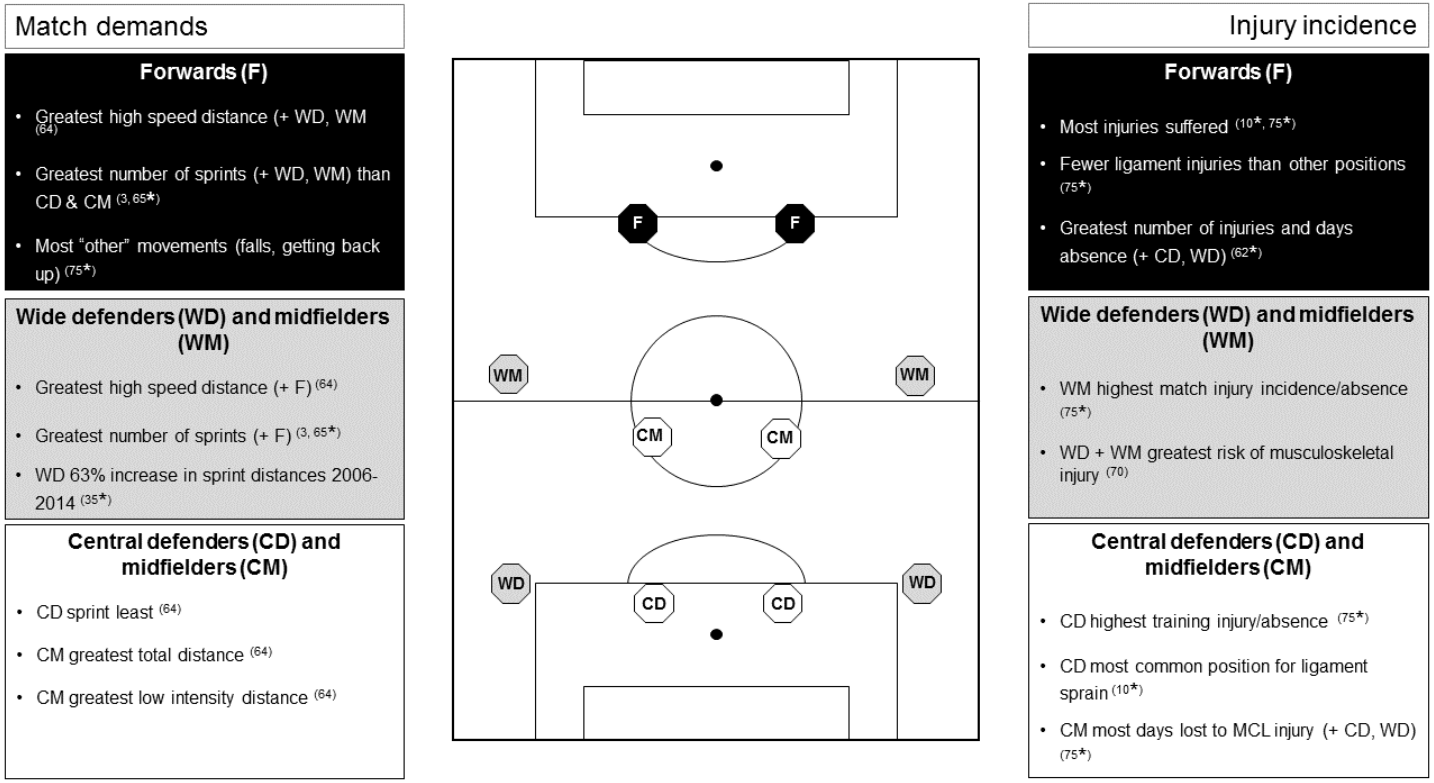

Figure 2.3. Differences in match demands and injury incidence according to playing position in professional (*) and youth soccer players

and injury prevention strategies (Al Haddad et al., 2015). Nevertheless, current literature describing injury and playing position in YSP is limited.

2.4.2 Effect of playing position on injury incidence in youth soccer

Evidence from English YSP suggests defensive and midfield players suffer the greatest proportion of injuries (Price et al., 2004; Cloke et al., 2011), with midfielders at greatest risk of musculoskeletal injury (Deehan, Bell and McCaskie, 2007). In professional players, some report that strikers suffer most injuries (Carling, Orhant and LeGall, 2010; Mallo and Dellal, 2012; Ryynänen et al., 2013), whilst others find no influence of position (Dauty and Collon, 2011) or suggest that some positions are more likely to suffer certain injuries. Specifically, strikers reportedly miss fewer days to ankle sprains than midfielders, with defenders and midfielders missing more days to knee ligament injuries than forwards (Leventer et al., 2016).

Despite evidence describing the influence of playing position on injury, a lack of distinction between central and wide players in many studies limits comparisons due to their varied demands (Towlson et al., 2017). Nevertheless, if playing position is an important risk factor for injury, it is most likely because of differences in match demands. In players performing more forceful muscle contractions when sprinting, such as those experienced by lateral players and strikers (Mohr, Krustrup and Bangsbo, 2003; Buchheit et al., 2010), more muscle strains may occur, whilst ligament injury may be more frequent in players who turn, jump and land more often, such as central defenders and strikers (Reilly, 2003). Knowledge of such differences merits the attention of coaches and practitioners, potentially informing training and

injury prevention strategies, though it is important to consider the limitations of adopting playing position as a risk factor for injury. The interpretation and definition of different playing positions itself can vary based on the philosophies and playing styles of different teams, making comparisons between teams difficult. These differences can also be present within the same team from one match to another depending on the behaviour or skill level of the opposing team. Specifically, a team's formation may change from one match to the next or within a single match, which will affect the demands imposed upon players seemingly occupying the same playing positions. For example, there is evidence that wide defenders perform up to 20% more decelerations when deployed as part of a 3-5-2 formation as opposed to a 4-4-2 (Tierney et al., 2016). Such fluidity amongst outfield positions means defined playing positions are likely to differ within and between different teams, players and matches. Not only does this highlight a limitation of grouping players by their on-field role, such data challenges the reliability of playing position as a risk factor for injury, especially when comparing players from multiple teams. Finally, there is evidence that maturation affects match running performance in YSP (Buchheit and Mendez-Villanueva, 2014), meaning any influence of playing position on injury in YSP may also be maturation dependent.

2.5 THE POTENTIAL GENETIC CONTRIBUTION TO THE AETIOLOGY OF SOCCER INJURIES

Inter-individual variation in the frequency and severity of injury suggest genetic differences between elite level players may play a role (Pruna et al., 2016).

Due to the complex aetiology of soccer injuries, any genetic influence is more likely to be polygenic, rather than due to a single polymorphism (i.e. gene variant). Specifically, variation between players in the genes facilitating the repair and regeneration of soft-tissues may contribute to differences in injury rate (September, Schwellnus and Collins, 2007; Collins, 2010). Although this research is in its infancy, gene polymorphisms (e.g. single nucleotide polymorphisms, or SNPs, where a single nucleotide within the DNA sequence of a gene is substituted with another nucleotide) have been associated with soft-tissue injury in professional soccer players (Table 2.1). However, no evidence currently exists regarding a genetic association with injury risk in YSP, so this review will focus on those studies that have identified genetic associations with soccer injuries in senior players, providing mechanistic information where possible. Knowledge of whether YSP injury risk is genetically influenced could help reduce injuries by identifying those players with greater susceptibility to injury, and altering their training/recovery strategies accordingly to enhance the prospect of them achieving professional careers.

Table 2.1. Genes and single nucleotide polymorphism (SNPs) associated with soft-tissue injury in professional male soccer players.

Gene	Encoded Protein	Chromosome	SNP rs number	Type	Substitution	Genotypes
ACTN3	α-actinin-3	11	rs1815739	Nonsense variant	C>T	CC, CT, TT
CCL2	Chemokine (C-C motif) ligand-2	17	rs2857656	Upstream variant	G>C	GG, GC, CC
COL1A1	α1(I) collagen chain	17	rs1800012	Intron variant	C>A	CC, CA, AA
COL5A1	α1(V) collagen chain	9	rs12722	UTR variant	C>T	CC, CT, TT
EMILIN-1	Elastin microfibril interfacer-1	2	rs2289360	Intron variant	T>C	TT, TC, CC
GEFT	Guanine nucleotide exchange factor	12	rs11613457	Upstream transcript variant	G>A	GG, GA, AA
HGF	Hepatocyte growth factor	12	rs5745697	Intron variant	T>G	TT, TG, GG
HGF	Hepatocyte growth factor	12	rs1011694	Intron variant	T>A	TT, TA, AA
HGF	Hepatocyte growth factor	12	rs5745678	Intron variant	A>G	AA, AG, GG
HIF1A	Hypoxia-inducible factor-1, α-subunit	14	rs11549465	Intron variant	C>T	CC, CT, TT
IGF-2	Insulin-like growth factor-2	11	rs3213221	Intron variant	C>G	CC, CG, GG
IL-6	Interleukin-6	7	rs1800795	Intron variant	C>G	CC, CG, GG
MCT-1	Monocarboxylate transporter 1	1	rs1049434	Missense variant	A>T	AA, AT, TT
MMP3	Matrix metalloproteinase-3	11	rs679620	Missense variant	T>C	TT, TC, CC
NOS3	Nitric oxide synthase-3	7	rs1799983	Missense variant	T>G	TT, TG, GG
SOX15	Sex-determining region-related HMG-box 15	17	rs4227	Downstream transcript variant	G>T	GG, GT, TT
TNC	Tenascin-C	9	rs2104772	Missense variant	T>A	TT, TA, AA
VDR	Vitamin D receptor	12	rs7975232	Intron variant	C>A	CC, CA, AA

ACTN3

The rs1815739 SNP is a C>T (R>X) substitution in the *ACTN3* gene (North and Beggs, 1996), where individuals homozygous for the X-allele are deficient of α-actinin-3, a structural protein found only in fast-twitch muscle fibres (Mills et al., 2001). Compared to XX homozygotes, RR homozygotes have a greater proportion of type II muscle fibres (Vincent et al., 2007) (that are larger and stronger than type I fibres (Bottinelli et al., 1996)), which is likely due to the inhibitory effect of α-actinin-3 on calcineurin signalling (Seto et al., 2013). Consequently, R-allele carriers have larger and stronger muscles (Erskine et al., 2014; Broos et al., 2016). As low muscle strength is a risk factor for soccer injuries (Timmins et al., 2016), it is interesting to note that soccer players with the XX genotype demonstrate greater exercise-induced muscle damage than RR homozygous players (Pimenta et al., 2012), and also suffer more frequent and severe muscle injuries (Myosotis et al., 2017a). However, others found no association between this SNP and hamstring injury risk in professional soccer players (Larruskain et al., 2018), which may reflect differences in sample size or the fact one study investigated muscle injuries and the other only hamstring injuries. It is possible that the benefit of the RR genotype is most evident during high-intensity actions such as sprints and jumps where type II fibres are most susceptible to damage (Macaluso, Isaacs and Myburgh, 2012). Therefore, if soccer players with type II fibres lacking α-actinin-3 experience greater risk of muscle damage (Pimenta et al., 2012), the influence of α-actinin-3 on skeletal muscle function may also affect their risk of muscle injury. Although research in YSP is required, *ACTN3* may influence muscle injury risk in soccer, possibly

by influencing muscle strength and/or by providing structural stability to the sarcomere during dynamic muscle contractions (Baumert et al., 2016).

MCT1

The rs1049434 SNP is a A>T substitution in the *MCT1* gene (Merezhinskaya et al., 2000). In skeletal muscle, lactate is removed by monocarboxylate transporter (MCT) and oxidized by fibres expressing MCT1 encoded by the *MCT1* gene (Massidda et al., 2015c). TT homozygotes exhibit reduced lactate transport rates (Merezhinskaya et al., 2000), suggesting the T allele affects MCT1 function. In professional soccer players, AA homozygotes had the greatest risk of muscle injury in one study (Massidda et al., 2015c) but not in another (Massidda et al., 2014). These dissimilarities may reflect differences in sample size, with an association evident in the larger sample. Despite TT homozygotes experiencing higher lactate concentrations during exercise (Cupeiro et al., 2010), it is unclear how TT genotype reduces muscle injury risk. It is possible that the T-allele impairs lactate transport and limits players' capacity to sustain intense contractile activity, causing premature metabolic fatigue (Thomas et al., 2005). Accordingly, TT homozygous players may become fatigued from intense activity at an earlier stage of a match and suffer fewer muscle injuries. Further investigation is required regarding the relationship between *MCT1* and injuries in YSP.

VDR

The *VDR* A*pa*I SNP (rs7975232) is a C>A substitution in the *VDR* gene encoding the vitamin D receptor (VDR) in skeletal muscle (Taymans et al., 1999; Habuchi et al., 2000). Vitamin D enhances the Ca^{2+} pool needed for

muscle contraction and is key to Ca^{2+} transport and muscle protein synthesis. Consequently, Vitamin D influences skeletal muscle function, with deficiency inhibiting strength and post-exercise recovery (Bischoff-Ferrari et al., 2004; Barker et al., 2013). In professional soccer players, the A*pa*I SNP accounted for 18% of the variance in muscle injury severity (AA and CC homozygotes were associated with the least and most severe injuries, respectively) (Massidda et al., 2015b) but was not associated with injury incidence (Massidda et al., 2014; Massidda et al., 2015b). This suggests this SNP influences injury severity via regulating the muscle regeneration process, which is supported by increased *VDR* expression at day 7 of muscle regeneration (Srikuea et al., 2012) and vitamin D enhancing post-injury strength recovery (Barker et al., 2013). Accordingly, it is possible that the A*pa*I CC genotype lowers skeletal muscle Vitamin D concentration, limiting the post-injury healing capacity of skeletal muscle. Further research is required to confirm influence of *VDR* on muscle injury severity in YSP.

CCL2

The rs2857656 SNP is a G>C substitution in the *CCL2* gene affecting plasma C-C motif chemokine ligand-2 (CCL2) levels (Guo et al., 2014). CCL2 is expressed within the interstitial space between myofibres after muscle-damaging activity (Hubal et al., 2008) and mediates systemic changes from chronic exercise (Catoire and Kersten, 2015). In elite soccer players, GG homozygotes had more severe muscle injuries than C-allele carriers (Pruna et al., 2013; Pruna et al., 2016). Plasma CCL2 is lowest in GG homozygotes (Guo et al., 2014), which might explain why those players suffered the most

severe injuries. Furthermore, the GG genotype is also linked to lower muscle strength than C-allele carriers (Hubal et al., 2010). It is possible that low CCL2 activity impairs the post-injury recovery of skeletal muscle in GG homozygous soccer players, contributing to more severe injury. Indeed, the potential role of CCL2 in satellite cell proliferation (Yahiaoui et al., 2008) points toward the benefits of higher CCL2 during recovery from muscle injury in C-allele carriers. Thus, *CCL2* could influence muscle injury severity in YSP.

HGF

Hepatocyte growth factor (HGF) is encoded by the *HGF* gene and activates quiescent satellite cells during skeletal muscle development and regeneration (Gutiérrez, Cabrera and Brandan, 2014). The HGF receptor, c-Met, and HGF mRNA are co-expressed during satellite cell activation and repressed during myoblast differentiation (Tatsumi et al., 1998) and a lack of c-Met activity hinders skeletal muscle formation (Gal-Levi et al., 1998). In professional soccer players, three *HGF* SNPs were associated with muscle injury (Pruna et al., 2016). Specifically, rs5745697 CC homozygotes and rs1011694 AA homozygotes had fewer injuries than their A- and T-allele carrying counterparts, respectively, whilst rs5745678 T-allele carriers had less severe injuries than CC homozygotes. The authors speculated that a well-established interaction between HGF and c-Met amongst wild-type rs5745697 and rs1011694 genotypes would support normal development of skeletal muscle and protect against injury (Pruna et al., 2016). Despite suffering fewer injuries, rs5745697 CC and rs1011694 AA homozygotes experienced greater injury severity. Whilst the reasons are unclear, only one player with the rare

genotype was present for each SNP, restricting comparison between genotype groups. Nevertheless, preliminary evidence suggests that *HGF* might influence injury incidence and severity in YSP.

SOX15

The rs4227 SNP is a G>T substitution within the *SOX15* gene, a member of the SOX family of transcription factors expressed by satellite cells (Béranger et al., 2000). Evidence in rodents confirms the role of SOX15 in regulating myogenic progenitor cells (Lee et al., 2004; Meeson et al., 2007) and demonstrates that the absence or inactivation of SOX15 perturbs skeletal muscle regeneration (Meeson et al., 2007). In professional soccer players, T-allele carriers suffered fewer muscle injuries than GG homozygotes (Pruna et al., 2016). However, no association was reported with non-contact soft-tissue injuries in another study (Pruna et al., 2013). This suggests that the rs4227 SNP influences risk of skeletal muscle injury, but not other soft tissues. Considering evidence that the correct form of SOX15 is required for muscle regeneration (Meeson et al., 2007), the previous authors speculated that players lacking the T allele would present “abnormal” skeletal muscle function, which would contribute to their higher rate of muscle injuries (Pruna et al., 2016). Therefore, preliminary evidence suggests *SOX15* might influence muscle injuries in YSP.

COL5A1

The rs12722 SNP is a C>T substitution within the *COL5A1* gene (Collins and Posthumus, 2011), which encodes the α1 chain of Type V collagen, a vital structural component of tendons and ligaments during collagen fibrillogenesis

(Wenstrup et al., 2004). One hypothesis proposes that the T allele increases *COL5A1* mRNA stability, increasing type V collagen production and leading to reduced tensile strength and increased collagen fibril stiffness in §musculoskeletal tissues (Collins and Posthumus, 2011). In soccer players, TC heterozygotes had more severe hamstring injuries in two studies (Pruna et al., 2013; Pruna et al., 2016), with others reporting no association with hamstring injury (Larruskain et al., 2018). In another investigation, TT homozygous players had more severe musculoskeletal injuries than C-allele carriers (Massidda et al., 2015a), whilst the T-allele was associated with ACL injury and Achilles tendinopathy in non-athletes (Posthumus et al., 2009b; September et al., 2009). The dissimilar findings between studies could relate to modest sample sizes, the small number of severe hamstring injuries recorded, or the absence of TT homozygotes in two studies (Pruna et al., 2013; Pruna et al., 2016). Nevertheless, this SNP has been associated with musculoskeletal injuries in several cohorts, suggesting that *COL5A1* could influence soft-tissue injury risk and severity in YSP.

EMILIN1

The rs2289360 SNP is a T>C substitution within the *EMILIN1* gene (Zacchigna et al., 2006) that encodes the elastin microfibril interfacer 1 (EMILIN-1) protein, which assists the fusion of elastin fibres during elastogenesis (Randell and Daneshtalab, 2017) and is a source of elasticity in ligament and tendon (Kannus, 2000). Moreover, elastin proteins have important load-bearing roles in musculosketetal tissues (Gosline et al., 2002). The intronic location of this SNP suggests that different alleles could influence expression of the gene

(Tabor, Risch and Myers, 2002), which could influence soft-tissue properties and have implication for injury risk. In professional soccer players, the TT genotype was associated with greater MCL injury severity, but not with injury incidence or severity of muscle or tendon injuries (Pruna et al., 2013). The TT genotype was associated with greater incidence and severity of MCL injuries in another study where no CC homozygotes suffered MCL injuries, though this was a pilot study with only 19 injuries recorded amongst 60 players (Artells et al., 2016). This SNP was not associated with hamstring injury in another professional soccer cohort (Larruskain et al., 2018), suggesting a tissue-specific influence in ligament but not skeletal muscle. It is plausible that this SNP influences tissue elasticity and specifically, that the TT genotype reduces ligament elasticity, increasing injury risk. Accordingly, investigation of *EMILIN1* and injury risk in YSP is warranted.

COL1A1

The rs1800012 SNP is a C>A substitution in the *COL1A1* gene (Mann et al., 2001), which encodes type I collagen, the major fibrillar collagen providing structural stability to ligaments and tendons (Boot-Handford and Tuckwell, 2003). The rare AA genotype is reportedly protective against injury to soft-tissues, such as the ACL (Posthumus et al., 2009a) and Achilles tendon (Posthumus et al., 2009c). In a study of professional soccer players, the AA genotype was underrepresented in players with ACL ruptures (Ficek et al., 2013). However, no association was found between this SNP and non-contact soft-tissue injuries in other soccer cohorts (Pruna et al., 2013; Larruskain et al., 2018). The conclusions of these studies may differ due to the investigation

of different soft-tissues, and it is likely that this SNP specificially influences the risk of ligament and/or tendon injury in soccer players, which may also be specific to anatomical location and function of the ligament/tendon. Evidence that the A-allele increases *COL1A1* transcriptonal activity (Jin et al., 2009) suggests a greater presence of type I collagen in the soft-tissues of AA homozygotes, and if this leads to greater tissue strength, it could explain the lower injury risk. Nevertheless, further research should aim to determine the influence of *COL1A1* on injuries in YSP.

MMP3

The rs679620 SNP is a T>C substitution within the *MMP3* gene (Posthumus et al., 2012). The MMPs are a family of collagen-degrading enzymes and are physiological regulators of ECM remodelling (Birkedal-Hansen et al., 1993), with an important role in skeletal muscle regeneration (Chen and Li, 2009). In professional soccer players, the T-allele was associated with hamstring injuries, with injury risk doubled amongst TT homozygotes (Larruskain et al., 2018). However, no asscociation was observed with non-contact muscle injury in another soccer cohort (Pruna et al., 2016). These differences may reflect the investigation of a single muscle group in one study and any non-contact muscle injury in the other, as well as modest sample sizes. Previous associations may be due to this SNP being in high linkage disequilibrium with the functional rs3025058 SNP, which causes differential MMP3 expression. Specifically, the rs679620 T-allele corresponds to the rs3025058 5A-allele, which decreases MMP3 expression (Medley et al., 2003). It is possible that lower MMP3 expression in T-allele carriers impairs muscle remodelling and

increases injury risk. Conversely, CC homozygosity is associated with Achilles tendinopathy risk in non-athletes (Raleigh et al., 2009), suggesting the influence of this SNP is tissue-dependent. Accordingly, *MMP3* is a candidate for soft-tissue injury in YSP.

IL6

The rs1800795 SNP is a C>G substitution within the *IL6* gene (Fishman et al., 1998a). The cytokine IL-6 is produced in skeletal muscle after exercise and is functionally related to muscle growth and atrophy (Muñoz-Cánoves et al., 2013) as well as collagen synthesis (Andersen et al., 2011). In elite Spanish soccer players, GG homozygotes had greater risk of hamstring injury than C-allele carriers (Larruskain et al., 2018), with the same genotype previously associated with Achilles tendinopathy in a non-athletes (September et al., 2011). The ability of IL-6 to act in both pro- *and* anti-(Pedersen and Febbraio, 2008) inflammatory capacities makes it plausible that IL-6 could increase or reduce the risk of injury. It is possible that the increase in plasma IL-6 associated with the G-allele (Fishman et al., 1998b) increases soft-tissue injury risk. Specifically, because eccentric contractions can induce pro-inflammatory IL-6 expression in skeletal muscle (Nieman et al., 1998), and because cytokines trigger tenocyte apoptosis and ECM degradation (Millar et al., 2009), GG homozygotes may experience these unfavourable changes via increased IL-6, heightening the risk of injury. Accordingly, the *IL-6* rs1800795 SNP appears a viable candidate for determining soft-tissue injury risk in YSP.

NOS3

The rs1799983 SNP is a T>G substitution in the *NOS3* gene (Leeson et al., 2002). Nitric oxide (NO) has several functions in skeletal muscle, including muscle contractility and injury repair, and NOS3 is the rate-limiting enzyme in NO production (Stamler and Meissner, 2001). This SNP was associated with hamstring injury in professional soccer players, with GG homozygotes at greatest risk (Larruskain et al., 2018). However, no association was observed with Achilles tendon injuries in non-athletes (Nell et al., 2012). The kinetics of NO synthesis do not differ between *NOS3* alleles, however the G-allele is less susceptible to proteolytic cleavage (Tesauro et al., 2000) and T-allele carriers present with lower NO levels (Luo et al., 2019). Due to the possibility that NO promotes exercise-induced muscle hypertrophy (Wang et al., 2001), and because GG homozygotes have been overrepresented in power athletes (Eider et al., 2014), GG homozygous soccer players may have a greater proportion of type II muscle fibres. With type II fibres being most susceptible to damage (Macaluso, Isaacs and Myburgh, 2012), it may be possible that the acute muscle damage suffered by *NOS3* GG homozygotes could also influence their risk of suffering hamstring injuries (Larruskain et al., 2018). Further work is required to determine whether the rs1799983 *NOS3* SNP influences injury risk in YSP.

HIF1A

The rs11549465 SNP is a C>T substitution in the *HIF1A* gene (Tanimoto et al., 2003). Hypoxia-inducible factor-1α (HIF1A) is a transcription factor regulating genes in response to hypoxia and mechanical loading, and is important during matrix remodelling and myogenesis (Lindholm and

Rundqvist, 2016). Interestingly, elite CC homozygous soccer players had double the risk of hamstring injury compared to CT heterozygotes (Larruskain et al., 2018). Due to the fact the T allele increases *HIF1A* transcriptional activity (Tanimoto et al., 2003), this may protect against injury because of the role of HIF1A during matrix remodelling and myogenesis. It is conceivable that lower *HIF1A* transcriptional activity in those lacking the T allele leads to impaired matrix remodelling and myogenesis in response to mechanical loading, thus compromising the acute recovery of soft tissue from soccer activity and increasing injury risk during subsequent bouts. Accordingly, *HIF1A* may influence injury in YSP.

TNC

The rs2104772 SNP is a T>A substitution within the *TNC* gene (Matsuda et al., 2005). Tenascin-C (TNC) is important during repair from muscle damage and is expressed in regenerating myofibres and at the myotendinous junction after mechanical loading (Flück et al., 2008). This SNP was not associated with non-contact soft-tissue injuries in one study of soccer players (Pruna et al., 2013), but the A-allele increased hamstring injury risk in another soccer cohort (Larruskain et al., 2018) and the risk of Achilles tendinopathy in two non-soccer cohorts (Saunders et al., 2013). These discrepancies may be due to differences in the type of injury analysed and the low number of players investigated, although because TNC is expressed at the myotendinous junction and is associated with muscle *and* tendon injuries, injuries to the muscle-tendon unit may be influenced by *TNC*, especially as many hamstring strains occur near the myotendinous junction (Small et al., 2009). Accordingly,

the possibility that different *TNC* alleles affect the expression of TNC offers a potential mechanism for the observed associations with soft-tissue injury, and such knowledge may assist the reduction of soft-tissue injuries in YSP.

IGF2

The rs3213221 SNP is a C>G substitution within the *IGF-2* gene (Devaney et al., 2007). Insulin-like growth factor II (IGF2) is involved in repair following muscle damage (Keller et al., 1999), with SNPs of the *IGF2* gene associated with the response to muscle damage (Devaney et al., 2007). In two studies of professional soccer players, GC heterozygotes suffered less severe non-contact muscle injuries compared to both homozygous genotypes (Pruna et al., 2013; Pruna et al., 2016). However, it is interesting that neither homozygote genotype was associated with injury severity, particularly because GG homozygotes previously demonstrated greater strength loss, soreness and CK activity after damaging exercise (Devaney et al., 2007). It is not clear why the heterozygous genotype would be associated with the least severe muscle injuries in professional soccer players, and caution regarding such results is encouraged. Nevertheless, some literature suggests SNPs within the *IGF-2* gene could influence muscle injury severity in YSP.

GEFT

The rs11613457 SNP is a G>A substitution within the *GEFT* gene. The GEFT protein is highly expressed in adult skeletal muscle (Bryan et al., 2004) with a potential role in regeneration (Bryan et al., 2005). This SNP was associated with recovery from non-contact muscle injury in elite soccer players (Pruna et al., 2016), where GG homozygotes recovered faster than the heterozygotes,

suggesting the A-allele blunts recovery from injury. Whilst no players in this study were AA homozygotes, having two A-alleles could increase muscle injury severity further. Evidence suggests that GEFT encourages post-injury healing via the promotion of myogenic progenitor cells (Bryan et al., 2004). Therefore, the rare A-allele may reduce GEFT function following skeletal muscle injury, potentially explaining why *GEFT* heterozygous soccer players had more severe injuries than GG homozygotes. Further work is required to ascertain the influence of this SNP on soft-tissue injury in YSP.

2.6 SUMMARY AND CONCLUSION

Despite advances in monitoring and treatment, injuries remain a notable burden in YSP (Parry and Drust, 2006; Renshaw and Goodwin, 2016; Read et al., 2018b), suggesting not all risk factors are fully understood. Understanding the risk and aetiology of injuries in YSP may assist their chances of success, and may benefit soccer clubs who invest heavily in talent development. Variation between players in the rate and timing of biological maturation has been suggested as an injury risk factor in YSP (Van der Sluis et al., 2015). Specifically, performance attributes are influenced by the rate and timing of maturation (Malina, Bouchard and Bar-Or, 2004) and might also affect the incidence and severity of injury. Based on existing evidence, players may experience greater risk of injury whilst undergoing maturation, and that players who undergo maturation later than their peers are less physically robust, and therefore at increased injury risk.

Playing position is one of several extrinsic injury risk factors in professional soccer players. However, whether an association is as

pronounced in YSP is not clear. Differences in match activity, such as a greater number of sprint or high-speed running actions in some positions, underpin the possibility that playing position influences injury risk in YSP.

Recent literature demonstrates genetic associations with muscle damage and recovery after exercise (Baumert et al., 2016; Baumert et al., 2017; Baumert et al., 2018), and in the composition of ligaments and tendons (Collins and Posthumus, 2011). This suggests that genetic variants could influence the incidence and severity of soccer injuries. Indeed, numerous studies have reported genetic associations with soccer injury risk but all of these studies have been performed in professional (senior) soccer players and several have limited sample sizes and injury data sets. No evidence currently exists regarding a genetic association with injury risk in YSP.

Individualised information on injury risk could help reduce injuries in YSP by identifying those with increased susceptibility and modifying their training/recovery/nutrition accordingly to enhance their chances of achieving a professional playing career. However, before injury risk can be reduced through preventative strategies targeted at specific risk factors, the problem of injury in YSP must be quantified. Despite previous audits describing the nature of injuries sustained by YSP (Price et al., 2004; Read et al., 2018b), the external validity of these findings is unknown. Accordingly, regular and population-specific injury audits are desirable prior to addressing risk factors within that population.

CHAPTER THREE

AN AUDIT OF INJURIES IN HIGH-LEVEL YOUTH SOCCER PLAYERS FROM ENGLISH, SPANISH, URUGUAYAN AND BRAZILIAN ACADEMIES

PRELUDE

The work described within Chapter Three seeks to describe the problem caused by injuries within the YSP population investigated within this thesis, and to offer validation that the injuries observed in this cohort are similar to what would be expected in a typical YSP population based on existing literature. Accordingly, Chapter Three provides an injury audit representative of the initial stage of several injury prevention models, to be followed by the investigation of proposed risk factors in later chapters. Whilst recognising that these stages reflect the first two steps of most injury prevention models, it should be noted that this thesis does not seek to follow any particular model, and that following the cyclic process contained within these models is beyond the scope of this thesis. Nevertheless, it is hoped that Chapter Three provides an overview of the injuries causing the greatest burden to YSP, and that preventative measures might be informed by the investigation of risk factors in subsequent experimental chapters.

STUDY 1: ABSTRACT

Background: Injury audits inform prevention strategies to reduce injury risk but the number of audits in high-level youth soccer players (YSP) is limited. Moreover, no audit has compared injuries in YSP from different countries. **Methods:** This study included 624 high-level YSP [Under 9 (U9) to U23 year-old age groups] from eight academies in England, Spain, Uruguay and Brazil. **Results:** Over one season, 443 injuries sustained in training and matches were prospectively recorded, giving an injury rate of 0.71 injuries per player. Non-contact injuries were most common (58.5%), with most resolved between 8 and 28 days (44.2%). Most injuries occurred in the lower limbs (75.4%), with muscle the most commonly injured tissue (29.6%). The U14 and U16 suffered a greater number of severe injuries relative to the U12 and U19/U20/U23/Reserves. Tendon injury rate was higher in Brazilian *vs.* Spanish players ($p<0.05$), while low back/sacrum/pelvis injury rate was higher in Spanish players *vs.* all other players ($p<0.05$), otherwise there were no differences. **Conclusion:** The proportion of severe injuries in the U14 and U16 age groups suggests injury risk in YSP is maturation-dependent. Minimal differences in injury frequency, type and location between high-level YSP from four different countries across two continents suggest injury rates in this population are universally similar.

3.1 INTRODUCTION

The epidemiological study of sports injuries is imperative for injury prevention, by assisting in the identification of common injuries and their aetiology (Van Mechelen, Hlobil and Kemper, 1992; Bahr and Krosshaug, 2005; Finch, 2006). Accordingly, an injury audit provides stakeholders with evidence to enable them to advocate which factors likely influence injury occurrence and explore which may be modified to reduce injury risk (Fuller and Drawer, 2004). An audit also forms the primary step in the preventative process described by a number of proposed injury prevention models (Van Mechelen, Hlobil and Kemper, 1992; Bahr and Krosshaug, 2005; Finch, 2006), identifying which injuries occur, how often, and the extent of their impact upon a player or team. Subsequently, the understanding of injury occurrence is challenged and risk factors assumed to contribute toward, or cause injury, are proposed. Only after this step can the design and implementation of preventative strategies be considered in an attempt to reduce injuries. The cyclic process should then revisit the initial audit phase to evaluate the effectiveness of preventative measures on injury occurrence.

Identifying common types, circumstances and anatomical locations of soccer injuries highlights which have the greatest impact on player availability (Parry and Drust, 2006). When many similar injuries are observed, it is logical that those injuries receive greatest attention compared to rare injuries affecting fewer players and teams. However, some infrequent injuries can be severe, causing the lengthiest absence to players, and may be career-threatening. Accordingly, the identification of severe yet less frequent injuries is also important, particularly as time lost to injury threatens the long-term

development of YSP (Price et al., 2004; Le Gall, Carling and Reilly, 2007). In addition, player availability is closely linked to team success (Arnason et al., 2004a), meaning injury reduction is of significance to numerous stakeholders within the sport (Brink et al., 2010; Faude, Rößler and Junge, 2013; Read et al., 2016).

A considerable body of literature describes injury in soccer, with a large proportion derived from professional adult players. However, research on injury in YSP is also available. Whilst existing evidence guides researchers toward the most commonly cited types, causes and locations of injury, it is important to perform regular injury audits to ensure injury prevention strategies remain focussed on those posing the greatest problem. Furthermore, in populations where the number of injury audits are limited, the novel outcomes of new audits can assist in the study of risk factors specific to those populations.

The majority of injury-related absence in professional players and YSP is typically caused by soft-tissue injury (Hawkins et al., 2001; Price et al., 2004) and a large proportion of soccer injuries occur through non-contact situations (Hawkins et al., 2001; Ekstrand, Hägglund and Waldén, 2011; Renshaw and Goodwin, 2016; Read et al., 2018b). Injuries primarily occur within the lower extremities (Ekstrand, Hägglund and Waldén, 2009), particularly in muscles such as those of the thigh (Nilsson, Östenberg and Alricsson, 2016; Renshaw and Goodwin, 2016), with ligament injury also common (Price et al., 2004). In YSP, contusions, bruises and tendinopathies are also present (Le Gall et al., 2006). Whilst the existing literature describes the most common types and locations of injuries in YSP, it was deemed appropriate to perform a new injury

audit specific to those YSP participating in this thesis. This would help define the problem caused by injury in the current cohort and to determine whether the injury data collected was representative of that in previous YSP injury studies. An audit of the injuries suffered by high-level YSP was performed over the course of one competitive season to address the hypothesis that the most frequently reported injury types would be muscle and ligament, and would primarily be non-contact. Furthermore, different coaching, playing and training styles may exist between countries and continents, which may influence the type and frequency of injuries suffered. However, despite the existence of studies of injuries in YSP from separate nations (Price et al., 2004; Le Gall et al., 2006; Van der Sluis et al., 2014), it is currently unknown if injuries in YSP differ when countries are compared with one another. Therefore, this study also sought to investigate for the first time whether differences existed between high-level YSP from four different nations with respect to the most common injury types suffered across a single soccer season. It was hypothesised that the lower limbs would incur the greatest proportion of injuries with minimal differences between nations, and that the thigh, knee and ankle would be among the most common locations. Finally, injuries reportedly peak in specific months of the season (Price et al., 2004; Read et al., 2018b) and this study sought to investigate whether a similar pattern existed in the current cohort.

3.2 METHODOLOGY

3.2.1 Participants and study period

The cohort included 624 high-level male YSP aged 9-23 years from the academies of eight professional soccer clubs from England, Spain, Uruguay and Brazil. Of the five English academies, two were categorised under the Premier League's Elite Player Performance Plan (EPPP) as Category 1 and two were Category 2. One English academy operated independently of the EPPP and competed regularly with Category 1 academies (Under 23 level). The Uruguayan academy was of the highest national category (Category A). There is no classification system for soccer academies in Spain or Brazil, however, the Spanish and Brazilian academies included in this audit are recognised as among the most successful in their respective countries. A further 49 players (U18 = 36; U23 = 13) from two more English Category 1 academies were recruited, but were excluded at this point due to non-receipt of injury data. Participant characteristics are described in Table 3.1. The three youngest age groups were combined due to small numbers, and the U17 and U18 age groups were combined because no U17 age group exists in England under the Premier League's EPPP. The U19, U20, Reserves and U23 groups were combined, as only the U23 age group exists in England, and because player ages in the U19, U20 and Reserve teams of non-English clubs were similar to that of the English U23 teams. All players participated in regular soccer training and competition, which was in accordance with the Premier League's EPPP for the English clubs. Injuries were prospectively recorded during the 2011/12 to 2017/18 seasons. The number of seasons per club within this period ranged from one to seven, with only one season per player,

per club included within the injury audit. The selected season corresponded to the season where the greatest number of players were available from each academy. This resulted in records for 223 players from the 2014/15 season (two clubs), 17 players from the 2016/17 season (one club) and 384 players from the 2017/18 season (five clubs). No player records contributed to more than one soccer season, in order to ensure equal comparison and reduce the influence of re-injuries. Written informed consent to participate in this audit was collected from club officials and players, with parental consent and player assent collected for all participants less than 16 years of age. The study received approval from Liverpool John Moores University Research Ethics Committee.

Table 3.1. Participant characteristics. Data are mean ± SD.

Age Group	Number of players (%)	Age (years)	Height (m)	Body mass (kg)
U9, U10, U11	66 (10.6)	10.3 ± 0.8	1.42 ± 0.06	34.5 ± 4.0
U12	47 (7.5)	11.6 ± 0.4	1.49 ± 0.05	38.9 ± 3.7
U13	43 (6.9)	13.1 ± 0.4	1.60 ± 0.08	46.3 ± 7.1
U14	62 (9.9)	14.0 ± 0.4	1.68 ± 0.07	56.7 ± 8.4
U15	67 (10.7)	15.0 ± 0.7	1.74 ± 0.08	63.6 ± 8.5
U16	61 (9.8)	16.2 ± 0.5	1.76 ± 0.06	68.2 ± 7.4
U17, U18	148 (23.7)	17.6 ± 0.8	1.79 ± 0.07	73.4 ± 8.2
U19, U20, U23, Reserves	130 (20.8)	19.6 ± 1.3	1.81 ± 0.07	76.4 ± 7.5

3.2.2 Injury recording and definitions

Injuries sustained by players were diagnosed and recorded by medical personnel at each club, in accordance with previously published guidelines (Fuller et al., 2006) and sent anonymised to researchers in a standardised electronic spreadsheet. Injuries were recorded when they had occurred during soccer-related activity (training or match-play) and resulted in a player being unable to participate in training or competition for 24 hours or more following

the incidence or onset of injury. A player was classified as injured until they were able to return to full training and become available for match selection, with the number of days absent calculated as the difference between the date of injury until the date of return to full training and selection availability. Categorisation of injury location and type were recorded according to previously published guidelines (Fuller et al., 2006). Severity of injury was classified according to the total number of days missed, including: minimal (1-3 days), mild (4-7 days), moderate (8-28 days) and severe (>28 days) (Fuller et al., 2006; Ekstrand, Hägglund and Waldén, 2009). Traumatic injury was defined as an injury with a clearly identifiable event leading to injury, whilst overuse injury was defined as an injury believed to result via gradual onset without a clear injury-inciting event. Injuries were classified as contact or non-contact depending on whether a clear incident involving contact with another player, the ball or another object was present or not. Injuries categorised as muscle rupture/strain/cramps, sprain/ligament injury or tendon injury/rupture/tendinosis/bursitis were grouped under “soft-tissue injury”. Injury rate was calculated by dividing the number of injuries by the number of participating players (Price et al., 2004; Read et al., 2018b).

3.2.3 Statistical and data analysis

Data are presented as means ± standard deviations (SD). The chi-square (χ^2) test of independence was used to compare the injury rate for the most common injury types and locations between the four nations and injury severity for each age group, while the Pearson’s χ^2 (goodness of fit) test compared the monthly distribution of injuries throughout the season for each country. Due to

English and Spanish soccer seasons starting in August and the Uruguayan and Brazilian seasons beginning in February, the 10 months of the season were normalised to month number, where Month 1 represented August for England and Spain, and February for Uruguay and Brazil. All statistical analyses were performed using SPSS Version 25.0 (IBM Statistics, Chicago, Illinois) and statistical significance was set at $p < 0.05$.

3.3 RESULTS

3.3.1 Summary of injuries

During the season, a total of 471 injuries were recorded. Twenty eight injuries were excluded because they occurred outside of soccer training or match play, leaving 443 injuries for analysis. The injury rate for all injuries in the entire cohort was 0.71 injuries per player, with 252 players (40.4%) from the cohort suffering at least one injury. A total of 12,143 days were lost to injury with an average of 28 (range 1 to 303) days of absence per single injury. The majority of injuries were non-contact (58.5%) and were mainly suffered in training (54.4%) compared to matches (40.9%), with 4.7% from unknown soccer origin. Traumatic and overuse injuries accounted for 46.3% and 26.6% of injuries, respectively, however, 27.1% were of unspecified origin due to lack of sufficient data.

3.3.2 Injury severity

“Moderate” injuries (8 to 28 days, 44.2%) represented the most frequent severity category, followed by “severe” (>28 days, 28.7%) and “mild” (4-7 days, 18.3%), with “minimal” injuries (1-3 days, 8.1%) contributing fewest.

There was a significant difference in the proportion of severe injuries according to chronological age group, $\chi^2 = 42.19$, $p = 0.001$ (Fig. 3.1). The U13, U14, U15, U16 and U17/U18 age groups had a significantly greater proportion of severe injuries than the U12 age group, whilst the U14 and U16 age groups also had a significantly greater proportion of severe injuries than the U19/U20/U23/Reserves age group (all $p < 0.05$).

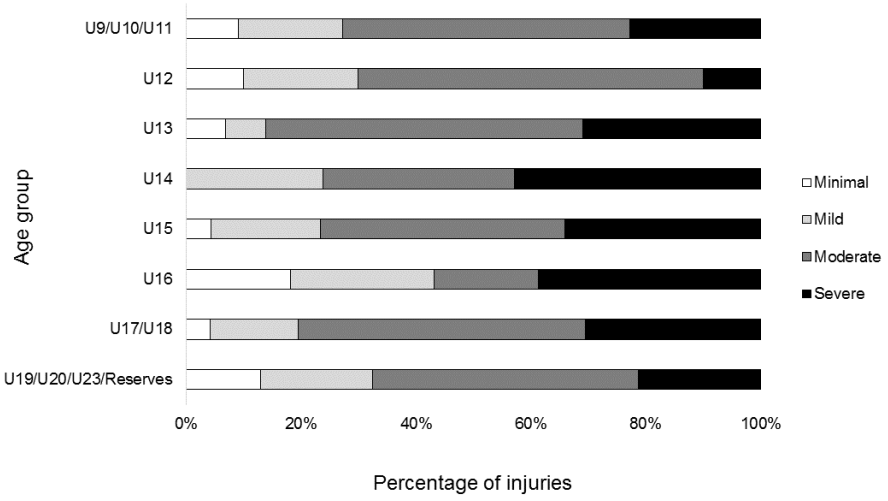

Figure 3.1. Distribution of injury severity according to age

3.3.3 Injury location and injury type

The most common locations were thigh, knee, ankle and low back/sacrum/pelvis (Fig. 3.2), with the most common types of injury being muscle strain/rupture/cramps and sprain/ligament injury (Fig. 3.3). Most injuries were to the lower limbs (75.3%), and over half of all injuries were classed as soft-tissue injuries (54.0%). Of these, muscle rupture/strain/tear/cramps was most common (54.8%), followed by

sprain/ligament injury (37.7%) and tendon injury/rupture/tendinosis/bursitis (7.5%). Most soft-tissue injuries were non-contact (65.3%), meaning 35.2% of all recorded injuries were non-contact soft-tissue (NCST) injuries.

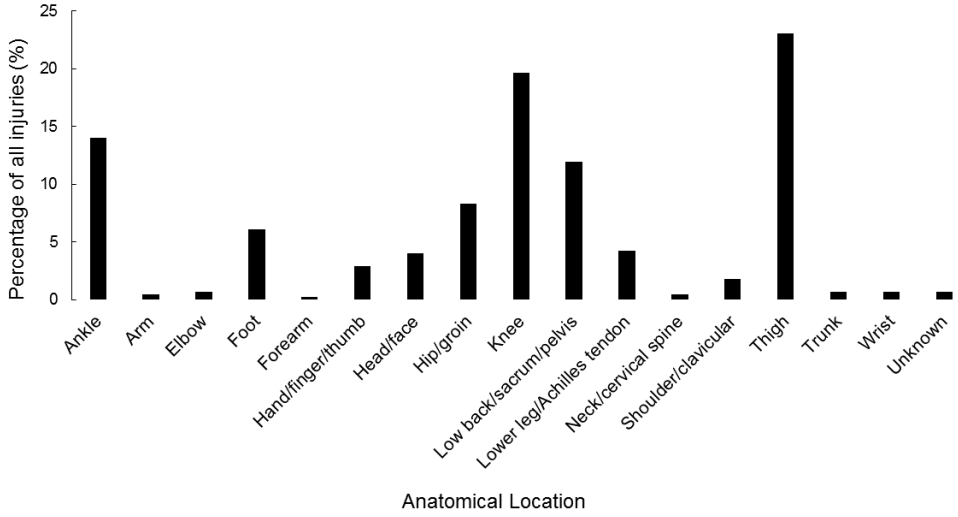

Figure 3.2. Distribution of all recorded injuries based on anatomical location

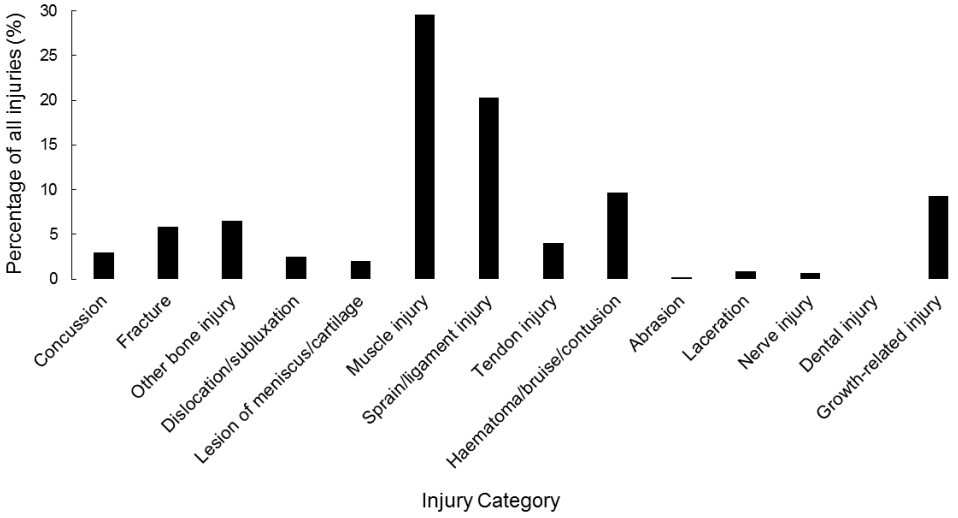

Figure 3.3. Distribution of all recorded injuries based on injury type

3.3.4 Muscle injuries

There were 131 muscle/rupture/strain/tear/cramps injuries incurring 2,285 days of absence and an average of 17 (range 1 to 91) days lost per injury. Of all injuries in this category, 77.1% occurred through non-contact situations, and were mainly from training (58.8%) compared to matches (38.2%), with 3.1% of unspecified origin. Most were traumatic (39.7%) compared to overuse (32.8%), though 27.5% were unspecified due to lack of sufficient data. Most muscle injuries were resolved between 8 and 28 days (48.9%), with only 17.6% requiring more than 4 weeks before return to play. The thigh was the most common site of muscle injury (59.5%), followed by the hip/groin (19.8%). Hamstring injuries were most frequent, accounting for 38.9% of muscle injuries and 11.5% of all injuries.

3.3.5 Ligament injuries

There were 90 sprain/ligament injuries over the course of the season, with a total absence of 3,251 days and an average of 36 (range 1 to 303) days missed per injury. Half of the ligament injuries were non-contact (50.0%) recorded during training (54.4%) and matches (40.0%), with 5.6% from unspecified activity. Ligament injuries were mainly traumatic (66.7%) compared to overuse (10.0%), with 23.3% unspecified due to lack of sufficient data. Injury severity in the sprain/ligament injury category was mainly moderate (47.8%), followed by severe (28.9%) and mild (18.9%), with few minimal injuries (4.4%). The ankle and knee were the most common sites, with 54.4% and 34.4% of all ligament injuries, respectively. Of the knee ligament injuries, 22.6% were to

the anterior cruciate ligament (ACL), representing 42.9% of all ligament injury absence.

3.3.6 Tendon injuries

Tendon injury/rupture/bursitis/tendinosis represented 4.1% of all injuries, leading to 561 days of absence with a mean absence of 31 (range 6 to 117) days per injury. More than half were non-contact (55.6%) with most during training (44.4%) compared to matches (33.3%), however 22.2% were during unspecified activity due to lack of sufficient data. Tendon injuries were mainly severe (44.4%) or moderate (38.9%), and were most common in the knee (44.4%) and the hip/groin (27.8%).

3.3.7 Injury rate between countries

Differences in injury rate were observed between countries ($\chi^2 = 76.61$, $p < 0.001$), with the rate of tendon injury being greater in the Brazilian cohort than the Spanish cohort (0.06 *vs* 0.01, $p < 0.05$), and the rate of low back/sacrum/pelvis injury being greater in the Spanish cohort compared to the English, Uruguayan and Brazilian cohorts (0.29 *vs* 0.01, 0.03 and 0.00, respectively, $p < 0.05$). No differences in injury rate were observed between countries for any other injury type/location (all, $p > 0.05$).

3.3.8 Seasonal distribution of injuries

A significant difference in the rate of injuries suffered per month of the season was observed when all countries were combined ($\chi^2 = 108.98$, $p < 0.001$) and

when each country was analysed separately ($\chi^2 \geq 91.50$, $p < 0.001$). Overall, Months 6, 2 and 10 had the highest injury rates. In English academies, Month 4 and Month 2 (November and September) had the greatest injury rates, whilst in the Spanish academy Months 6 and 7 (January and February) had equally high injury rates. For the Uruguayan academy, Month 6 (July) had the highest injury rate with months 2 (March) and 10 (November) equal second. In the Brazilian academy, Months 5 and 8 (June and September) shared the highest injury rates.

3.3.9 Identification of injuries for analysis in experimental chapters

Based on these results, the injury categories (types and locations) to be investigated in Chapters Four, Five and Six are defined in Table 3.2.

Table 3.2. Injury categories and definitions for analysis throughout Chapters Four, Five and Six

Injury category	Definition
Injury	Any recorded injury
Non-contact injury	Injuries recorded as occurring without physical contact with an opponent, ball or other object
Muscle injury	Injuries recorded as muscle rupture/strain/tear/cramps
Ligament injury	Injuries recorded as sprain/ligament injury
Tendon injury	Injuries recorded as tendon injury/rupture/tendinosis/bursitis
Soft-tissue injury	Injuries defined as occurring in muscle, ligament or tendon tissue
Non-contact soft-tissue (NCST) injury	Injury to muscle, ligament or tendon tissue occurring without physical contact with an opponent, ball or other object
Low back/sacrum/pelvis injury	Injuries recorded as low back/sacrum/pelvis injury
Knee injury	Injuries located at the knee
Ankle injury	Injuries located at the ankle
Thigh muscle injury	Muscle injuries located in any muscle of the thigh
Hamstring muscle injury	Muscle injuries located within the hamstrings

3.4 DISCUSSION

The primary purpose of this injury audit was to identify the most common injuries in YSP from four high-level soccer nations across two continents, and which injuries caused the longest absences from training and match play. It was hypothesised that muscle and ligament injuries would be most prevalent and that the lower limbs would incur a considerable proportion of non-contact injuries, particularly to the thigh, knee and ankle. The main findings of this audit confirmed these hypotheses, as well as the hypothesis that minimal differences would exist between the four nations regarding injury type and location. Importantly, these novel findings suggest that the most common types and locations of injuries in YSP do not differ according to the country or continent where they are recorded.

In general, the commonly recorded injury locations and types did not differ significantly between the four nations. However, there were differences in the rates of tendon injuries and low back/sacrum/pelvis injuries. Specifically, players in the Brazilian academy had a higher rate of tendon injury compared to players in the Spanish academy, who had a higher rate of low back/sacrum/pelvis injuries compared to players from English, Uruguayan and Brazilian academies. The reasons for these differences are unclear, though there were a small number of tendon injuries recorded within the current audit. Nevertheless, it is possible that different interpretations or diagnoses of injuries between Brazil and Spain contributed to these results. In addition, the mean age of the Spanish cohort was lower than the Brazilian cohort. It is possible that chronologically older players amongst the Brazilian cohort might influence the number of tendon injuries recorded, as they are likely to have accumulated

greater soccer exposure and thus have suffered previous tendon injuries (Gajhede-Knudsen et al., 2013), though there are many possible factors which might explain the observed differences. Further comparison between nations may offer further insight into whether the observed differences are most likely to exist between individual clubs or between countries.

In attempting to explain the higher rate of low back/sacrum/pelvis injuries in Spanish players, it was observed that the U12 to U15 age groups contributed more than two thirds of these injuries. This injury location comprises a broad range of possible injury types, which may be related to maladaptation of under-developed tissues/structures to loads experienced during training/match play. Interestingly, the Spanish cohort had a relatively higher number of players (51.7%) in the U12 to U15 age groups in comparison to the English, Uruguayan and Brazilian clubs who had 31.9%, 34.7%, and 0.0% respectively. Therefore, a greater relative number of U12 to U15 players in the Spanish cohort might have contributed to the differences observed. It is also possible that injury diagnosis and recording differs between the medical staff of different clubs or countries, based on the interpretation of injury location. Another possibility is differences in strength training practices between countries. In players performing limited strength training, these injuries could be due to low relative maximum strength or stability in players frequently required to run, jump and rotate (Purcell and Micheli, 2009). The opposite may also occur, where players undertaking high volumes of soccer and strength training are more likely to be injured due to added stress on the lower back region. Most low back injuries in the present audit occurred through overuse, as previously reported (Purcell and Micheli, 2009), suggesting low

back/sacrum/pelvis injuries may be linked to insufficient rest and recovery. Nevertheless, further research on low back/sacrum/pelvis injury in YSP is warranted.

Most injuries in the present sample were non-contact, as previously reported in youth (Renshaw and Goodwin, 2016; Read et al., 2018b) and senior players (Hawkins et al., 2001; Ekstrand, Hägglund and Waldén, 2011) and 75.3% of injuries were in the lower limbs, supporting previous work (Hawkins et al., 2001; Price et al., 2004; Deehan, Bell and McCaskie, 2007; Nilsson, Östenberg and Alricsson, 2016). The thigh was the most common site of injury, followed by the knee and the ankle, with muscle and ligament the most frequently injured tissues, meaning the injuries observed in this study were typical of a soccer population (Price et al., 2004; Le Gall et al., 2006; Read et al., 2016; Renshaw and Goodwin, 2016). hamstring muscle injuries were recorded as the single most common injury, which has been documented elsewhere (Price et al., 2004; Le Gall et al., 2006; Ekstrand, Hägglund and Waldén, 2011). Tendon injuries typically led to absences greater than a week, despite representing a small fraction of injuries, which is also commonly observed (Price et al., 2004; Deehan, Bell and McCaskie, 2007; Ekstrand, Hägglund and Waldén, 2009). This is considered a justification for further investigation of their occurrence, particularly as injured tendons are unlikely to ever regain their pre-injured condition (Tozer and Duprez, 2005). These data suggest further study of soft-tissue injury in high-level YSP, particularly addressing the risk factors that lead to their occurrence.

The percentage of severe injuries was greater in the U14 and U16 age groups compared to U12 and U19/U20/U23/Reserves age groups. Crucially,

this would suggest that players close to the age of 14 and 16 years old miss more days per injury than other age groups. This is particularly interesting as these are the ages where biological maturation typically occurs in adolescent males, often coinciding with increments in training volume (Elferink-Gemser et al., 2012). Further investigation is merited to determine whether there is an association between biological maturation and injury severity, with some authors suggesting the rate and timing of skeletal maturation affect injury incidence and severity in YSP (Le Gall, Carling and Reilly, 2007; Van der Sluis et al., 2014; Van der Sluis et al., 2015).

Recovery from soccer injury varies considerably by the type and location of the injury, with injury severity categorised based on the number of days missed (Hawkins and Fuller, 1999; Fuller et al., 2006; Le Gall et al., 2006; Read et al., 2016). Moderate and severe injuries represented a combined 72.9% of all injuries in the present audit, meaning less than 30% of injuries were resolved within a week. It is therefore abundantly clear that the significant problem caused by injury to player availability (Parry and Drust, 2006) extends to YSP. Absence periods could be influenced by coach attitudes, and whether some players are given additional time to recover compared to others who may be inadequately recovered but cleared as fit. Severe injuries represented more than a quarter of all injuries in the present audit, a finding similar to some literature (Hawkins et al., 2001; Nilsson, Östenberg and Alricsson, 2016; Read et al., 2018b) but higher than others (Ekstrand, Hägglund and Waldén, 2009; Ekstrand, Hägglund and Waldén, 2011). Notably, studies with fewer severe injuries involve elite level professional (senior) teams, where medical assistance and facilities are likely to be superior, and players may be

encouraged to return to play sooner. Conversely, YSP may be afforded greater recovery time due to attitudes prioritising athletic development, which may supersede the desire for success. Nevertheless, a similar distribution of injury severity to that observed in this audit was evident amongst comparable cohorts (Nilsson, Östenberg and Alricsson, 2016; Read et al., 2018b).

When collectively analysing all players, the rate of injury was dependent on the month of the season. Specifically, months 6, 2 and 10 of the playing season demonstrated the highest rate of injury. In players from English academies, month 4 and month 2 had the highest injury rates, which is in part agreement with previous literature describing an injury peak in month 2 in English academy players (Read et al., 2018b). The same study also found another injury peak in month 6, which is reflected in the findings that Spanish players had similarly high injury rates in months 6 and 7. In Uruguay, the greatest peak was observed in month 6 of the season, similar to the peak within the English and Spanish seasons. It is thought that higher injury rates occur in certain months following a return to activity after acute deconditioning during summer or winter break periods (Read et al., 2018b). However, the months with the highest injury rates in Uruguayan and Brazilian academies do not follow such periods. Nevertheless, months within the second and third quarters of the season generally appear to demonstrate higher injury rates in each country, though the specific months when injuries peaked differed between countries. Not all studies report monthly differences in injury rates (Carling, Orhant and LeGall, 2010) and between-season variation has also been demonstrated (Carling, Orhant and LeGall, 2010). Not every season is expected to be identical, thus it is not clear if the same pattern of injuries would

exist amongst the same players in another season. Whilst practitioners should remain cognisant of the reasoning for elevated injury risk in periods following breaks from activity, this audit suggests this might affect some academies more than others.

There are some limitations in the present injury audit. Firstly, lack of data regarding soccer activity (exposure) restricts the ability to provide accurate injury incidence data, which is typically reported per 1000 hours of soccer activity (Fuller et al., 2006). However, exposure records can lack clarity regarding the nature and intensity of activity, which also limits comparison between research studies even when it is available. Nevertheless, information regarding the training schedules and practices in each country could offer greater insight into the observed differences in the present study. Secondly, nearly half of the cohort were above the U16 group, meaning much of the injury data may be more representative of post-pubertal players. Older players will have accrued greater soccer exposure since they began playing, which will increase their risk of injury (Nédélec et al., 2013), with older players more likely to have suffered one or more previous injuries due to the length of their career. It could be argued that including several soccer academies from different countries could introduce more variability from potentially different training styles, training volumes and coaching philosophies between countries. In addition, there may be differences in diagnosis and reporting of injuries between different countries. However, one of the main aims of this audit was to investigate whether injury rates differed between YSP from England, Spain, Uruguay and Brazil, which has not been investigated before. Furthermore, only small differences in injury rate in only two injury types/locations were observed

between countries, demonstrating that injuries were broadly equivalent in academies from these countries. Moreover, including fewer academies would limit the sample size considerably and restrict the external validity of findings, particularly if the data had come from a single academy, or a single country. Indeed, the majority of previous injury audits include several academies but from just one country (Hawkins et al., 2001; Price et al., 2004; Read et al., 2018b). It is also acknowledged that training schedules and off-season periods may differ between clubs and countries and between age groups within the same clubs, which could be influential to the occurrence of injury, and that these are not described in the present audit. Finally, information concerning the playing positions of the players was not provided in this audit, which is recognised as a risk factor for soccer injury (Carling, Orhant and LeGall, 2010). Future studies should include this important variable in their injury risk analyses.

3.5 CONCLUSION

The present study concludes that injuries are prevalent in YSP, are most often suffered in the lower limbs, and that non-contact injuries to soft-tissue structures constitute a substantial proportion of injuries. Interestingly, players from the Spanish academy suffered more low back/sacrum/pelvis injuries than players from English, Uruguayan or Brazilian academies, which may be due to there being relatively more U14-U16 players in the Spanish cohort (the ages at which more low back/sacrum/pelvis injuries tended to occur). Apart from a higher rate of tendon injuries in players from Brazil than Spain, data were similar between countries concerning the main injury types/locations,

suggesting injury risk in this population is similar between countries. Furthermore, specific months demonstrated peaks in injury rate, suggesting certain periods of the season when YSP may be at a higher risk of injury (e.g. following off/mid-season breaks). Finally, a key finding from this audit is that players in the U14 and U16 age groups suffered a greater percentage of severe injuries compared to players of other age groups. This suggests that maturation status influences injury risk. Therefore, there is a need to understand whether there is an association between the maturation status of YSP and the injuries they suffer. Such information would be beneficial to practitioners working with YSP in order to limit the prevalence and/or severity of injuries.

CHAPTER FOUR

INJURY RISK IN HIGH-LEVEL YOUTH SOCCER PLAYERS DEPENDS ON MATURATION STATUS

PRELUDE

Following the observation in Chapter Three that the proportion of severe injuries appeared to be greater in the U14 and U16 age groups, it was deemed appropriate to investigate the potential influence of maturity status on injuries in YSP. Importantly, the observations contained within Chapter Three suggested that players close to the age of PHV may be at greater likelihood of suffering severe injuries, and therefore missing more crucial development time. Chapter Four considered these findings, and the findings of previous authors (see Literature Review) and sought to investigate whether the maturity status of YSP was associated with the prevalence and severity of injuries in this population, specifically investigating the types and locations of injuries identified by Chapter Three as being the most commonly observed in this cohort.

STUDY 2: ABSTRACT

Background: Soccer injuries negatively affect player availability and, therefore, athletic development in YSP. As musculoskeletal growth rate changes during adolescence, the aim of this study was to determine whether maturation influences injury risk in high-level YSP. **Methods:** The present study investigated 597 male YSP from Under 9 (U9) to U23 year-old age groups from eight high-level soccer academies in England, Spain, Uruguay and Brazil. Using anthropometry, players were grouped according to years from predicted peak-height-velocity (PHV), i.e. pre-, mid- and post-PHV, to determine maturation status. Injury frequency, type, location and severity sustained during training and matches were prospectively recorded over one season and analysed according to PHV group. **Results:** Relatively more post-PHV players suffered soft-tissue, muscle, ligament/tendon and thigh injuries than pre-PHV players, and relatively more post-PHV players suffered soft-tissue, ligament/tendon and thigh-muscle injuries than mid-PHV players. Of all the injured players, post-PHV suffered more soft-tissue injuries than pre-PHV and missed more days due to injury compared to pre-PHV. Post-PHV players also had greater injury-severity scores for soft-tissue injuries and ligament/tendon injuries than pre-PHV. **Conclusion:** These findings demonstrate that post-PHV, high-level male YSP are at greater risk of injury to soft-tissue, skeletal muscle, ligament/tendon and the thigh, compared to pre-/mid-PHV players, and that post-PHV players miss more of the season through injury than pre-PHV players. Injury prevention strategies should, therefore, be developed to target these specific tissues/locations in physically mature players.

4.1 INTRODUCTION

Chapter Three demonstrated that skeletal muscles and ligaments of the lower limbs are commonly injured in YSP, in agreement with previous literature (Wong and Hong, 2005; Renshaw and Goodwin, 2016). These injuries occur during actions such as running, kicking and turning (Price et al., 2004) and are partly due to the intermittent nature of soccer, which involves repeated high intensity actions (Stølen et al., 2005). The influence of injury on player availability and team success means strategies aimed at injury prevention are important within elite soccer (Arnason et al., 2004a; Price et al., 2004) and, for YSP, time lost to injury may also reduce the chance of maximising skill development (Price et al., 2004). Consequently, understanding why and when YSP may be more susceptible to specific injuries may help practitioners enhance their players' chances of success.

In high-level YSP, 6-11% of development time is lost to injury (Price et al., 2004; Le Gall, Carling and Reilly, 2007) with a peak around the age of 14 years old, as demonstrated in Chapter Three and by others (Le Gall et al., 2006; Le Gall, Carling and Reilly, 2007). This coincides with the age at which most adolescent males undergo their fastest rate of somatic growth, known as peak height velocity (PHV) (Malina et al., 2005a), when they can grow up to 9.7 cm in height per year (Malina et al., 2005b; Philippaerts et al., 2006). Although differences in body size exist from a young age, they intensify during adolescence. Furthermore, some studies suggest that the number of injuries suffered by YSP increases in the 12 months surrounding PHV, potentially attributable to rapid somatic change (Van der Sluis et al., 2014; Van der Sluis et al., 2015). Indeed, Kemper *et al.* suggested that injury may be 1.63 and 1.61

times more likely in YSP who grow at least 0.6 cm in height or gain 0.3 kg/m^2 in body mass index in a single month, respectively (Kemper et al., 2015). Some authors have suggested that the advanced trunk and limb growth, which precedes skeletal muscle adaptation, creates an “adolescent awkwardness”, characterised by impaired motor task performance that can alter movement mechanics. This may increase injury risk, particularly in combination with an imbalance between strength and flexibility (Beunen and Malina, 1988; Malina, Bouchard and Bar-Or, 2004). Besides these physical changes, YSP often experience increased external load through greater training volume and match intensity, which may augment the internal load experienced by their soft tissues, such as muscles, tendons and ligaments (Wrigley et al., 2012). When combined with perturbations from adolescent growth, external loads may lead to varied internal loads dependent on a player’s stage of maturation, which may in turn influence injury risk (Van der Sluis et al., 2015). Consequently, it is possible that players between the ages of 12 and 16 years are at increased risk of injury and that this is influenced by the rate and timing of PHV.

The chronological age at which PHV occurs varies considerably between individuals (Mirwald et al., 2002). In some cases, players of the same chronological age are up to 3.7 years apart in skeletal age, characterised by differences of up to 15 cm in height and 21 kg in mass (Figueiredo et al., 2010). Nonetheless, YSP compete according to their chronological age, and those players who mature later potentially face the disadvantage of being comparatively smaller, lighter, and weaker than their peers. This can affect the performance of soccer-specific attributes such as power and agility, often influencing players’ chances of selection (Figueiredo, Coelho e Silva and

Malina, 2011; Johnson, Farooq and Whiteley, 2017). These differences may also affect their risk of injury around the age of PHV (Malina et al., 2005a).

Improving current appreciation of how biological maturation affects injury risk would assist injury prevention strategies, helping to reduce the number of injuries suffered by YSP and the time lost to injury, thus increasing their chances of achieving a successful soccer career. Despite their merits, previous investigations are limited by sample size (Van der Sluis et al., 2014; Van der Sluis et al., 2015) and the investigation of a single club or age group (Le Gall, Carling and Reilly, 2007) with the external validity of those findings unknown, and there is currently a lack of literature concerning the influence of biological maturity on injury occurrence in YSP from multiple nations. Accordingly, the purpose of this study was to quantify the influence of maturation status on injury risk across a cohort of high-level male YSP across multiple age groups and from multiple academies in several countries and across two continents. In Chapter Three, players in the Under 14 (U14) to U16 age groups suffered the greatest proportion of severe injuries compared to other age groups. Therefore, it was hypothesised that maturation status would influence injury risk, with a greater injury frequency/severity in players close to PHV, i.e. mid-PHV.

4.2 METHODOLOGY

4.2.1 Participants and study design

The present study used a mixed prospective cohort design to report soccer injuries in 597 high-level male YSP aged 9-23 years, who were registered with one of eight professional soccer clubs previously described in Chapter Three.

Injury data for each player was recorded for a minimum of one soccer season between the 2014-15 and 2017-18 seasons, with anthropometric measures taken during that season. This resulted in 220 player records for the 2014-15 season, 17 player records for the 2016-17 season, and 360 player records for the 2017-18 season, with one season per player. All players participated in regular soccer training and competitive match play, which was in accordance with the EPPP for the English clubs. Written informed consent to participate in this study was obtained from club officials and players, with parental consent and player assent collected for all participants less than 16 years of age. The study received approval from Liverpool John Moores University Research Ethics Committee and complied with the Declaration of Helsinki.

4.2.2 Anthropometry and biological maturation

Body mass (kg) was measured on a calibrated physician scale (Seca, Birmingham, UK). Standing and sitting height (m) were recorded on a stadiometer (Holtain Limited, Crosswell, United Kingdom), with players sitting on a standardised anthropometry box (Holtain Limited, Crosswell, United Kingdom). Participants' stage of maturation was calculated via non-invasive methods using a previously validated regression equation consisting of age, body mass, standing height and sitting height (Mirwald et al., 2002). This allowed calculation of maturity offset, providing a prediction of years from PHV. Players with a maturity offset greater than -1.0 years were categorised as pre-PHV; players with a maturity offset between -1.0 and +0.5 years were categorised as mid-PHV; with all others categorised as post-PHV. Previous paediatric research using this method indicates that maturity offset can be

estimated within 1 year 95% of the time (Mirwald et al., 2002). Participant characteristics according to maturation group are described in Table 4.1. The proportions of players at each stage of maturation within each chronological age group are displayed in Fig. 4.1.

Table 4.1. Participant characteristics according to maturation group. Data are mean ± SD.

Maturation Group	*n* (% cohort)	Age	Height (m)	Mass (kg)
Pre-PHV	132 (22.1)	11.2 ± 1.2	1.47 ± 0.07	37.1 ± 4.8
Mid-PHV	59 (9.9)	13.7 ± 0.8	1.65 ± 0.06	51.7 ± 6.2
Post-PHV	406 (68.0)	17.5 ± 2.0	1.78 ± 0.08	71.8 ± 8.9

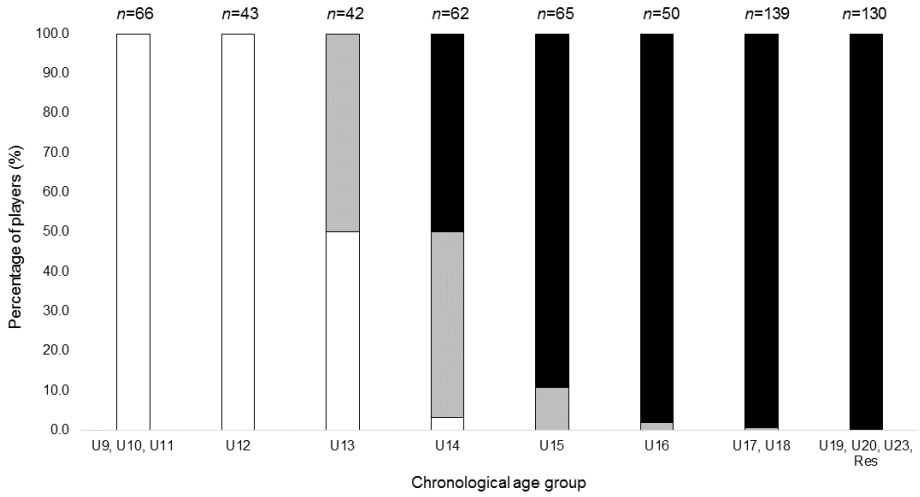

Figure 4.1. Relative contribution of maturation status [pre- (white), mid- (grey), or post-PHV (black)] to each chronological age group.

4.2.3 Injury recording and definitions

The methodology adopted for the recording and definition of injuries in this study has been previously described in Chapter Three. The injury-burden score for each player represented the sum of severity classifications allocated to each injury (Fuller et al., 2006). For example, a player with one muscle injury

categorised as 2 (mild, 4-7 days) would have an injury-burden score for muscle injuries of 2, whilst a player with two separate ankle injuries categorised as 4 (severe, > 28 days) would have an ankle injury-burden score of 8. Injury rates were calculated by dividing the total number of injuries by the number of participating players (Price et al., 2004; Read et al., 2018b). Due to small numbers of injuries recorded and similarities in tissue structure and injury aetiology (Tozer and Duprez, 2005), ligament and tendon injuries were grouped as 'ligament/tendon injuries'.

4.2.4 Statistical and data analysis

Data are presented as mean and standard deviation (SD). The types and locations of injuries analysed were selected according the findings of Chapter Three (see Table 3.2). For each injury category, players were grouped according to whether they had suffered one or more injury, or no injury. A Chi-square (χ^2) test of independence was then used to assess whether the proportion of injured and uninjured players for each injury category was independent of maturation group. For those players, who had suffered at least one injury for each injury category, frequency and severity of injury were compared between PHV groups using a Kruskal-Wallis H test of variance (because data were not normally distributed). Statistical significance was accepted at $p < 0.05$, with a post-hoc Bonferroni adjustment applied to post-hoc pairwise comparisons. All statistical analyses were performed using SPSS version 25.0 (Chicago, Illinois).

4.3 RESULTS

4.3.1 Total injuries and injury rate

A total of 388 injuries were recorded over the course of one season, resulting in an injury rate of 0.65 injuries per player and an absence period of 26.2 ± 37.8 days per injury. The pre-, mid- and post-PHV groups had injury rates of 0.47, 0.64 and 0.71 injuries per player, respectively. Of the injuries recorded, 9.8% were classed as growth-related injuries.

4.3.2 Injured vs. uninjured players

There was a difference in the proportion of players who suffered one or more soft-tissue injuries between maturation group, with post-PHV being greater than pre- and mid-PHV (33.7% *vs* 16.7% and 16.9%, respectively, OR = 2.5 and 2.5), χ^2 = 18.30, p = 0.000115 (Fig. 4.2a). To demonstrate that this study was statistically powered, we performed a power analysis calculation based on these data, which demonstrated a power of 0.893. Similarly, the proportion of post-PHV players suffering at least one ligament/tendon was greater than that of the pre- mid-PHV groups (17.8% *vs* 10.6% and 6.8%, respectively, OR = 1.8 and 3.0), χ^2 = 7.59, p = 0.022. Relatively more post-PHV players suffered one or more thigh-muscle injuries compared to pre- and mid-PHV (15.3% *vs* 3.8% and 5.1%, respectively, OR = 4.7 and 3.4), χ^2 = 15.62, p = 0.001 (Fig. 4.2b). The proportion of players with one or more muscle injuries was greater in post-PHV players than in pre-PHV (20.3% vs 6.1%, OR = 3.9), χ^2 = 16.63, p = 0.000300. The proportion of players with one or more growth-related injury was lower in post-PHV players than pre- and mid-PHV (1.0% vs 15.2% and 10.2,%, respectively, OR = 17.7 and 11.2), χ^2 = 45.52, p = < 0.001.

No differences in any other injury category were found between maturation groups.

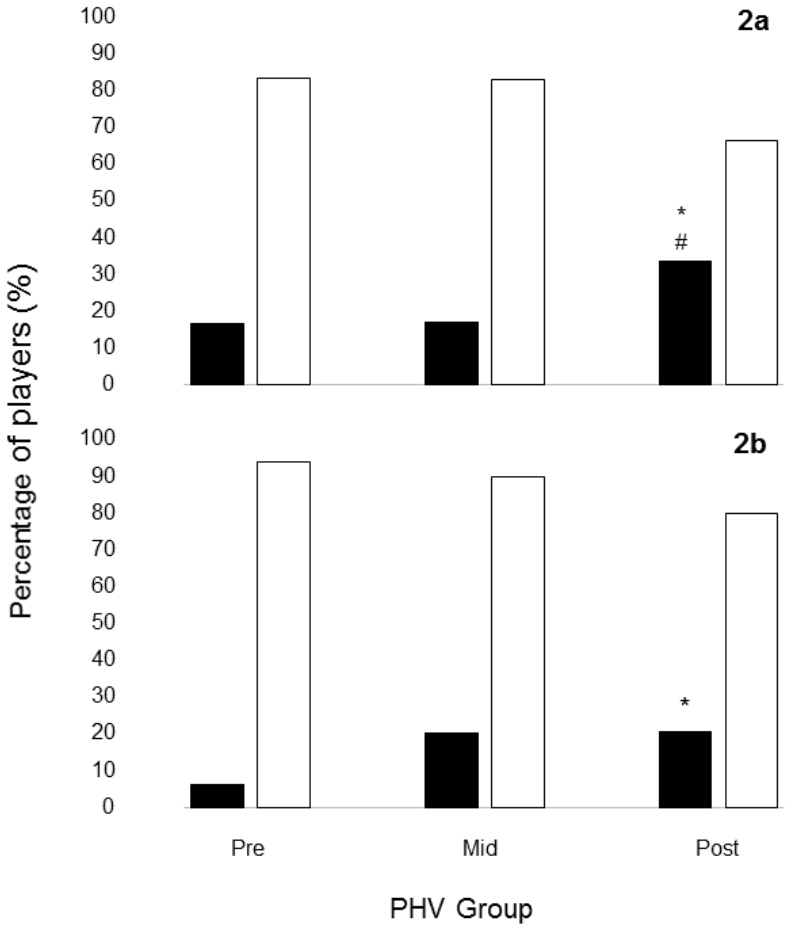

Figure 4.2. a) Proportion of players in each maturation group (pre-, mid-, and post-PHV) having suffered at least one (black bars) or no (white bars) soft-tissue injury. * significantly greater than pre ($p < 0.05$); # significantly greater than mid ($p < 0.05$). Figure 4.2 b) Proportion of players in each maturation group (pre-, mid-, and post-PHV) having suffered at least one (black bars) or no (white bars) muscle injury. * significantly greater than pre ($p < 0.05$).

4.3.3 Number of injuries

Of players who suffered at least one soft-tissue injury, the mean number of injuries suffered per player was greater amongst injured post-PHV players than injured pre-PHV players ($p = 0.020$, Table 4.2). No difference between

maturation groups in the number of injuries was evident for any other injury category.

4.3.4 Days of absence

When all injuries were combined, the mean number of days missed per player from injuries was significantly greater amongst injured post-PHV players than injured pre-PHV players (p = 0.045) but not compared to injured mid-PHV players (p = 0.936, Table 4.3). To demonstrate that this study was statistically powered, we performed a power analysis calculation based on these data, which demonstrated a power of 87.6%. No between group differences were evident for any other injury category regarding days of absence.

4.3.5 Injury-burden score

Post-PHV players with at least one soft-tissue injury had a greater median injury-burden score compared to pre-PHV (p = 0.027) but not mid-PHV (p = 1.000, Table 4). Post-PHV players with at least one ligament/tendon injury also had a greater injury-burden score than injured pre-PHV players (p = 0.043) but not mid-PHV (p = 1.000, Table 4.4). No difference between maturation groups was found for any other injury category regarding injury-burden score.

Table 4.2. Number of injuries per injured player for each category according to maturation group (pre-, mid- and post-PHV), expressed as mean and standard deviation (SD).

Injury category	Pre-PHV	Mid-PHV	Post-PHV	
	Mean (SD)	Mean (SD)	Mean (SD)	Total number of injuries
Injury	1.5 (0.6)	1.4 (0.6)	1.7 (1.1)	388
Non-contact injury	1.5 (0.6)	1.3 (0.6)	1.6 (0.9)	244
Muscle injury	1.0 (0.0)	1.0 (0.0)	1.3 (0.7)	121
Ligament/tendon injury	1.0 (0.0)	1.3 (0.5)	1.2 (0.3)	103
Growth-related injury	1.3 (0.5)	1.3 (0.6)	1.0 (0.0)	38
Soft-tissue injury	1.0 (0.0)	1.1 (0.3)	1.4 (0.8)*	242
Non-contact soft-tissue injury	1.5 (0.6)	1.3 (0.6)	1.5 (0.9)	220
Low back/sacrum/pelvis injury	1.2 (0.4)	1.3 (0.5)	1.3 (0.6)	50
Knee injury	1.1 (0.4)	1.0 (0.5)	1.1 (0.2)	79
Ankle injury	1.2 (0.4)	1.0 (0.0)	1.3 (0.6)	55
Thigh muscle injury	1.0 (0.0)	1.0 (0.0)	1.4 (0.8)	93
Hamstring muscle injury	1.0 (0.0)	- (-)	1.4 (0.9)	46

* Significantly higher than pre-PHV ($p < 0.05$).

Table 4.3. Number of days absent per injured player for each category according to maturation group (pre-, mid- and post-PHV), expressed as mean and standard deviation (SD).

Injury category	Pre-PHV	Mid-PHV	Post-PHV	
	Mean (SD)	Mean (SD)	Mean (SD)	Total Number of Days
Injury	29.1 (31.9)	42.9 (38.3)	45.9 (52.2)*	10,178
Non-contact injury	24.9 (21.2)	39.3 (30.4)	43.5 (52.5)	6.304
Muscle injury	17.6 (19.1)	34.7 (36.4)	21.3 (22.0)	2,097
Ligament/tendon injury	16.5 (18.1)	30.5 (22.7)	45.8 (65.9)	3,647
Growth-related injuries	28.0 (37.3)	21.5 (14.1)	10.3 (4.0)	1,935
Soft-tissue injury	16.9 (18.1)	23.5 (23.1)	35.0 (48.6)	5,931
Non-contact soft-tissue injury	25.2 (20.7)	37.3 (30.0)	40.0 (49.5)	5,366
Low back/sacrum/pelvis injury	21.3 (19.8)	66.8 (28.6)	46.9 (56.8)	1,642
Knee injury	26.5 (24.2)	18.6 (24.8)	51.1 (76.5)	3,154
Ankle injury	13.8 (10.2)	31.0 (10.9)	37.1 (47.9)	1,529
Thigh muscle injury	25.8 (21.3)	13.0 (5.6)	24.5 (24.5)	1,688
Hamstring muscle injury	25.8 (21.3)	- (-)	25.4 (20.5)	885

* Significantly higher than pre-PHV ($p < 0.05$). **Table 4.4.** Injury-burden score (sum of severity classification scores) according to maturation group (pre-, mid- and post-PHV), expressed as mean and standard deviation (SD).

Injury category	Pre-PHV	Mid-PHV	Post-PHV
	Mean (SD)	Mean (SD)	Mean (SD)
Injury	4.2 (2.2)	4.4 (2.4)	4.9 (2.9)
Non-contact injury	4.1 (2.1)	4.1 (1.9)	4.5 (2.5)
Muscle injury	2.5 (1.1)	3.3 (0.8)	3.6 (2.0)
Ligament/tendon injury	2.7 (0.7)	4.3 (1.9)	3.6 (1.6)*
Growth-related injury	3.5 (1.5)	3.8 (1.5)	2.8 (0.5)
Soft-tissue injury	2.8 (0.8)	3.2 (1.8)	4.0 (2.2)*
Non-contact soft-tissue injury	4.5 (2.7)	4.2 (1.9)	4.4 (2.3)
Low back/sacrum/pelvis injury	3.3 (1.4)	4.8 (1.7)	3.9 (2.0)
Knee injury	3.5 (1.3)	2.9 (1.7)	3.5 (0.9)
Ankle injury	3.0 (1.9)	3.8 (0.5)	3.9 (2.3)
Thigh muscle injury	3.2 (0.8)	3.0 (0.0)	3.7 (2.1)
Hamstring muscle injury	3.2 (0.8)	- (-)	3.9 (2.3)

* Significantly higher than pre-PHV ($p < 0.05$).

4.4 DISCUSSION

The aim of the present study was to investigate whether the frequency and/or severity of injuries in high-level YSP was dependent on maturation status. Contrary to the original hypothesis, a key finding was that when all injuries were analysed collectively, injured post-PHV players missed more days of the season than injured pre-PHV players (45.9 days *vs* 29.1 days). When specific injury categories were analysed, relatively more post-PHV players suffered at least one soft-tissue injury, at least one muscle injury, at least one ligament/tendon injury or at least one thigh-muscle injury in a season compared to pre-PHV players. Additionally, relatively more post-PHV players suffered soft-tissue injuries, ligament/tendon injuries and thigh-muscle injuries than mid-PHV players. Injured post-PHV players suffered more soft-tissue

injuries during the season than injured pre-PHV players, and post-PHV players had higher injury-burden scores for soft-tissue injuries and ligament/tendon injuries than pre-PHV players. Although there were no maturity-dependent differences between *injured* players regarding the number of growth-related injuries or days absent per player, the proportion of *total* players with one or more growth-related injury was lower in post-PHV compared to pre- and mid-PHV players. Together, these findings demonstrate that injury risk and absence is greater in post-PHV players, suggesting injury prevention strategies are more applicable to this sub group of high-level YSP.

There are a number of factors with the potential to explain the findings of this study and importantly, many such factors are likely to be linked to one another. Post-PHV (i.e. more physically mature) players are likely to have played soccer for longer, thus accumulating more training and match exposure, and may also undertake greater acute volumes of training and matches (Read et al., 2018b). In higher standards of soccer, greater risk-taking behaviours are linked to external influences such as increased competition and reward (Keller, Noyes and Buncher, 1988), which may affect the risk of certain injuries amongst post-PHV players. Furthermore, larger body masses and faster play are observed in older players (Orchard, 2001; Arnason et al., 2004a) and might contribute to the higher frequency of muscle, ligament/tendon and thigh-muscle injuries in post-PHV players compared to pre-PHV and mid-PHV players. The findings of the present study are supported by the documented rise in the frequency of youth soccer injuries with chronological age (Inklaar, 1994; Price et al., 2004), and the increased frequency of muscle and thigh muscle injuries in older players (Hawkins et al.,

2001; Ekstrand, Hägglund and Waldén, 2011; Cloke et al., 2012). An overall increase in injury rate was observed from pre-, to mid-, to post-PHV groups and accordingly, these findings contest suggestions, and reject the initial hypothesis, that mid-PHV players are at greatest risk of injury (Van der Sluis et al., 2014). Instead, these findings demonstrate a greater frequency of common soccer injuries in post-PHV players compared to pre- and mid-PHV players, with evidence that injured post-PHV players miss more days of the season than injured pre-PHV when considering all injuries together. It is likely that a combination of differences between pre- and post-PHV players influence the variance in injury frequency and severity between groups more than the impact of a single factor.

Previous injury is a notable risk factor for soccer injury (Carling, Orhant and LeGall, 2010) and it is possible that post-PHV players had suffered previous injuries during years of soccer activity prior to participating in the present study. Importantly, recurrent injuries often result in absence periods that are longer than the original injury (Ekstrand, Hägglund and Waldén, 2011), which might explain why post-PHV players were absent for more of the season and had a greater frequency of soft-tissue injuries than pre-PHV players. It is not known why recurrent injuries often incur longer absence periods than original injuries in soccer, though it is possible that aggressive rehabilitation, extensive scar tissue formation, or underestimation of the original injury are contributory factors (Croisier, 2004). Post-PHV players suffered more soft-tissue injuries than pre-PHV in the present study, which was reflected by a greater injury-burden score. This suggests that post-PHV players are more likely to suffer numerous soft-tissue injuries during a season. Those injuries

could be related to differences in the nature of play (Arnason et al., 2004a) or the increased quantity and regularity of soccer activity compared to younger players (Le Gall et al., 2006; Read et al., 2018b). Further research is required to determine other factors that may influence the occurrence of soft-tissue injuries, and why these injuries may be more severe in biologically mature players.

The findings of this study are supported by previous research that has identified muscle and ligament injuries as the most prevalent types of injury in YSP, with a considerable proportion of muscle injuries to the thigh (Price et al., 2004; Renshaw and Goodwin, 2016). In the present study, ligament and tendon injuries were combined due to relatively few injuries involving each tissue type, and because of similarities in tissue structure and injury aetiology (Tozer and Duprez, 2005; Solomonow, 2009). Nevertheless, the contribution of ligament and tendon injuries is similar to previous literature in youth (Price et al., 2004) and professional players (Ekstrand, Hägglund and Waldén, 2009). The risk of recurrent injuries to ligaments and tendons is high as a consequence of the fact that, once injured, these collagenous tissues are unlikely to ever fully restore their original properties (Yeung et al., 1994; Tozer and Duprez, 2005). This may explain why relatively more post-PHV players suffered ligament/tendon injuries than pre- and mid-PHV players, and may contribute to the greater injury burden from ligament/tendon injuries amongst post-PHV players. Poor recovery from previous injury might also contribute to relatively more post-PHV players suffering muscle injuries than pre-PHV players. As well as the possible contribution of recurrent injuries in soft-tissues, it is possible that a greater frequency of training and matches in chronologically

older players (Le Gall et al., 2006) influences the occurrence of muscle and ligament/tendon injuries in post-PHV players. Furthermore, ligaments and tendons have slow healing rates, and the fact that chronic inflammation leads to weakness and functional impairment and arises from insufficient rest and recovery (Yeung et al., 1994; Tozer and Duprez, 2005; Solomonow, 2009) leads us to suggest that inadequate recovery from soccer activity might contribute to the relatively high prevalence of muscle and ligament/tendon injuries amongst post-PHV players.

This study is the first to demonstrate the influence of maturation status on injury risk in a cohort of high-level YSP from multiple academies in several countries. Previously, two studies tracked YSP to quantify whether the timing of PHV affects injury rates (Van der Sluis et al., 2015) and if the number of traumatic and overuse injuries differs as players progress through PHV (Van der Sluis et al., 2014). Traumatic injuries were more evident during PHV, and players who matured at a 'late' chronological age were at greater risk of injury than those classed as 'early' or 'normal', whilst others observed differences in the location, type and severity of injury according to skeletal age of U14 players (Le Gall, Carling and Reilly, 2007). Comparison with the present study is limited by differences in sample size, methodology and injury reporting, however, this study is the first to assess the association of maturation status on injury risk across a broad range of age groups in a large cohort of high-level YSP from multiple academies in different countries. Furthermore, previous studies have reported injury according to chronological age group, and have not discussed the potential for skeletal maturity to affect injury around the ages of PHV (Price et al., 2004; Deehan, Bell and McCaskie, 2007;

Renshaw and Goodwin, 2016; Read et al., 2018b). Our evidence that post-PHV players suffer fewer growth-related injuries than pre- and mid-PHV is likely to reflect the fact that chronologically older players have surpassed peak somatic change, and are therefore less likely to experience injuries intrinsically linked to growth. The findings of this study concerning the prevalence of injuries between maturation groups, and that injured post-PHV players had significantly higher injury-burden scores for soft-tissue and ligament/tendon injuries than less mature players, suggest that post-PHV players have a greater risk of experiencing these injuries over the course of one season. Moreover, the finding that the days missed through injuries of any description was greatest in post-PHV players merits further investigation to elucidate if injuries are more severe in older YSP and, if so, what the reasons are for this. While senior players probably benefit from greater medical support and might suffer fewer severe injuries when defined by days of absence, it is possible that older players are encouraged to return to play quicker due to their importance to their team, or through their desire to resume playing (Loose et al., 2018). In this instance, returning to activity following inadequate recovery may further increase the risk of re-injury, particularly to ligaments and tendons (Gajhede-Knudsen et al., 2013). As data were analysed from a single season, it is not possible to define the number of injuries linked to past injuries in earlier seasons. Accordingly, longitudinal research to track a sufficient number of adolescent players from pre- to post-PHV recording all injuries would be advantageous.

Despite previous authors demonstrating an elevated injury risk during PHV (Van der Sluis et al., 2014), the findings of this study suggest that post-

PHV, not mid-PHV, soccer players suffer more soft tissue injuries during a single season. It appears that these injuries are more likely to be to muscle tissue than ligament or tendon, with the thigh a common location. Whilst previous authors followed players before, during and after PHV and observed differences in injury occurrence at different stages, the tissue types and locations of those injuries were not clear (Van der Sluis et al., 2014). The present study includes considerably more players and provides details of specific injury categories, which adds to the current understanding of injuries in pre-, mid- and post-PHV players. In addition, the present study includes players from several clubs in different countries and across two continents, which strengthens the external validity of the results. Based on these novel observations, it is suggested that the influence of maturation status on injury frequency and severity is dependent on both the type and location of injury.

There are some limitations relating to the current study. Firstly no maturation-related differences were evident for the majority of injury variables studied and this may relate to the low number of recorded injuries in some categories, which became further reduced when separating players by maturation status. Also, this study used an estimation of PHV as opposed to a direct measurement of pubertal status. Whilst with limitations, this validated (Mirwald et al., 2002; Malina and Kozieł, 2014) and widely used (Van der Sluis et al., 2014; Van der Sluis et al., 2015) method aided the measurement of a large sample of players and was favourable considering the invasive nature of other procedures (Malina, Bouchard and Bar-Or, 2004; Johnson, Farooq and Whiteley, 2017). It is acknowledged that differences in the point of the season where anthropometric measures were collected from players from different

clubs and/or countries could increase the chances of player and/or injury misclassification. For example, players who had maturity offset calculated in the pre-season may be pre-PHV at this point but may develop to be mid-PHV during in the season. Accordingly, future studies of this scale should seek to perform repeated measures throughout the season to detect changes in the maturation of individual players. The ability to link injuries to previous injuries would assist in the understanding of their aetiology, and it is acknowledged that this requires analysis of several consecutive seasons and is a limitation of this study. Finally, the lack of exposure records, and therefore detailed injury incidence data, in this investigation limits context regarding the quantity of soccer activity undertaken by players and restricts comparison with certain studies. However even when available, exposure data does not describe the nature or intensity of the activity, which limits comparisons between soccer academies.

4.5 CONCLUSION

The novel findings of the present study demonstrate that injured post-PHV players missed more than two weeks more of the soccer season on average than pre-PHV players, and that relatively more post-PHV players suffer soft-tissue injuries, (thigh) muscle injuries, ligament/tendon injuries and thigh (muscle) injuries compared to pre- and mid-PHV players. Thus, it is suggested that specific prevention strategies should target these injury types according to the maturation status of the player. The data in the present study are from a larger and more diverse cohort than any previous study of this kind and provide important and relevant information concerning the types and locations

of soccer injuries in high-level YSP at different stages of maturation. Whilst demonstrating that the intrinsic influence of maturation status is associated with a greater prevalence and severity of injury in post-PHV YSP, this is likely to involve an interaction with extrinsic factors, such as the demands imposed upon players by different playing actions. These actions are likely to contribute to injury-inciting events, where susceptible players encounter scenarios that potentially lead to injury. For example, playing actions such as sprints, high speed running, tackling, jumping and landing may be more intense in post-PHV players due to increased competition and match speed. It follows that any factor influencing the frequency of these actions in YSP may indirectly affect the risk of injury. Whilst there are a number of factors with the potential to influence player activity during competition, such as opposition behaviour, one factor suggested as a key determinant of match activity in YSP is playing position. Accordingly, investigating whether there is an association between playing position and injury in YSP may provide greater context to the findings of this chapter.

CHAPTER FIVE

INVESTIGATING THE ASSOCIATION BETWEEN PLAYING POSITION AND INJURY RISK IN YOUTH SOCCER

PRELUDE

Chapter Five sought to investigate whether the on-field playing position occupied by YSP was associated with the type, location or severity of injuries sustained. Following the observations of Chapter Four, where it was evident that there are differences between players based on their maturity status, it was deemed appropriate to investigate only post-PHV players within this chapter. This decision was also informed by the fact that younger players often play shorter matches on smaller pitches and can occupy several playing positions before settling on one position at older ages. Based on existing literature describing the match activities of each playing position, players were grouped by their playing position to investigate the potential association with injury in YSP.

STUDY 3: ABSTRACT

Background: Different tactical and physiological requirements of soccer playing positions mean that certain positions demand a greater frequency of actions considered to increase injury risk (e.g. sprinting, jumping/landing), although it is unclear whether this is the case in youth soccer. The aim of this study was therefore to determine if playing position was associated with injury risk in high-level youth soccer players (YSP). **Methods:** This study investigated 404 male YSP from the Under 14 (U14) to U23 age groups from six academies in England, Spain, Uruguay and Brazil. Using anthropometry, the maturation status of all YSP was defined as 'post-peak height velocity'. Based on their main playing position, YSP were categorised as either goalkeepers, central players, lateral players or forwards. Injury frequency, type, location and severity (days missed and injury-severity score) sustained during training and matches were prospectively recorded over one season and analysed according to playing position. **Results:** Relatively more lateral and forward players combined suffered thigh injuries than goalkeepers (18.8% *vs.* 2.9%, p = 0.046). However, no differences existed between any playing position in injury frequency, days missed per injury or in injury-severity score for any other injury category. **Conclusion:** Post-peak height velocity YSP in lateral and forward positions have a greater risk of suffering thigh injuries than goalkeepers. This is probably due to the greater distances covered at high speeds in lateral/forward positions compared to all other positions. Injury prevention strategies in this population should, therefore, pay particular attention to the thigh in players of lateral/forward positions.

5.1 INTRODUCTION

A standard soccer team comprises eleven players occupying different playing positions, which generally reflect their location on the pitch and different tactical roles assumed during matches. Variation within the tactical and physiological requirements of playing positions means that players often display differences in stature and in their ability to perform specific skills to meet the tactical demands of their role (Bloomfield, Polman and O'Donoghue, 2007). During the development of YSP, possessing specific skills or physical qualities may lead to players being selected to occupy certain playing positions (Towlson et al., 2017). Goalkeepers have a unique playing position, which permits them to use their hands to control the ball within the penalty box and a team can only have one goalkeeper on the field of play. In addition, goalkeepers perform the greatest proportion of low-intensity activity compared to other positions (Clemente et al., 2013). The general activity profile of the remaining ten outfield players is distinctly different to goalkeepers and involves more running, ball possession and high-intensity activity. However, between outfield positions there are differences in the amount of distance covered, the variety of playing actions and the proportion of playing time spent standing, walking, running or sprinting (Bangsbo, 1993; Reilly, 1997). These differences also contribute to positional variance in the physical demands experienced by outfield players (Abbott, Brickley and Smeeton, 2018), meaning some players may experience different match intensities and fatigue. Knowledge of whether playing position, and specifically the demands of different outfield playing positions, influences soccer injury could assist the training and management of YSP.

Playing position has been linked to injury in other team sports such as American football (McCunn et al., 2017) and both rugby codes (Gabbett, 2005; Brooks and Kemp, 2010). Whilst the collision-based nature of these sports accounted for much of the position-based injury variance in contact injuries, positions performing greater quantities of sprinting and high-speed running were linked to a greater number of non-contact thigh and hamstring injuries in the rugby union cohort (Brooks and Kemp, 2010). In soccer, there is evidence of differences in the type and frequency of match actions performed between playing positions in professional and YSP (Bloomfield, Polman and O'Donoghue, 2007; Di Salvo et al., 2007; Al Haddad et al., 2015). Some playing demands, such as high speed running, induce fatigue (Nedelec et al., 2014) and muscle damage (Byrne, Twist and Eston, 2004) and may affect the risk of non-contact injury to players in certain playing positions (Dupont et al., 2010). Similarly, players in positions involved in a greater quantity of tackles might be at higher risk of contact injury, whilst those who jump and land more often may have a greater risk of injury to the ligaments of the ankle or knee (Ekstrand et al., 1983; Alentorn-Geli et al., 2009). Accordingly, the quantity, intensity and duration of different playing actions may explain the positional differences in injury occurrence reported in some studies of professional players (Reilly, 1997; Buchheit et al., 2010; Carling, Orhant and LeGall, 2010; Leventer et al., 2016). However, other studies concerning injury and playing position in professional players report equivocal findings (Dauty and Collon, 2011; Mallo and Dellal, 2012) and the literature in YSP is limited (Price et al., 2004; Cloke et al., 2011).

Recently, the way in which outfield players are grouped into different playing positions has been suggested to influence whether differences in playing actions are observed in YSP (Towlson et al., 2017). For example, solely grouping defenders overlooks the fact that central and wide defenders perform different quantities of sprints (Withers et al., 1982; Bloomfield, Polman and O'Donoghue, 2007), and grouping midfielders together does not account for the differences between wide and central midfielders in the distances run at low and high speeds (Mendez-Villanueva et al., 2013). Furthermore, wide players are known to perform more accelerations and decelerations than central players (Vigh-Larsen et al., 2018), which has implications for fatigue, acute muscle damage and recovery (Howatson and Milak, 2009; Nedelec et al., 2014). Consequently, grouping players as 'lateral' and 'central' appears to be more accurate in reflecting the distinct activity profiles of those positions and may be better suited to detecting any differences in the injuries suffered by players occupying different outfield positions (Towlson et al., 2017). Literature describing the different match activities of YSP also reports that high-speed running and sprint activities in forwards and lateral players are similar (Buchheit et al., 2010; Saward et al., 2015), suggesting these positions may also experience similar frequencies and/or types of injuries. Understanding whether the playing position of YSP influences injury could help the identification of 'at-risk' athletes and assist strategies aimed at reducing the frequency and quantity of time lost to injury.

The aim of the present study was to investigate if playing position was associated with the prevalence (proportion of injured players), frequency and severity of common injury types/locations in a large cohort of high-level YSP

over the course of a single season. Outfield players were grouped to reflect their activity profiles in order to address the hypothesis that players occupying lateral and forward positions, typically associated with more high-intensity activities such as high-speed running and sprints, would exhibit a greater proportion of injuries compared to centrally-positioned players. Finally, it was hypothesised that goalkeepers would suffer fewer injuries than outfield players due to their unique activity profile involving fewer high-intensity actions.

5.2 METHODOLOGY

5.2.1 Participants and study design

The present study used a prospective cohort design to report soccer injuries in 404 high-level male YSP (age: 17.5 ± 2.0 years, height: 1.78 ± 0.08 m, body mass: 71.8 ± 8.8 kg) who were registered with one of eight professional soccer clubs previously described in Chapter Three. As maturation status is associated with injury risk (Chapter Four) and YSP do not typically compete 11 *vs.* 11 on a full-sized pitch until they reach ~13 years of age (Carling et al., 2009), only YSP classified as post-peak height velocity (PHV) were included in this study. The method for estimating maturation status has been described in Chapter Four. The present study included one season's injury record per player, comprising 163 player records for the 2014-15 season, 17 for the 2016-17 season, and 224 for the 2017-18 season. All players participated in regular soccer training and competitive match-play, which was in accordance with the Premier League's EPPP for the English clubs. Written informed consent was obtained from club officials and players, with parental consent and player assent collected for all participants less than 16 years of age. The study

received approval from Liverpool John Moores University Research Ethics Committee.

5.2.2 Playing position

Playing position was self-recorded by each player via questionnaire. Subsequently, players were grouped as goalkeepers (GK, *n*=35), central players (CENT, *n*=176), and lateral and forward players (LAT/FWD, *n*=193). Central players included central defenders and central midfielders, while lateral players included wide defenders and wide midfielders. These groups were adopted to reflect previous literature describing differences in match activity between central and lateral playing positions (Di Salvo et al., 2007; Towlson et al., 2017).

5.2.3 Injury recording and definitions

The methodology adopted for the recording and definition of injuries in this study has been previously described in Chapter Three (see Table 3.2). The injury-burden score represented the sum of the severity classifications allocated to each separate injury per player based on the previously described severity classification system (Fuller et al., 2006). Injury rates were calculated for each playing position group by dividing the total number of injuries by the number of players (Price et al., 2004; Read et al., 2018b).

2.4 Statistical and data analysis

Injuries were categorised and analysed according to those most frequently recorded in Chapter Three. For each category, players were grouped

according to whether they had suffered one or more injury, or no injury, with the Chi-Squared (χ^2) test of independence used to assess whether the proportion of injured and uninjured players for each injury category was independent of playing position. For those players, who had suffered at least one injury for each injury category, between playing position group differences in injury frequency and severity (i.e. days absent and injury-burden score) were analysed using a Kruskal-Wallis H test of variance (due to the non-normal distribution of injury data). Statistical significance was accepted at $p < 0.05$, with Bonferroni adjustment applied to post-hoc pairwise comparisons. All statistical analyses were performed using SPSS version 25.0 (Chicago, Illinois)

5.3 RESULTS

5.3.1 Total injuries and injury rate

A total of 288 injuries were recorded during the season, contributing to an injury rate of 0.71 injuries per player. The absence per injury category ranged from 1 to 303 days with a mean of 27.2 days across the cohort. A total of 30 injuries were suffered by GK, 124 injuries by CENT, and 134 by LAT/FWD players, providing injury rates of 0.86, 0.70 and 0.69 injuries per player, respectively. The majority of injuries were classified as being moderate (43.2%) or severe (29.6%). More than half (60.4%) were non-contact, whilst, 21.5% were contact injuries and the remaining 18.1% were not specificied as being contact or non-contact. Training injuries accounted for slightly more than match injuries (51.8% *vs.* 42.5%), with 5.7% occurring during unspecified soccer activity. More injuries were traumatic (48.6%) than overuse (23.4%),

with the cause of the remaining 28.0% of injuries unspecified. Lower limb injuries were highly prevalent (79.6%), with the thigh, knee and ankle being the most common locations (Fig. 5.1).

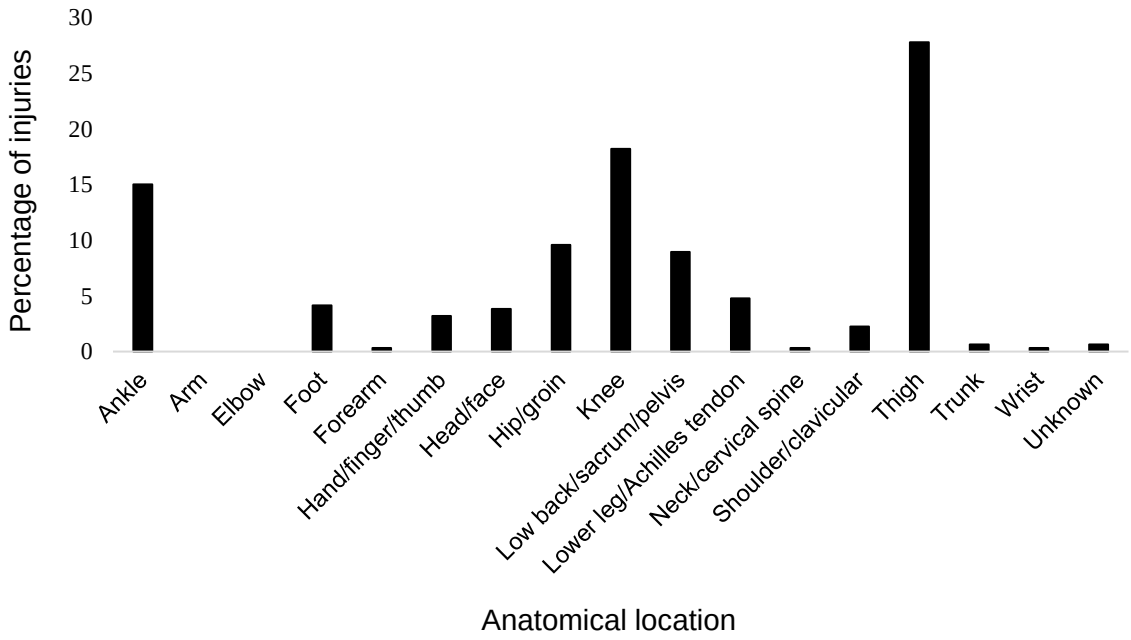

Figure 5.1. Distribution of all recorded injuries based on anatomical location

5.3.2 Injured vs. uninjured players

There was a difference in the proportion of players who suffered one or more thigh injury between playing positions, with relatively more LAT/FWD suffering injuries than GK (18.8% *vs* 2.9%, OR = 7.8), $\chi^2 = 6.04$, $p = 0.046$ (Fig. 5.2). To demonstrate that this study was statistically powered, we performed a power analysis calculation based on these data, which demonstrated a power of 81.2%. No other differences were evident between playing position groups for any other injury category.

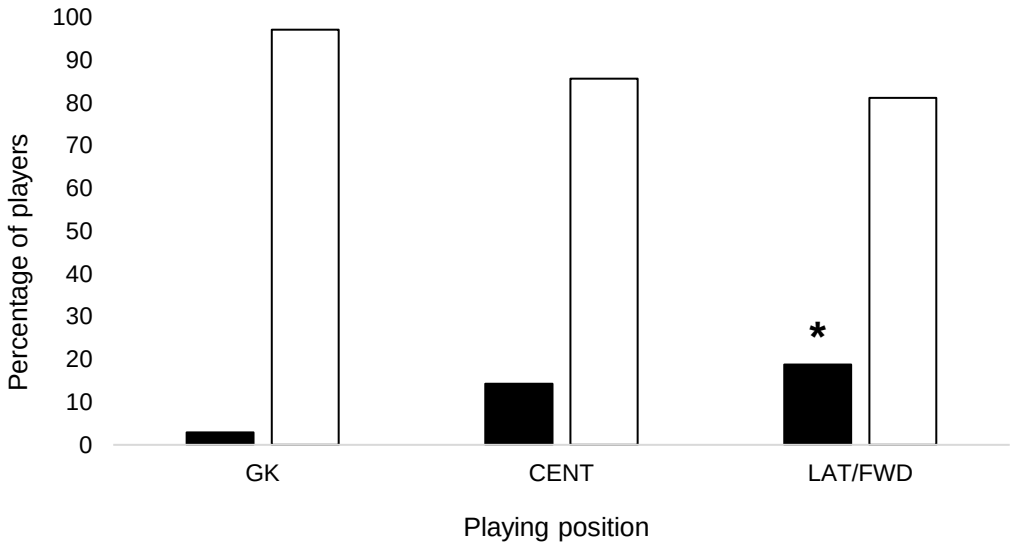

Figure 5.2. Percentage of players with (black bars) and without (white bars) one or more thigh injury according to playing position. *Greater than GK ($p < 0.05$).

5.3.3 Number of injuries, days missed and injury severity

There were no differences between playing position groups regarding the number of injuries suffered by injured players in any injury category (Table 5.1). No differences were detected between playing positions regarding the number of days missed through injuries within any injury category (Table 5.2). Finally, no differences were observed between playing positions regarding injury-burden scores from any injury category (Table 5.3).

Table 5.1. Number of injuries per injured player for each injury category according to playing position. Data are expressed as mean and standard deviation (SD).

Injury category	GK	CENT	LAT/FWD	
	Mean (SD)	Mean (SD)	Mean (SD)	Total number of injuries
Injury	1.9 (0.8)	1.7 (1.1)	1.7 (1.1)	288
Non-contact injury	1.8 (0.9)	1.5 (0.9)	1.6 (1.0)	174
Overuse injury	0.3 (0.0)	0.2 (0.1)	0.3 (0.1)	67
Muscle injury	1.2 (0.4)	1.4 (0.6)	1.3 (0.7)	107
Ligament/tendon injury	1.6 (0.9)	1.2 (0.5)	1.1 (0.3)	72
Soft-tissue injury	1.4 (0.7)	1.4 (0.8)	1.3 (0.7)	179
Non-contact soft-tissue injury	1.8 (0.9)	1.5 (1.0)	1.5 (0.9)	171
Low back/sacrum/pelvis injury	- (-)	1.4 (0.7)	1.2 (0.4)	28
Knee injury	1.2 (0.4)	1.0 (0.2)	1.0 (0.2)	54
Ankle injury	- (-)	1.5 (0.9)	1.1 (0.3)	45
Thigh muscle injury	- (-)	1.4 (0.8)	1.4 (0.8)	85
Hamstring muscle injury	- (-)	1.5 (0.7)	1.3 (0.9)	40

GK, goalkeepers; CENT, central players; LAT/FWD, lateral/forward players

Table 5.2. Number of days absent per injured player for each injury category according to playing position. Data are expressed as mean and standard deviation (SD).

Injury category	GK	CENT	LAT/FWD	
	Mean (SD)	Mean (SD)	Mean (SD)	Total Number of Days
Injury	41.8 (40.6)	43.5 (49.9)	47.2 (56.5)	7,828
Non-contact injury	46.0 (47.6)	41.4 (54.1)	44.8 (52.9)	4,830
Overuse injury	23.4 (19.1)	25.5 (21.4)	25.8 (16.8)	2,417
Muscle injury	12.8 (7.9)	17.3 (12.8)	25.0 (26.9)	1,748
Ligament/tendon injury	28.6 (27.5)	45.6 (66.8)	27.5 (70.0)	3,294
Soft-tissue injury	20.7 (20.8)	35.5 (52.8)	36.6 (48.0)	4,759
Non-contact soft-tissue injury	45.0 (46.7)	35.6 (46.4)	42.4 (52.9)	4,477
Low back/sacrum/pelvis injury	- (-)	67.3 (77.1)	31.5 (32.4)	985
Knee injury	29.8 (25.1)	47.3 (77.1)	60.1 (84.3)	2,608
Ankle injury	- (-)	44.8 (28.4)	33.4 (58.1)	1,336
Thigh muscle injury	- (-)	20.6 (17.1)	27.5 (28.6)	1,520
Hamstring muscle injury	- (-)	23.5 (15.8)	26.4 (23.0)	737

GK, goalkeepers; CENT, central players; LAT/FWD, lateral/forward players

Table 5.3. Injury-burden score (sum of severity classification scores) per injury category according to playing position. Data are expressed as mean and standard deviation (SD).

Injury category	GK	CENT	LAT/FWD
	Mean (SD)	Mean (SD)	Mean (SD)
Injury	5.4 (2.8)	4.8 (2.6)	4.8 (3.2)
Non-contact injury	5.4 (3.3)	4.2 (3.3)	4.6 (2.5)
Overuse injury	3.9 (2.9)	4.1 (3.2)	4.1 (4.0)
Muscle injury	3.2 (1.6)	3.6 (1.7)	3.6 (2.3)
Ligament/tendon injury	4.4 (2.9)	3.6 (1.8)	3.5 (1.2)
Soft-tissue injury	3.8 (2.3)	4.0 (2.0)	4.0 (2.4)
Non-contact soft-tissue injury	5.4 (3.3)	4.1 (2.0)	4.3 (2.3)
Low back/sacrum/pelvis injury	- (-)	4.2 (2.5)	3.4 (1.5)
Knee injury	3.8 (1.9)	3.4 (0.8)	3.5 (0.7)
Ankle injury	- (-)	4.9 (3.1)	3.3 (1.4)
Thigh muscle injury	- (-)	3.6 (2.1)	3.7 (2.2)
Hamstring muscle injury	- (-)	4.2 (2.3)	3.7 (2.4)

GK, goalkeepers; CENT, central players; LAT/FWD, lateral/forward players

5.4 DISCUSSION

The aim of this study was to investigate whether playing position was associated with injury risk in a large cohort of high-level, male, post-PHV YSP. This is the first study to investigate the influence of playing position in YSP from multiple nations, thereby providing valuable information from a larger and more diverse cohort of high-level YSP than previously studied. The main findings were that proportionately more lateral and forward players suffered thigh injuries (inclusive of quadriceps and hamstrings) compared to goalkeepers, but that the frequency and severity of injuries did not differ between playing positions in any injury category. The lack of difference in injury risk between outfield positions contradicts the primary hypothesis, whilst the secondary hypothesis that injury risk would be lower in goalkeepers compared to outfield players is partially supported by the observed findings.

These data demonstrate that a greater proportion of high-level YSP occupying forward and lateral positions, such as wide defenders and wide midfielders, suffer injuries to the upper leg compared to goalkeepers. Accordingly, similar injury prevention strategies should be adopted for all outfield players, and separate injury prevention training should be prescribed for goalkeepers.

Current data are similar to the conclusions of Hägglund *et al.*, where fewer lower-limb muscle injuries were evident in professional goalkeepers, with no difference between outfield positions (Hägglund, Waldén and Ekstrand, 2013). Goalkeeper is a unique playing position with distinct competitive demands. For example, professional goalkeepers cover ~50% less distance than outfield players during a match and perform the least high intensity activity compared to other playing positions (Clemente et al., 2013; White et al., 2018). This implies that goalkeepers are less likely to be involved in as many crucial runs, sprints or changes in direction, and are less likely to experience neuromuscular fatigue, as supported by similar performance levels in both halves of the match (Di Salvo et al., 2008). Furthermore, lateral and forward players perform more sprints (Buchheit et al., 2010) that involve high-intensity eccentric contractions of the hamstrings (e.g. during the late swing phase (Opar, Williams and Shield, 2012)) and the quadriceps (e.g. during rapid decelerations following sprints or short pressing actions). These actions are thought to cause muscle damage (Howatson and Milak, 2009), which together with insufficient recovery, could increase the susceptibility to muscle strain injuries. It is also likely that lateral and forward positioned players perform a greater quantity of pressing actions when attempting to win the ball back from the opposition, and that these actions involve considerable eccentric loading,

potentially increasing the risk of injury to the quadriceps. Despite observing fewer thigh injuries in goalkeepers compared to lateral and forward outfield players, the frequency and severity of any injuries amongst the injured players was not influenced by their playing position. This suggests that whilst the occurrence of thigh injuries is influenced by playing position, the recovery time for any player who suffers a thigh injury is similar, regardless of their playing position.

This study focussed solely on post-PHV players to remove the influence of maturation status. This was based on the findings of Chapter Four where it was demonstrated that injury prevalence and severity differed between pre-, mid- and post-PHV players. Whilst eliminating the variability in injuries attributed to biological maturation, it is not possible to extend the external validity of these findings to pre- and mid-PHV YSP. Nevertheless, some younger age groups play with fewer players on the field than older age groups (Moore et al., 2011), which limits comparison of playing positions between older and younger players. This is the first study to include players from eight different academies in four different countries across two continents, thus increasing the external validity of the present findings compared to previous investigations where playing position is considered, which typically involve players from a single club (Deehan, Bell and McCaskie, 2007; Cloke et al., 2012). In previous studies of similarly aged participants, English academy midfield players had significantly more, and defenders and goalkeepers significantly fewer, thigh muscle injuries than would be expected by chance (Cloke et al., 2012). Another investigation reported that midfield players had the greatest risk of musculoskeletal injury relative to goalkeepers, who had

fewer injuries, in an English youth academy where all other positions exhibited similar likelihood of injury (Deehan, Bell and McCaskie, 2007). Previous investigations in professional soccer players reach equivocal conclusions, with some reporting differences in injury frequency between outfield playing positions (Ryynänen et al., 2013; Leventer et al., 2016) and others describing no difference (Dauty and Collon, 2011; Hägglund, Waldén and Ekstrand, 2013).

The conflict between the findings of ours and previous studies may be explained by the fact that some studies group players as central or lateral; and others as defensive, midfield or forward players. The latter could be considered as the 'traditional' method of grouping players, predating the literature describing differences between wide and central defenders, and wide and central midfielders (Buchheit et al., 2010; Dellal et al., 2010). Despite using a similar method of grouping playing positions (separating lateral and central players) to Tourny *et al.* (Tourny et al., 2014), who observed differences in injury risk between playing positions in YSP, the present study found no direct differences in injury risk between outfield positions. Whilst previous studies investigated players ranging from age 9 to 20 years (Deehan, Bell and McCaskie, 2007; Cloke et al., 2012; Tourny et al., 2014), the present study included only post-PHV players, thus eliminating chronologically younger players. The rationale to divide outfield players as lateral, central and forward players stems from their different match activities documented by previous authors (Bloomfield, Polman and O'Donoghue, 2007; Di Salvo et al., 2007; Al Haddad et al., 2015; Towlson et al., 2017). Nevertheless, differences in injuries between playing positions are also reported by studies which divide

outfield players as defensive, midfield and forward positions (Deehan, Bell and McCaskie, 2007; Cloke et al., 2012) as opposed to lateral and central. Such equivocal conclusions, regardless of the way in which player positions are grouped, lead us to suggest that factors other than playing position are more influential to injury risk in YSP.

It is possible that players who occupy the same playing position over many years are familiarised with the physiological demands of that position and adapt to the required activity profile. In these instances, other factors may influence injury occurrence, such as deviation from a player's typical positional role, should this significantly alter the type and/or quantity of activities performed. For example, moving a central defender to play as a lateral defender may drastically increase the quantity and distance of high speed runs performed (Buchheit et al., 2010), which could increase the risk of muscle strain injury (Ekstrand, Hägglund and Waldén, 2011). Player activity is also externally influenced by the behaviour and quality of opponents. Teams may adopt specific tactical roles or formations for different matches, which could increase the demands imposed on their opponents and elevate injury risk if players are exposed to intensities beyond their capacity. It is also important to consider that team formations can change from one game to another and within a single match, which will affect the demands imposed upon players seemingly occupying the same playing positions. For example, despite having different in-game demands to central players, wide defenders perform 20% more decelerations when utilised in a 3-5-2 formation compared to a 4-4-2 (Tierney et al., 2016). Such differences will likely increase eccentric loading and muscle damage and will probably affect recovery (Howatson and Milak,

2009; Nedelec et al., 2014), and potentially the likelihood of muscle injury. This example of within-position variance highlights a limitation of using playing position to assess injury risk, because the definition and utilisation of different playing positions may differ according to the tactical strategies of coaching staff. Coaching styles may also directly affect the loading demands experienced by players, such as when issuing instructions for certain positions to attack or to press their opponents. In such scenarios, which are likely to be influenced by external factors such as the score during a match or the importance of the match, external circumstances may exert a greater influence on player activity than their playing position. Accordingly, fluidity amongst outfield positions means defined playing positions are difficult to apply to all teams, players and matches, and challenges the reliability of playing position as a risk factor for injury. Due to the aforementioned limitations, there appears to be a complexity regarding the distinction of playing positions, which is further confounded by external factors such playing styles, opposition behaviour and match importance. Consequently, it is suggested that the differences in injuries observed between playing positions reported by some studies are likely to be underpinned by variance in the quantity and intensity of playing actions, which may be influenced by the dynamic nature of external and match-related factors as opposed to a direct influence of predefined playing positions.

It is pertinent to acknowledge the limitations of this study. Firstly, the inclusion of only post-PHV players limits the external validity of these findings in relation to pre- and mid-PHV YSP, meaning it is not clear whether similar results would be observed in those players. However, as explained in the

Methods section of this chapter, the main rationale for excluding pre- and mid-PHV players from these analyses was (i) that maturation status is associated with injury risk (Chapter Four) and (ii) YSP do not typically compete 11 *vs.* 11 on a full-sized pitch until they reach ~13 years of age (Carling et al., 2009), thus playing position is unlikely to be accurate until the player is post-PHV. Secondly, whilst hypothesising that the presence of position-based differences in injury prevalence and severity may relate to match/training intensity/volume (e.g. distance covered at high-speed), we acknowledge that this study does not provide information that quantifies either match activity or the training schedules of the analysed players, meaning this link remains speculative. Accordingly, future studies should seek to include players' match/training activity to investigate associations between these variables and injury risk. Finally, the lack of exposure records for each player limits context surrounding the quantity of soccer activity undertaken and whether this relates to injury based on playing position. However, exposure records do not typically account for differences in activity and intensity, meaning their use is not without limitation when comparing players from different clubs and countries.

5.5 CONCLUSION

The novel findings of this study in high-level, male, YSP demonstrate that lateral and forward players have a higher risk of thigh injuries than goalkeepers. This is probably due to the greater number of high-intensity actions (particularly rapid accelerations, sprints and decelerations) performed by lateral and forward players compared to other positions. The lack of difference in injury risk between outfield positions could be influenced by

external factors such as playing styles, opposition behaviour and the importance of individual matches. Nevertheless, this study is the first to investigate an association between playing position and injury risk in high-level YSP from multiple academies across four nations and two continents, thus increasing the external validity of these findings. However, the lack of association between playing position, which represents an environmental influence, suggests that there are other risk factors affecting the prevalence and severity of injury in YSP. Such factors could include differences in genetic make-up, which might lead to intrinsically weaker/stronger soft-tissue, thus predisposing certain players to particular types of injury independent of playing position and maturation status.

CHAPTER SIX

THE GENETIC ASSOCIATION WITH SOFT TISSUE INJURY RISK IN HIGH-LEVEL YOUTH SOCCER PLAYERS DEPENDS ON MATURATION STATUS

PRELUDE

As the final experimental chapter of this thesis, Chapter Six sought to investigate the association of genetic variants with injuries in YSP. Recent evidence in professional (adult) soccer players, and in non-athletic populations, suggests that genetic variants are associated with physiological characteristics and injuries to tissues such as muscles, ligaments and tendons. With these associations yet to be investigated in YSP, who could potentially lose valuable development time to such injuries, Chapter Six represents a key step toward understanding the genetic influence on injury prevalence and severity in YSP. Following the differences in injury prevalence and severity between maturity groups described within Chapter Four, and because of the small number of mid-PHV players in the cohort, only Pre- and Post-PHV players were included within Chapter Six.

STUDY 4: ABSTRACT

Background: The aetiology of injuries in youth soccer players (YSP) is poorly understood, however, evidence in professional players suggests genetic differences are important. This study investigated the individual and combined association of multiple single nucleotide polymorphisms (SNPs) with injury prevalence and severity in high-level YSP. **Methods:** Saliva samples and injury records were collected from 538 male YSP aged 9 to 23 years from high-level academies in England, Spain, Uruguay and Brazil. DNA was genotyped for nine SNPs using real-time PCR, and injury prevalence and severity were analysed according to genotype and maturation status, defined as years from/to predicted peak-height-velocity (PHV), i.e. pre- or post-PHV. **Results:** *EMILIN1, VEGFA* and *MMP3* SNPs were associated with general and non-contact, muscle, and ligament injury prevalence, respectively, in pre- and post-PHV combined. In pre-PHV alone, *IL6* and *MMP3* SNPs were associated with general and ligament injury prevalence, respectively, and in post-PHV alone, *EMILIN1* was associated with general, non-contact, soft-tissue and NCST injury prevalence, *VEGFA* was associated with non-contact, NCST and muscle injury prevalence, and *COL5A1* was associated with thigh and hamstring injury prevalence. *MYLK* and *MMP3* SNPs were associated with injury severity. A higher total genotype score was associated with greater non-contact and non-contact soft-tissue injury prevalence. **Conclusion:** This study is the first to demonstrate the association of multiple SNPs with injury risk in high-level YSP. This provides valuable insight into the inter-individual variation in injury frequency and absence in YSP, with the potential to aid the identification and management of ‘at-risk’ players.

6.1 INTRODUCTION

Soccer injuries negatively influence player availability and team success (Arnason et al., 2004a; Parry and Drust, 2006) and for YSP, injuries may affect the process of skill acquisition and athletic development (Price et al., 2004). Furthermore, previous injury represents a major risk factor, meaning that players who suffer injuries at younger ages increase their risk of subsequent injury (Brink et al., 2010). Accordingly, the prevention of injuries is an important consideration for soccer players of all ages, particularly YSP. Before prevention strategies are considered, the factors that influence injury risk must be identified and understood (Van Mechelen, Hlobil and Kemper, 1992). However, despite the fact that there are several known risk factors for soccer injury (Arnason et al., 2004b), inter-individual variation in the frequency and severity of injuries suggests not all factors are fully understood.

The risk of injury in soccer involves the interaction of intrinsic and extrinsic factors (Brink et al., 2010) relating to the individual player and their surrounding environment, respectively. From Chapter Two, it is known that these factors include previous injury, chronological age (intrinsic) and opponent behaviour (extrinsic). In Chapter Four, it was demonstrated that maturation status (biological age) was associated with soft tissue injury risk in YSP. Relatively more post-peak height velocity (PHV, the point at which children undergo their highest growth rate, which is associated with puberty) players suffered soft-tissue, muscle and ligament/tendon injuries than pre-PHV players, and the absence from injury in general was greater in post- compared to pre-PHV players. Genetic variation is an intrinsic factor known to be associated with physical attributes (phenotypes), such as aerobic capacity

(Bouchard et al., 1999) and the size and strength of skeletal muscle (Erskine et al., 2014). Thus, common genetic variants have the potential to influence the structure, function or expression of proteins within tissues, which can in turn affect the tissue phenotype (Lamb et al., 2006). Notably, there is evidence that the risk of soft-tissue injury is 40% heritable (Hakim et al., 2003), highlighting the potential extent to which genetic variation may influence the occurrence of injury. It therefore follows that in high-level YSP, where it is shown that skeletal muscle, ligament and tendon are amongst the most commonly injured tissues (Chapter Three)(Hawkins et al., 2001; Price et al., 2004), genetic differences between players have the potential to alter the mechanical properties of these tissues (Collins and Posthumus, 2011), thereby contributing to inter-individual differences in injury occurrence and severity (Collins, 2010). For example, SNPs have been shown to affect the regulation (Mann et al., 2001) and mRNA stability (Laguette et al., 2011) of collagen genes, which could influence ligament compliance, joint laxity and the risk of rupture under load (Bell et al., 2012). Recent studies report the association of certain genetic variants with musculoskeletal injury in professional (adult) soccer players (Ficek et al., 2013; Pruna et al., 2013; Massidda et al., 2015a; Massidda et al., 2015b; Massidda et al., 2015c; Artells et al., 2016; Pruna et al., 2016; Myosotis et al., 2017b; Larruskain et al., 2018), though most studies are limited by modest sample sizes. In addition to the fact that injury incidence involves a multifactorial interaction between intrinsic and extrinsic factors, any genetic influence on injury risk is likely to involve a number of SNPs as opposed to a single variant. However, no study has sought to investigate the genetic association with injuries in high-level YSP, or the

combined association of multiple SNPs on injury risk in YSP. Moreover, as injury risk in this population differs according to biological age (Chapter Four), it is crucial that any genetic association with injury risk be investigated according to maturation status.

The aim of the present study was to determine whether variations in genes encoding key proteins in the structure and/or function of musculoskeletal tissues were associated with injury risk in high-level YSP from four nations. Nine genetic variants were selected based on previous associations with musculoskeletal injury, and it was hypothesised that high-risk genotypes of each variant would be associated with greater injury prevalence and severity in YSP. It was also hypothesised that there would be a combined influence of multiple gene variants on the prevalence and severity of injuries in this population.

6.2 METHODOLOGY

6.2.1 Participants and study design

This prospective cohort study investigated the relationship between SNPs and injury in 538 high-level male YSP aged 9-23 years (white = 78.0%, black = 9.9%, black/white = 10.8%, Asian = 0.6%), who were registered with one of eight professional soccer clubs previously described in Chapter Three. Due to the influence of maturation status on injury in this population (Chapter Four), players were grouped and analysed according to maturation status, as detailed in Chapter Four. Briefly, maturation status was determined by documenting chronological age, body mass, standing height and sitting height, and using regression equations to calculate a maturity offset (Mirwald et al.,

2002). As there was a limited number of mid-PHV players, only pre- and post-PHV players were included for analysis. Player characteristics according to PHV group are described in Table 6.1. Injuries for each player were recorded for a minimum of one season between the 2014-15 and 2017-18 soccer seasons. This resulted in 204 player records for the 2014-15 season, 17 player records for the 2016-17 season, and 317 player records for the 2017-18 season, with one season per player. All players participated in regular soccer training and competitive match play, which was in accordance with the Premier League's EPPP. Written informed consent to participate in this study was obtained from club officials and players, with parental consent and player assent collected for all participants less than 16 years of age. The study received approval from Liverpool John Moores University Research Ethics Committee and complied with the Declaration of Helsinki.

Table 6.1. Player characteristics according to PHV group. Data are means ± SD.

Group	*n* = (% cohort)	Age (years)	Height (m)	Mass (kg)
Pre-PHV	132 (24.5)	11.2 ± 1.2	1.47 ± 0.07	37.1 ± 4.8
Post-PHV	406 (75.5)	17.5 ± 2.0	1.78 ± 0.08	71.8 ± 8.9

6.2.2 Injury recording and definitions

The methodology adopted for the recording and definition of injuries in this study has been previously described in Chapter Three.

6.2.3 Saliva samples and DNA isolation

Saliva samples were collected following abstinence from food or drink for period of least 30 minutes. Participants were instructed to add at least 2 mL of

saliva to a sterile collection tube containing 2 mL of stabilisation buffer (GeneFix, Isohelix, Kent, UK). Samples were transported to Liverpool John Moores University and stored at -80ºC. The extraction of DNA was performed using a genomic DNA extraction kit (PureLink Genomic DNA Mini Kit, Invitrogen, UK) according to the manufacturer's instructions. All DNA samples were stored at 4ºC following extraction.

6.2.5 Genotyping

Genotyping for nine single nucleotide polymorphisms (SNPs, Table 6.2) was performed using real-time polymerase chain reaction (PCR) on a Rotor-Gene Q PCR machine (Qiagen, Manchester, UK). Reactions were completed on a 72-well rotor disc, with each reaction containing 5 µL Genotyping Master Mix (Applied Biosystems, Foster City, California, USA), 3.5 µL nuclease-free H_2O (Qiagen), 0.5 µL genotyping assay containing SNP-specific TaqMan primers and probes (Applied Biosystems), and 1.0 µL of participant DNA. For negative controls, DNA was replaced by 1 µL nuclease-free H_2O and positive controls for each genotype were used to provide extra confidence in results. The PCR protocol involved 50 denaturation cycles of incubation at 92ºC for 15 s, followed by annealing and extension at 60ºC for 1 min. Genotype was determined using Rotor-Gene Q Software 2.3.1. All samples and controls (positive and negative) were analysed in duplicate with 100% agreement. The alleles of each SNP described in this study refer to the sequence read in the forward (5' to 3') direction.

Table 6.2. List of single nucleotide polymorphisms (SNPs) analysed in this study

Gene	Encoded protein	rs number	Substitution	Minor allele
ACTN3	α-actinin-3	rs1815739	C>T	T
CCL2	Chemokine (C-C motif) ligand-2	rs2857656	G>C	C
COL1A1	α1 (I) collagen chain	rs1800012	C>A	A
COL5A1	α1 (V) collagen chain	rs12722	C>T	T
EMILIN1	Elastin microfibril interface-1	rs2289360	T>C	T
IL6	Interleukin-6	rs1800795	C>G	C
MMP3	Matrix metalloproteinase-3	rs679620	T>C	T
MYLK	Myosin light chain kinase	rs28497577	G>T	T
VEGFA	Vascular endothelial growth factor-A	rs2010963	C>G	C

6.2.6 Total genotype score (TGS) calculation

Those SNPs that were individually associated with injury prevalence and/or severity (following correction for multiple comparisons, see below) were included in a TGS model (Williams and Folland, 2008). Based on the results of this study, the genotype associated with the highest injury prevalence/severity for each SNP was given a score of 2, with a linear trend applied to the remaining genotypes so that the genotype with the next highest prevalence/severity was given a score of 1 and the genotype with the lowest injury prevalence/severity was given a score of 0. Accordingly, a higher TGS is indicative of increased injury risk.

6.2.7 Statistical and data analysis

Data are presented as mean ± standard deviation (SD). The injuries selected for analysis were those recorded occurring most frequently in Chapter Three (see Table 3.2). For each category, players were initially grouped according to PHV status and then by whether they had suffered at least one injury, or no injury. The Chi-square (χ^2) test of independence was then used to assess

whether injury prevalence (proportion of injured players) for each injury category was independent of genotype group. All SNPs were analysed in a co-dominant model (AA *vs* Aa *vs* aa) in the first instance. Where there was a tendency toward a statistically significant association, dominant (AA *vs* Aa+aa) or recessive (AA+Aa *vs* aa) models were used depending on which homozygous group tended to exhibit the higher or lower injury prevalence. A dominant model was used in the first instance for all SNPs with a minor allele frequency (MAF) lower than 0.35. Odds ratios (ORs) were calculated where the prevalence of injured players differed significantly between genotype groups. For those players who had suffered at least one injury for each injury category, differences in injury severity between genotype and PHV groups were analysed using a two-way between groups ANOVA. Differences in TGS between pre- and post-PHV, and also between injured and uninjured players, were analysed by independent samples t-test. To control for multiple comparisons, a false discovery rate (FDR) of 0.2 was applied to the Chi-square and ANOVA analyses using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). Incidentally, following FDR, all statistically significant p-values were < 0.05 but not all p-values < 0.05 were deemed statistically significant. All statistical analyses were performed using IBM SPSS version 25.0 (Chicago, Illinois).

6.3 RESULTS

6.3.1 Hardy-weinberg equilibrium (hwe) and genotype distribution

Genotype distributions of all SNPs were in HWE ($\chi^2 \leq 3.044$, $p \geq 0.081$) except for *COL5A1* ($\chi^2 = 9.412$, $p = 0.002$). When YSP were segregated according to

maturation status, genotype distributions were in HWE for all nine SNPs amongst pre-PHV ($\chi^2 \leq 2.785$, $p \geq 0.095$) and for all SNPs amongst post-PHV ($\chi^2 \leq 2.027$, $p \geq 0.154$) except *COL5A1* ($\chi^2 = 11.754$, $p = 0.001$) and *EMILIN1* ($\chi^2 = 6.001$, $p = 0.014$). The genotype distribution of all nine SNPs is in Table 6.3.

Table 6.3. Genotype distribution of single nucleotide polymorphisms (SNPs) analysed in pre- and post-PHV players, and pre- and post-PHV players combined (All)

SNP	Genotype	Pre-PHV *n* = (%)	Post-PHV *n* = (%)	All *n* = (%)	MAF (All)
ACTN3 rs1817539	CC	42 (32.8)	140 (34.7)	182 (34.2)	0.42
	CT	59 (46.1)	191 (47.3)	250 (47.0)	
	TT	27 (21.1)	73 (18.1)	100 (18.8)	
CCL2 rs2857656	GG	65 (49.2)	182 (44.8)	247 (45.9)	0.32
	GC	50 (44.7)	174 (42.9)	233 (43.3)	
	CC	8 (6.1)	50 (12.3)	58 (10.8)	
COL1A1 rs1800012	CC	91 (69.5)	282 (69.6)	373 (69.6)	0.17
	CA	36 (27.5)	107 (26.4)	143 (26.7)	
	AA	4 (3.1)	16 (4.0)	20 (3.7)	
COL5A1 rs12722	CC	31 (23.8)	118 (29.1)	149 (27.9)	0.50
	CT	64 (49.2)	168 (41.5)	232 (43.4)	
	TT	35 (26.9)	119 (29.4)	154 (28.8)	
EMILIN1 rs2289360	CC	46 (35.1)	128 (31.6)	174 (32.5)	0.45
	CT	68 (51.9)	177 (43.7)	245 (45.7)	
	TT	17 (13.0)	100 (24.7)	117 (21.8)	
IL6 rs1800795	GG	51 (38.9)	194 (47.9)	245 (45.7)	0.32
	GC	66 (50.4)	172 (42.5)	238 (44.4)	
	CC	14 (10.7)	39 (9.6)	53 (9.9)	
MMP3 rs679620	CC	40 (30.5)	124 (30.6)	164 (30.6)	0.45
	CT	63 (48.1)	199 (49.1)	262 (48.9)	
	TT	28 (21.4)	82 (20.2)	110 (20.5)	
MYLK rs28497577	GG	105 (80.8)	297 (73.5)	402 (75.3)	0.13
	GT	22 (16.9)	98 (24.3)	120 (22.5)	
	TT	3 (2.3)	9 (2.2)	12 (2.2)	
VEGFA rs2010963	GG	46 (35.4)	181 (44.8)	227 (42.5)	0.34
	GC	70 (53.8)	177 (43.8)	247 (46.3)	
	CC	14 (10.8)	46 (11.4)	60 (11.2)	

MAF, minor allele frequency.

6.3.2 Prevalence of injury

6.3.2.1 General injuries

Injury prevalence did not differ between *IL6* genotype in pre- and post-PHV combined but, in pre-PHV alone, C-allele carriers were 2.8 times more likely to be injured than GG homozygotes (χ^2 = 5.389 p = 0.027, Table 6.4). Injury

prevalence differed between *EMILIN1* genotype in pre- and post-PHV combined, with CC homozygotes 2.1 times more likely to be injured than TT homozygotes (χ^2 = 8.514 p = 0.014, Table 6.4). Similarly, in post-PHV alone, CC homozygotes were 1.3 times more likely to be injured than CT heterozygotes and 2.7 times more likely to be injured than TT homozygotes (χ^2 = 12.837, p = 0.002, Table 6.4). To demonstrate that this study was statistically powered, we performed a power analysis calculation based on these data, which demonstrated a power of 92.7%..

6.3.2.2 Non-contact injuries

Non-contact injury prevalence did not differ between *VEGFA* genotype in pre- and post-PHV combined but, in post-PHV alone, GG homozygotes and CC homozygotes were 1.8 and 2.0 times more likely to be injured than GC heterozygotes (χ^2 = 6.797 p = 0.034, Table 6.4). Non-contact injury prevalence differed between *EMILIN1* genotype in pre- and post-PHV combined, with C-allele carriers 1.8 times more likely to be injured than TT homozygotes (χ^2 = 4.063, p = 0.046, Table 6.4). In post-PHV alone, CC homozygotes were 2.5 times more likely to be injured than TT homozygotes (χ^2 = 8.795, p = 0.013, Table 6.4).

6.3.2.3 Soft-tissue injuries

Soft-tissue injury prevalence did not differ between *EMILIN1* genotype in pre- and post-PHV combined. However, in post-PHV alone, CC homozygotes were

1.6 times more likely to be injured than CT heterozygotes and 1.5 times more likely to be injured than TT homozygotes (χ^2 = 11.713 *p* = 0.003, Table 6.4).

6.3.2.4 NCST injuries

NCST injury prevalence did not differ between *VEGFA* genotype in pre- and post-PHV combined. However, in post-PHV alone, GG homozygotes and CC homozygotes were 1.7 and 2.1 times more likely to be injured than GC heterozygotes (χ^2 = 6.560 *p* = 0.038, Table 6.4). NCST injury prevalence did not differ between *EMILIN1* genotype in pre- and post-PHV combined. However, in post-PHV alone, CC homozygotes were 2.3 times more likely to be injured than injured than TT homozygotes (χ^2 = 8.082, *p* = 0.018, Fig. 6.1).

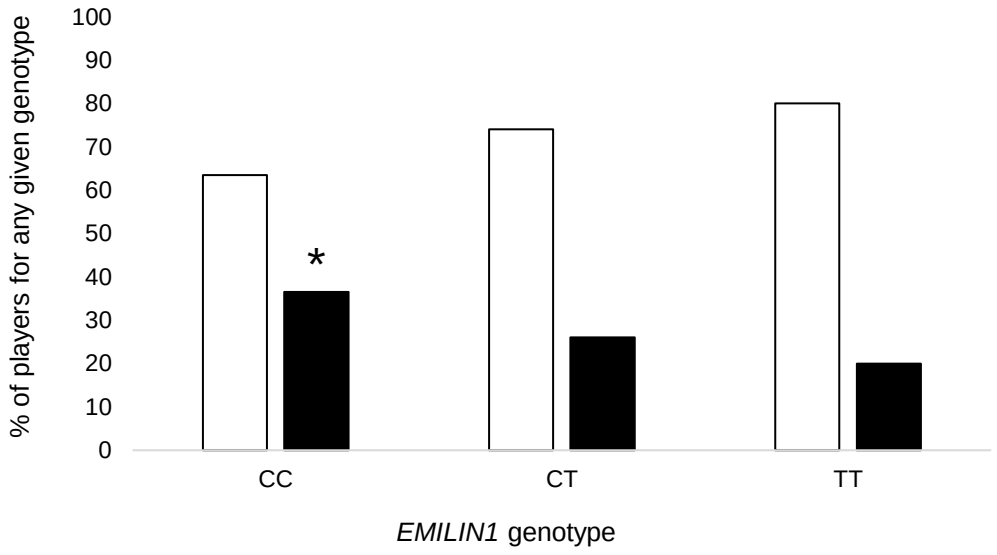

Figure 6.1. Proportion of post-PHV players according to *EMILIN1* rs2289360 genotype group having suffered at least one (black bars) or no (white bars) non-contact soft-tissue injury. *greater than CT and TT ($p < 0.05$).

6.3.2.4 Muscle injuries

Muscle injury prevalence differed between *VEGFA* genotype in pre- and post-PHV combined, with GG homozygotes and CC homozygotes each 2.2 times more likely to be injured than GC heterozygotes (χ^2 = 9.964, p = 0.007, Table 6.4). Similarly, in post-PHV alone, GG homozygotes and CC homozygotes were 2.2 and 2.3 times more likely to be injured than GC heterozygotes (χ^2 = 10.401, p = 0.006, Table 6.4).

6.3.2.5 Ligament injuries

Ligament injury prevalence differed between *MMP3* genotype in pre- and post-PHV combined (χ^2 = 8.123, p = 0.005, Fig. 6.2), with T-allele carriers 2.6 times more likely to be injured than CC homozygotes. The same pattern was seen in pre-PHV (χ^2 = 5.807, p = 0.018, Table 6.4). When combining ligament and tendon injuries, injury prevalence differed between *EMILIN1* genotype in post-PHV alone (χ^2 = 9.188, p = 0.010).

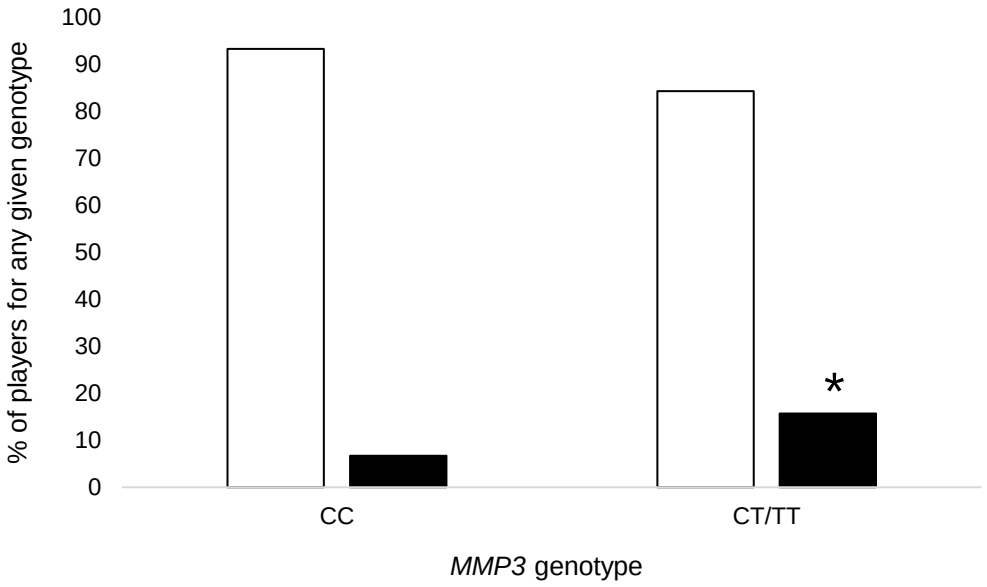

Figure 6.2. Proportion of all players (pre- and post-PHV combined) according to *MMP3* rs679620 genotype having suffered at least one (black bars) or no (white bars) ligament injury. *greater than CC ($p < 0.05$).

6.3.2.6 Thigh injuries

Thigh injury prevalence did not differ between *COL5A1* genotype in pre- and post-PHV combined. In post-PHV alone, TT homozygotes were 2.5 times more likely to be injured than CT homozygotes ($\chi^2 = 6.424$, $p = 0.022$, Table 6.4).

6.3.2.7 Hamstring injuries

There was a tendency for hamstring injury prevalence to differ between *COL5A1* genotype in pre- and post-PHV combined ($\chi^2 = 5.820$, $p = 0.061$), with relatively more TT homozygotes injured than CT heterozygotes. In post-PHV alone, TT homozygotes were 2.9 times more likely to be injured than both CC homozygotes and CT heterozygotes ($\chi^2 = 7.769$, $p = 0.021$, Fig. 6.3).

Table 6.4. Single nucleotide polymorphisms (SNPs) associated with injury prevalence in pre- and post-PHV players.

Group	Injury Type	Gene	SNP	Genotype	Prevalence (%)	*p* value
Pre-PHV	General injury	*IL6*	rs1800975	GG†	17.4	0.027
				GC/CC*	36.9	
	Ligament	*MMP3*	rs679620	CC†	0.0	0.018
				CT/TT*	13.2	
Post-PHV	General injury	*EMILIN1*	rs2289360	CC*	51.6	0.002
				CT†	40.7	
				TT†	28.0	
	Non-contact	*VEGFA*	rs2010963	GG*	31.8	0.034
				GC†	20.9	
				CC*	34.8	
	Non-contact	*EMILIN1*	rs2289360	CC*	35.7	0.013
				CT	27.1	
				TT†	18.0	
	Soft-tissue	*EMILIN1*	rs2289360	CC*	43.7	0.003
				CT†	33.3	
				TT†	33.7	
	NCST	*EMILIN1*	rs2289360	CC*	36.5	0.018
				CT	26.0	
				TT†	20.0	
	NCST	*VEGFA*	rs2010963	GG*	31.3	0.038
				GC†	21.5	
				CC*	37.0	
	Muscle	*VEGFA*	rs2010963	GG*	22.5	0.006
				GC†	11.8	
				CC*	23.6	
	Thigh	*COL5A1*	rs12722	CC	16.5	0.022
				CT†	11.3	
				TT*	24.3	
	Hamstring	*COL5A1*	rs12722	CC†	5.4	0.021
				CT†	5.3	
				TT*	14.0	
All players	General injury	*EMILIN1*	rs2289360	CC*	51.6	0.014
				CT	40.7	
				TT†	28.0	
	Non-contact	*EMILIN1*	rs2289360	CC/CT*	29.0	0.046
				TT†	19.7	

Muscle	*VEGFA*	rs2010963	GG GC† CC*	22.5 11.8 23.6	0.007
Ligament	*MMP3*	rs679620	CC† CT/TT*	6.7 15.7	0.005

NCST, non-contact soft-tissue; *genotype with greater injury prevalence; †genotype with the lower injury prevalence.

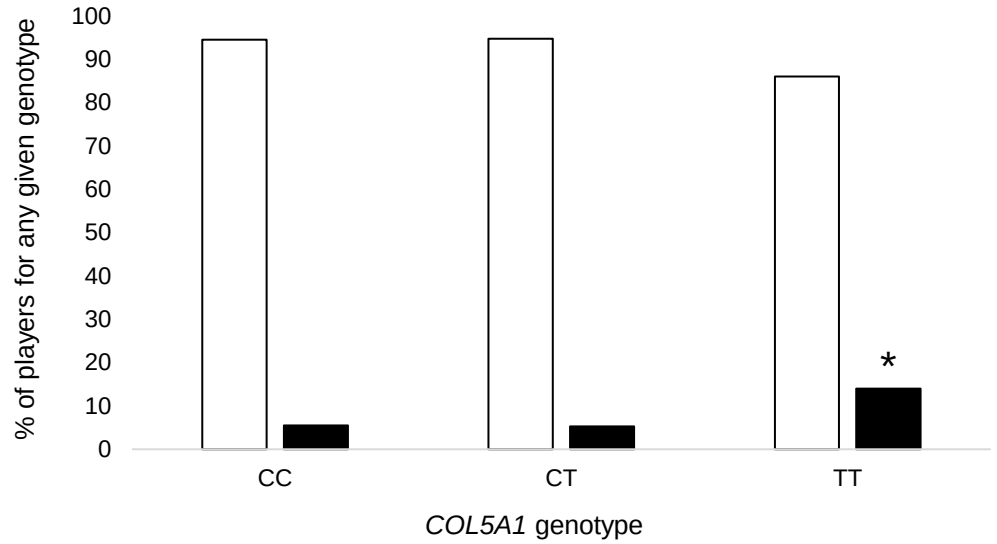

Figure 6.3. Proportion of post-PHV players according to COL5A1 *rs12722* genotype group having suffered at least one (black bars) or no (white bars) hamstring injury. *greater than CC and CT ($p < 0.05$).

6.3.3 Absence and injury burden score

6.3.3.1 General injuries

There was an interaction between *MYLK* genotype and PHV group on absence due to injury [F (1, 201) = 7.34, p = 0.007]. In post-PHV, GG homozygotes were absent for fewer days compared to T-allele carriers (p = 0.012), whilst in pre-PHV, GG homozygotes tended to be absent for more days compared to T-allele carriers (p = 0.051).

6.3.3.2 Knee injuries

There was a main effect of *MMP3* genotype on knee injury-burden score [F (2, 60) = 3.61, p = 0.033], where CC homozygotes had lower scores compared to CT genotype (p = 0.029) regardless of maturation status. To demonstrate that this study was statistically powered, we performed a power analysis calculation based on these data, which demonstrated power of 80.1%. There was also a main effect of *MYLK* genotype on knee injury-burden score [F (1, 60) = 6.41, p = 0.014], where GG homozygotes had lower scores than T-allele carriers regardless of maturation status.

6.3.4 Total genotype score

The SNPs associated with the prevalence, absence and/or injury-burden score for one or more injury category on an individual basis were included in the TGS model. The TGS model included the *COL5A1, EMILIN1, IL6, MMP3, MYLK* and *VEGFA* SNPs. There was no difference in TGS between pre- and post-PHV (37.8 ± 12.5 *vs* 38.4 ± 13.6, t(524) = 11.732, p = 0.634). Players who had suffered one or more non-contact injury had a higher TGS than players who had suffered no non-contact injuries (40.4 ± 12.6 *vs* 37.4 ± 13.5, t(522) = 2.337 , p = 0.020). Similarly, players who had suffered one or more NCST injury had a higher TGS than players who had suffered no NCST injuries (41.1 ± 12.4 *vs* 36.9 ± 13.4, t(522) = 2.295 , p = 0.022).

6.4 DISCUSSION

The aim of this study was to investigate the influence of nine candidate SNPs on injury prevalence, absence and severity in high-level, male YSP. The main findings were that, in pre- and post-PHV combined, general and non-contact injuries were more prevalent in *EMILIN1* C-allele carriers, muscle injuries were less prevalent in players with the *VEGFA* GC genotype, and ligament injuries were more prevalent in *MMP3* T-allele carriers. Interestingly, more SNPs were individually associated with injury in post- *vs* pre-PHV. In pre-PHV, general and ligament injury prevalence were greater in *IL6* C- and *MMP3* T-allele carriers, respectively. In post-PHV, general, non-contact, soft-tissue and NCST injuries were more prevalent in *EMILIN1* CC homozygotes, non-contact and muscle injuries were less prevalent for *VEGFA* GC heterozygotes, and muscle and hamstring injuries were more prevalent in *COL5A1* TT homozygotes. Additionally, *MYLK* and *MMP3* were associated with injury absence and severity, regardless of PHV group. There was no difference in TGS between pre- and post-PHV but players who had suffered one or more non-contact or NCST injury had a higher TGS than players suffering no non-contact or NCST injuries, respectively. This is the first study to provide evidence that genetic variation is associated with the prevalence and severity of injuries in YSP, and the first to report the combined association of multiple SNPs with soccer injury risk.

Injury prevalence was greater for *EMILIN1* rs2289360 CC homozygotes than TT homozygotes for general and non-contact injuries in pre- and post-PHV players combined, and for general, non-contact, soft-tissue and NCST injuries in post-PHV alone. The *EMILIN1* gene encodes the elastin microfibril

interfacer 1 (EMILIN-1) protein, which assists the fusion of elastin fibres (Randell and Daneshtalab, 2017) to provide elasticity to ligaments and tendons (Kannus, 2000). When combining ligament and tendon injuries, prevalence was greatest for CC homozygotes in post-PHV. The intronic location of this SNP suggests it could influence ligament/tendon injury risk by altering *EMILIN1* expression and/or mRNA stability (Tabor, Risch and Myers, 2002). Greater injury prevalence in CC homozygotes may therefore be due to the C-allele overexpressing EMILIN-1 in ligament/tendon, potentially leading to increased compliance and reduced tensile strength, ultimately increasing joint laxity and risk of tissue rupture. However, these findings are not supported by previous studies linking *EMILIN1* to knee ligament injuries in professional soccer players, where TT homozygotes had the greatest risk and severity (Pruna et al., 2013; Artells et al., 2016). This disparity may be due to fewer players in previous studies (n = 60-71) than ours (n = 538). These data suggest that pre- and post-PHV players with the *EMILIN1* CC genotype are at greater risk of general and non-contact injuries, extending to soft-tissue injuries in post-PHV. Interestingly, other SNPs linked to ligament and tendon injuries were associated with injury in the present study.

Injury prevalence was lower in *VEGFA* rs2010963 GC homozygotes for muscle injury in pre- and post-PHV combined, and for non-contact, NCST and muscle injury in post-PHV alone. The VEGF family of growth factors are key signalling proteins involved in angiogenesis and the A-isoform has the greatest angiogenic potency (Lulińska-Kuklik et al., 2019). Previously, more CC homozygous soccer players suffered anterior cruciate ligament (ACL) ruptures (Lulinska-Kuklik et al., 2019) despite fewer ACL injuries in non-athletic C-allele

carriers (Rahim et al., 2018). This study is the first to associate *VEGFA* with muscle injury in soccer following no link to hamstring muscle injuries in elite professional players (Larruskain et al., 2018). The indication of a heterozygous advantage merits attention, because risk/protective genotypes are typically homozygous (Nell et al., 2012). A *"Goldilocks effect"* suggests too much or too little expression of a protein can increase risk, and that heterozygosity provides an advantageous balance between both. This phenomenon is yet to be reported for injury (Nell et al., 2012) but is not unusual in the context of infectious disease (Chapman et al., 2010). Accordingly, evidence of enhanced VEGF-A expression in CC homozygotes (Schneider et al., 2008) leads us to hypothesise that for GG homozygotes, VEGF-A levels would be diminished, inferring greater injury risk for each homozygous group. Whilst speculative, this could affect the findings observed for *VEGFA*, though precisely how the angiogenic effects of *VEGFA* influence injury risk is unclear. In addition to the associations of *VEGFA* and *EMILIN1*, this study describes the influence of another SNP previously linked to ligament and tendon, with injury in YSP.

Relatively more *MMP3* rs679620 T-allele carriers suffered ligament injuries than CC homozygotes in pre- and post-PHV combined, with a similar finding in pre-PHV alone. In addition, knee injury-burden score was greater for T-allele carriers than CC homozygotes, irrespective of PHV group (3.7 ± 1.1 *vs* 3.1 ± 0.8). The MMPs mediate extracellular matrix degradation and remodelling (Birkedal-Hansen et al., 1993) and in non-athletes, this *MMP3* SNP has been associated with Achilles tendinopathy (Raleigh et al., 2009) and ACL rupture (Posthumus et al., 2012). The CC genotype was associated with increased Achilles tendinopathy risk and reduced ACL injury risk, the latter in

line with the findings of this study. The C-allele reportedly increases *MMP3* gene expression, and because MMP3 is involved in ECM degradation, greater gene expression may increase MMP3 activity, leading to more ECM degradation. This could assist post-injury regeneration, potentially underpinning the lower knee injury-burden score in CC homozygotes, though mechanistic experiments are required to determine the effect of increased *MMP3* expression on tendon/ligament regeneration. In professional soccer, hamstring injury risk in was greatest in TT homozygotes (Larruskain et al., 2018), whilst others found no association with muscle injuries (Pruna et al., 2016). It is possible that YSP, who are T-allele carriers, are at greater risk of ligament injury, and that the burden of knee injuries, most of which are to ligament in this population (Moore et al., 2011), is greater in T-allele carriers. These findings suggest that SNPs involved in tissue regeneration influence injury risk in YSP. Indeed, other variants related to tissue recovery and adaptation were associated with injury in this study.

Carriers of the *IL6* rs1800795 C-allele suffered relatively more general injuries than GG homozygotes in pre-PHV alone. The cytokine IL-6 is produced by skeletal muscle with pro- and anti-inflammatory functions (Pedersen and Febbraio, 2008), suggesting that the increased IL-6 associated with the G-allele (Fishman et al., 1998b) could increase or decrease injury risk. The findings of this study are in contrast with those linking *IL6* genotype to hamstring injury in professional soccer players (Larruskain et al., 2018), though ours concern general injuries, constituting many injury types and mechanisms, which could explain differences to findings in hamstring muscle injuries, in addition to the difference in populations investigated (pre-PHV YSP

vs elite professional players). Because the GG genotype is associated with power athlete status (Weyerstraß et al., 2018) and the G-allele with sprint performance in YSP (Pickering et al., 2019), it is suggested that pre-PHV C-allele carriers are less robust and less likely to reach the elite level of sports such as soccer, where powerful actions are key determinants of success (Murtagh et al., 2019). *IL6* C-allele carriers have elevated creatine kinase responses to eccentric exercise (Yamin et al., 2008), thus it is proposed that pre-PHV YSP who are GG homozygotes benefit most from the anti-inflammatory action of IL-6, and are more robust to injury than C-allele carriers. No association was evident for post-PHV, suggesting the influence of *IL6* on injury prevalence is maturation dependent. Similarly, some SNPs appear to only be associated with injuries post-PHV.

In post-PHV alone, proportionately more *COL5A1* rs12722 TT homozygotes suffered thigh and hamstring injuries. The *COL5A1* gene encodes the α1 chain of Type V collagen, a vital structural component of ligaments and tendons during collagen fibrillogenesis (Wenstrup et al., 2004). The T-allele is linked to slower recovery from exercise-induced muscle damage in non-athletes (Baumert et al., 2018), with the CT genotype linked to hamstring injury severity in professional soccer players (Pruna et al., 2013; Pruna et al., 2016). Such data link the T-allele to more severe muscle injuries and are supported by a tendency for TT homozygotes to suffer more severe injuries in a separate soccer cohort (Massidda et al., 2015a), but contested by a lack of association with hamstring injury in another (Larruskain et al., 2018). Nevertheless, the T-allele is linked to ACL injury and Achilles tendinopathy in non-athletes (Wenstrup et al., 2004; September et al., 2009), and this study is

the first to associate TT homozygotes with injury in YSP. The capacity for the TT genotype to increase *COL5A1* mRNA stability may result in decreased fibril diameter, increased fibril density and reduced tensile strength (Collins and Posthumus, 2011). Accordingly, the rs12722 SNP may affect the mechanical properties of the muscle-tendon unit and lead to variance in the risk of injury to muscles such as the hamstrings (Baumert et al., 2018). In older players, who play at a higher intensity (Arnason et al., 2004a) and greater running velocities than younger players (Nikolaidis et al., 2016), this could increase hamstring injury risk in TT homozygotes, with many such injuries occurring close to the myotendinous junction (Small et al., 2009). The findings of this study support the association of this *COL5A1* SNP with musculoskeletal injury and are the first to associate this SNP with injury in YSP. As well as investigating the association of SNPs with injury prevalence, the present study also sought to determine if these SNPs influence injury-induced absence and injury severity.

Absence due to general injuries and the injury-burden score from knee injuries were associated with *MYLK* genotype. The interaction effect of *MYLK* and maturation on general injury absence suggests that the influence of *MYLK* is maturation dependent. Indeed, the median days missed by GG homozygotes was lower than T-allele carriers for post-PHV (median = 25.0, IQR = 37.0 *vs* median = 38.0, IQR = 57.8) but tended to be greater than T-allele carriers for pre-PHV (median = 19.0, IQR = 41.0 *vs* median = 9.0, IQR = 14.0). Regardless of PHV group, T-allele carriers exhibited greater knee injury-burden scores than GG homozygotes. The T-allele was associated with elevated creatine kinase and greater muscle strength deficit post-exercise

(Clarkson et al., 2005), whilst exertional rhabdomyolysis is more common in T-allele carriers (Deuster et al., 2013), suggesting muscle damage is influenced by *MYLK* genotype. Whilst a link to the severity of knee injuries is less clear, this potentially involves the influence of *MYLK* on skeletal muscle, whereby T-allele carriers experience greater acute muscle damage, which may impair the contractile capacity of musculature surrounding the knee. Nevertheless, the novel associations of *MYLK* genotype with injury absence and severity require further investigation.

It is possible that differences between pre- and post-PHV in the individual SNPs associated with soccer injuries are due to differences in the intensity of match play and levels of competition (Arnason et al., 2004a), causing genotype-environment interactions to vary. For example, a pre-PHV player with a less favourable genotype or polygenic profile may encounter fewer inciting events, such as forceful muscular contractions during acceleration or deceleration, meaning they are less likely to be injured even though they are genetically more predisposed to injury. Accordingly, the influence of some SNPs might be diminished in the absence of certain extrinsic factors, meaning the risk associated with those SNPs may be moderate until a player reaches an age or competitive level where exposure to inciting events is increased.

The interaction of intrinsic and extrinsic factors (Van Mechelen, Hlobil and Kemper, 1992) characterises the multifactorial nature of soccer injuries (Volpi et al., 2016) and similarly, genetic associations are likely to involve multiple SNPs. Despite the fact that a greater number of SNPs were individually associated with injury prevalence in post-PHV than pre-PHV, TGS

did not differ between groups. Consequently, it is suggest that the combined influence of ‘risk’ alleles does not differ for players at different stages of maturation, and that the evidence of higher injury prevalence and days missed by post-PHV (Chapter Four) implicates other factors. In post-PHV, the interaction of genotype with extrinsic factors may be more prominent than for pre-PHV, augmenting the influence of ‘unfavourable’ genotypes and the ability to detect positive associations. This may explain why a greater number of SNPs were individually associated with injury in post-PHV in the present study. It was hypothesised that YSP reaching post-PHV are more robust to injury, however, a lack of difference between pre- and post-PHV in TGS suggests this may not be determined solely by genetic variation. However, it is possible that other SNPs, which were not investigated in this study, are associated with injury. It is also possible that other genetic variants unrelated to injury, such as those that influence the performance attributes required in soccer, impact a player’s ability to succeed. Indeed, SNPs are associated with elite soccer player status (Eynon et al., 2012), the physical capacity of professional players (Pimenta et al., 2013) and recently, sprint performance in YSP (Pickering et al., 2019). Future studies should seek to increase sample size and the number of SNPs investigated to address this possibility. Nonetheless, the present study is the first to demonstrate that this TGS model (comprising six SNPs in the following genes: *COL5A1, EMILIN1, IL6*, *MMP3, MYLK* and *VEGFA*) is associated with non-contact injuries in YSP.

A higher TGS was observed in players who had suffered at least one non-contact, or at least one NCST injury, than those who had suffered no non-contact or NCST injuries, respectively. This novel data demonstrates the

additive effect of ‘risk’ alleles on injury prevalence in YSP. Several studies have linked individual SNPs to injury in adult players (Ficek et al., 2013; Pruna et al., 2013; Massidda et al., 2015a; Massidda et al., 2015b; Massidda et al., 2015c; Pruna et al., 2016; Myosotis et al., 2017b; Lulinska-Kuklik et al., 2019), with only one attempting to quantify the combined influence of multiple SNPs (Larruskain et al., 2018). A multivariate model including SNPs associated with hamstring injury in the same study was used to estimate injury risk, however, the model was unable to accurately predict future injuries, highlighting the need for caution when attempting to predict injury risk from candidate gene studies. Nevertheless, associating candidate SNPs with injury provides important novel information regarding the factors underpinning their occurrence, and the present study is the first to demonstrate the combined influence of candidate SNPs on injury prevalence in soccer.

It is important to mention the differences between our findings and those of previous studies regarding the alleles and/or genotypes associated with greater/reduced risk of injury. Whilst some have suggested that associations of SNPs with injury that are in opposing directions may imply a complex mechanism at a particular locus (El Khoury, Ribbans and Raleigh, 2016), there remains the possibility that one or both studies report false positives. Due to the presence of conflicting results between ours and previous studies, as well as differences between existing studies, we urge caution regarding the interpretation of each study and recommend further research to scrutinise these findings. Nevertheless, our use of the FDR to control for multiple comparisons and therefore reduce the risk of finding false positives represents an attempt to implement greater scientific rigour than previous

literature, where such measures are absent. In addition, the use of sample sizes that are similar to, or greater than, that investigated in the present study can also help to increase confidence in the findings of candidate gene studies. Accordingly, we suggest the use of the FDR and large sample sizes be adopted in future candidate gene studies of injury risk. It is also important to note that this is the first study to investigate the genetic association with injury risk in this unique population of high-level *youth* soccer players. Therefore, any discrepancy between previous results and ours may also be due to differences in the populations studied (e.g. YSP vs. senior/professional players).

The present study reports, for the first time, that individual SNPs are associated with the prevalence, absence and severity of injuries in YSP before and after PHV, and that the combination of multiple SNPs is associated with the prevalence of non-contact injuries, which pose a considerable burden to YSP (Chapter Three). However, there are some acknowledged limitations in this study. Firstly, mid-PHV players were excluded due to limited numbers, which would have been reduced further when separating by genotype (thus reducing statistical power), and it is possible that the investigated SNPs could affect mid-PHV players differently. Secondly, it is recognised that genetic variability is one of numerous risk factors, and that quantifying other factors (in addition to maturation status) may have provided greater context to the present findings. However, the current sample size is substantially greater than any previous study investigating a genetic association with soccer injury risk and these findings are the first of their kind in the field of *youth* soccer. Furthermore, these results offer greater external validity than previous studies

due to the inclusion of players from several clubs in four different countries across two continents.

6.5 CONCLUSION

The novel findings of this study demonstrate an association between multiple SNPs (*COL5A1* rs12722, *IL6* rs1800795, *VEGFA* rs2010963, *EMILIN1* rs2289360, *MMP3* rs679620 and *MYLK* rs28497577) and injury prevalence and/or severity in YSP. These results provide important information regarding the genetic factors underpinning common injuries in this under investigated population. Further, this study provides original evidence that *MYLK* rs28497577 is associated with absence due to general injuries, which differs according to maturation status, and that the association between the *MYLK* rs28497577 and *MMP3* rs679620 SNPs and knee injury severity occurred regardless of maturation status. Importantly, the present study demonstrates that the combined effect of individual SNPs influences the prevalence of non-contact injury. However, although the polygenic risk profile of YSP does not appear to differ between maturation group, more SNPs were associated with injury in post- than pre-PHV YSP, which suggests that environmental factors are more influential at post- *vs* pre-PHV. Whilst replication from independent groups is necessary to provide support for these results, the data reported here have the potential to *aid* the future identification and management of 'at-risk' players (in combination with traditional injury prevention strategies), to help reduce injury risk and maximise development in this important population.

CHAPTER SEVEN

SYNTHESIS OF FINDINGS

7.1 SYNTHESIS

The purpose of this chapter is to offer a theoretical interpretation of the current findings in relation to the aims and objectives of the PhD thesis. This chapter will also seek to interpret the findings of each experimental chapter in the context of current knowledge surrounding injury risk factors in YSP. The limitations of this thesis will also be discussed, in order to identify and recommend directions for future research in this important field of injury epidemiology in YSP.

7.2 ACHIEVEMENT OF AIMS AND OBJECTIVES

The aim of this thesis was to investigate the epidemiology of injuries in high-level YSP from different countries, with particular reference to maturation status, playing position and genetic variation. This was achieved via the completion of four objectives:

Objective 1: To identify the most common types and locations of injuries sustained in a cohort of high-level YSP from multiple nations and to determine if any differences in injury rate exist between countries. This was achieved through the injury audit performed in Chapter Three.

Objective 2: To ascertain whether the maturation status of high-level YSP affects the type, frequency and severity of injuries suffered. This was achieved through the completion of Chapter Four.

Objective 3: To determine whether playing position affects the type, frequency and severity of injuries suffered in physically mature, high-level YSP. This was achieved through the completion of Chapter Five.

Objective 4: To identify specific gene polymorphisms that are associated with the type, prevalence and/or severity of common injuries in high-level YSP. This was achieved through the completion of Chapter Six.

7.3 GENERAL DISCUSSION

The overarching aim of this thesis was to investigate the epidemiology of injuries amongst high-level YSP in different countries across two continents, and to examine the association of selected risk factors in relation to the most common injuries. Due to the limited research on injury risk in YSP, it was hoped that the conclusions of this thesis would enhance the understanding of the investigated risk factors in relation to the inter-individual variability in injury prevalence and severity in high-level YSP.

The injury audit embodied in Chapter Three described the types and locations of injuries to be studied in Chapters Four, Five and Six, and found that there were minimal differences between nations regarding the rate of injuries recorded. A total of 443 injuries were prospectively recorded in 624 players throughout a single season. Chapter Three illustrates the considerable burden created by injuries in YSP, specifically highlighting the problem elicited by non-contact and skeletal muscle injuries, which should be the target of specific injury prevention strategies. Importantly, these data demonstrate that the most common injuries in YSP are consistent with those recorded in similar

populations over a decade ago (Price et al., 2004; Le Gall et al., 2006), signifying the challenge faced by practitioners working with YSP in controlling and managing injuries. The relatively greater number of severe injuries in U14 and U16 players suggests injury severity is maturation-dependent, providing justification for the work of Chapter Four. Furthermore, similarities between these findings and other injury audits in this population (Price et al., 2004; Renshaw and Goodwin, 2016; Read et al., 2018b) suggest that the injuries recorded in Chapter Three represent a typical YSP population and increase the external validity of the results. Accordingly, practitioners working with YSP should anticipate similar injury patterns during one season to those described in Chapter Three. As well as these findings, Chapter Three suggested that biological maturation may affect injury severity within high-level YSP.

Existing studies linking biological maturation to injury risk in YSP have relatively small sample sizes (Van der Sluis et al., 2014; Van der Sluis et al., 2015) or a narrow chronological age range (Le Gall, Carling and Reilly, 2007). It was hoped that by comparing injury risk in YSP at different stages of maturity across several chronological age groups, Chapter Four would improve the understanding of maturation status as an injury risk factor in YSP. Using anthropometric measures, YSP were grouped according to predicted years from peak height velocity (PHV) as pre-, mid or post-PHV, with injuries analysed according to PHV group. The results showed that relatively more post-PHV suffered soft-tissue, ligament/tendon and thigh injuries than pre- and mid-PHV, and relatively more muscle injuries than pre-PHV. Together, this highlights post-PHV players as being at greatest risk of common injuries, and indicates that monitoring and injury prevention strategies may have the

greatest benefit in post-PHV. The secondary observation that injured post-PHV players miss more days due to injury than injured pre-PHV players means post-PHV miss more training and matches in one season than pre-PHV. Accordingly, practitioners who are able to successfully reduce injury in post-PHV players will have a positive impact on player availability. These findings contradict the hypothesis that injuries would be more prevalent and severe in mid-PHV players than pre- and post-PHV. Consequently, it is suggested that grouping YSP between the ages of 11 and 15 by maturation status rather than chronological age (known as bio-banding) may not have a practical benefit on injury risk, despite perceptions of YSP that maturity-matched competition reduces the likelihood of injury (Bradley et al., 2019). Nevertheless, Chapter Four provides useful information concerning the prevalence of injuries suffered by YSP at different stages of maturation, which may be useful to practitioners considering the value of maturation-specific injury prevention strategies.

Importantly, Chapter Four questions whether the period surrounding PHV, i.e mid-PHV, negatively affects injury risk as has been previously suggested (Van der Sluis et al., 2014). Post-PHV players are likely to have participated in soccer for longer and to have accumulated greater quantities of training and match exposure, which means they are more likely to have suffered previous injuries (Arnason et al., 2004b). It is proposed that these factors, in combination with more intensive training and match schedules (Read et al., 2018b), contribute to the observation that relatively more post-PHV players experience injury. The lack of difference between pre- and mid-PHV suggests the problem of injury does not increase for YSP as they progress toward PHV. Therefore, it is suggested that the higher proportion of

severe injuries in U14 and U16 players reported in Chapter Three relates to programmed increments in the volume of soccer activity (Read et al., 2018b) or more intense match-play and increased competitiveness as YSP mature (Wong and Hong, 2005). It is also possible that the intensity of match play is affected by the tactical roles of YSP, thus the influence of playing position on injury risk in YSP warranted exploration. It is hoped that practitioners interpret these findings as demonstrating the need for specific injury prevention practices targeted toward muscle and ligament/tendon injuries in post-PHV YSP. Successful reduction these injuries in post-PHV YSP would reduce the number of days missed per season due to injury, which is notably higher than pre-PHV in the current cohort. In doing so, the availability of post-PHV players for matches and training would be improved, which is likely improve a team's overall success (Arnason et al., 2004a).

Evidence describing the influence of playing position on injury risk in YSP is limited (Price et al., 2004; Moore et al., 2011), and the ability to infer differences from studies of professional players is constrained by equivocal findings (Dauty and Collon, 2011; Mallo and Dellal, 2012). The purpose of Chapter Five was, therefore, to investigate the relationship between playing positon and injury in YSP. To eliminate the influence of maturation on injury observed in Chapter Four, only post-PHV players were included in Chapter Five. Post-PHV were selected rather than pre-PHV due to the number of available players. It was also perceived that post-PHV were more likely to have permanent playing positions than pre-PHV, who may experiment in numerous playing positions. Players were grouped as either goalkeepers, central players, lateral players or forwards, with injuries recorded during one season

analysed according to playing position group. It was hypothesised that the prevalence and severity of injuries would differ between outfield playing positions, and that goalkeepers would suffer fewer injuries than outfield players. Instead, the results demonstrated no difference between outfield playing positions in the prevalence and severity of injury in YSP, except for the prevalence of thigh injuries, which was greater in lateral/forward players than goalkeepers. These data question whether playing position should be recognised as a risk factor for injury in YSP and suggest that other factors, such as those related to the variability in match circumstances (Bengtsson et al., 2013) and player behaviour (Schwebel, Banaszek and McDaniel, 2006), may have more influence on the frequency of injury-inciting events than playing position. In practical terms, it is proposed that training modalities designed to limit the risk of injury should be similar across outfield positions, with perhaps more focus on preventing thigh injuries in lateral/forward players. Due to the protective benefit of muscle strength on injury (Keller, Noyes and Buncher, 1988), specific training of the thigh (quadriceps and hamstring) musculature could be beneficial to these players. However, muscle strength (Hubal et al., 2010) and the response to strength training (Erskine et al., 2014) are genetically influenced, suggesting that intrinsic inter-individual variation could influence injury risk in YSP.

Literature concerning genetic variation and soccer injuries is limited to adult professional players (Ficek et al., 2013; Pruna et al., 2013; Massidda et al., 2015a; Massidda et al., 2015c; Pruna et al., 2016; Myosotis et al., 2017b; Larruskain et al., 2018). Accordingly, the association of SNPs with injury in YSP was addressed in Chapter Six and accounted for the effect of maturation

status described in Chapter Four. In pre-PHV, post-PHV and pre- and post-PHV combined, players were grouped by genotype to explore differences in injury prevalence and severity. Mid-PHV were not included due to a relatively low number of players (and therefore even lower number of injuries). The results suggest that the individual associations of six SNPs (*COL5A1* rs12722*, EMILIN1* rs2289360*, IL6* rs1800795*, MMP3* rs679620*, MYLK* rs28497577 and *VEGFA* rs2010963) with injury risk in YSP are dependent on maturation status. Despite the polygenic profile for injury risk being similar for both pre- and post-PHV, more SNPs were individually associated with injury in post- than pre-PHV players. This is probably a consequence of more prominent genotype-environment interactions in physically mature players. Specifically, post-PHV players are more prone to extrinsic injury risk factors, such as faster and more intense playing styles (Arnason et al., 2004a), greater cumulative training and match exposure as a product of their training age, which increases the risk of a previous injury (Arnason et al., 2004b), and busier training and match schedules (Read et al., 2018b). These factors are less likely to influence pre-PHV injuries, meaning less interaction with genetic predisposition, resulting in fewer injury-inciting events and injuries suffered in pre-PHV. Therefore, a key message from Chapter Six is the proposition that maturation-dependent genotype-environment interactions contribute to the greater number of SNPs individually associated with injury in post-PHV players. It is therefore likely that the key findings in Chapter Four, i.e. that injury prevalence and severity were greater in post- compared to mid- and pre-PHV, can be explained by these maturation-dependent genotype-environment interactions. In spite of differences in the individual SNPs associated with injury in pre- and

post-PHV, the risk of injury indicated using the TGS was similar between maturation groups, and was linked to injury regardless of maturation.

A higher TGS in players suffering non-contact and NCST injuries provides evidence for the combination of multiple 'risk' alleles having an additive effect on non-contact injury risk in YSP. As this thesis (Chapters Three, Four and Five) and others (Deehan, Bell and McCaskie, 2007; Renshaw and Goodwin, 2016; Read et al., 2018b) have demonstrated, the majority of injuries in YSP are non-contact. Therefore, for the first time, this study presents evidence that a polygenic profile can affect player availability in YSP. As mentioned above, TGS did not differ between pre- and post-PHV, indicating that the genetic predisposition to injury in YSP is similar regardless of maturation. This supports the theory that maturation-dependent genotype-environment interactions contribute to the differences in injury prevalence between pre- and post-PHV, and challenges the notion that YSP are able to progress because they are intrinsically robust to injury. If this were the case, pre-PHV would be expected to have a higher TGS than post-PHV under the assumption that YSP with fewer 'risk' alleles would remain injury-free, miss fewer days due to injury and consequently be overrepresented in post-PHV. Accordingly, research is encouraged to investigate whether the progression of YSP is affected by their genetic profile, or whether a player's chances of reaching the elite level of soccer is underpinned to a greater extent by factors such as technical ability or physical attributes, such as running speed and aerobic endurance (Vaeyens et al., 2006). Despite some of the SNPs associated with injury in Chapter Six also being associated with injury in studies of professional players, it is not clear whether the genetic profile of

YSP is different to that of professional players. Whilst the authors of this thesis support the current consensus against genetic testing for the purposes of talent identification, training design and injury screening (Webborn et al., 2015), it is hoped that the information provided by Chapter Six adds value to the field of sports genomics and to the understanding of intrinsic, athlete-specific factors that underpin the incidence and severity of sports injuries. With both practitioners and elite athletes of the belief that data describing the genetic susceptibility to injury would be valuable (Varley et al., 2018), the findings of this thesis also represent an important step toward understanding the impact of genetic variation on injury in elite sport.

7.4 PROJECT LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The research described within this thesis provides novel information regarding selected risk factors for injuries suffered by high-level YSP. Whilst reaching these conclusions, there are some recognised limitations in the work contained within the present thesis, which contribute to recommendations for future research conducted in this area. This section aims to discuss the limitations and recommendations for future research related to each specific chapter of this thesis.

Suggestions arising from Chapter Three

Chapter Three reported the type, location and severity of the most common injuries suffered by high-level YSP aged 9-23 years from the academies of eight professional soccer clubs from England, Spain, Uruguay and Brazil.

Future studies should aim to report injuries from additional nations to increase external validity, and should aim to describe the quantity of soccer exposure undertaken by YSP in order to calculate injury incidence rates. Such information would provide context regarding the frequency of injuries in YSP relative to the time spent training and/or playing matches, which increases as players progress through chronological age groups (Read et al., 2018b). It would also be advantageous to study YSP prospectively over consecutive seasons to investigate the impact of recurrent injuries on development time.

Suggestions arising from Chapter Four

Chapter Four compared pre-, mid- and post-PHV YSP to determine if maturation status was associated with injury prevalence and/or severity. Players were categorised based on a maturity offset calculated using anthropometric measures, which was obtained at a single time point. Whilst this method was considered the most ethically and logistically appropriate relating to the age and number of participating YSP, more precise methods to determine biological maturation are available. Future studies should also aim to quantify maturity offset at regular intervals over the course of a soccer season in order to detect advances in maturity during the study period. In concordance with suggestions relating to Chapter Three, a prospective study quantifying injuries and maturation over several seasons would allow injuries to be aligned to advancing skeletal maturation, in a similar manner to one previous study (Van der Sluis et al., 2014). Such investigations would be further enhanced by simultaneous quantification of training and competitive

schedules to investigate how the potential change in volume of soccer activity affects injury throughout maturation.

Suggestions arising from Chapter Five

Chapter Five compared the prevalence and severity of injuries in post-PHV YSP according to playing position. There are a number of studies in YSP describing the activity profile of playing positions (Mendez-Villanueva et al., 2011; Mendez-Villanueva et al., 2013; Al Haddad et al., 2015) and a much more conservative number comparing injury according to playing position (Moore et al., 2011; Cloke et al., 2012). Future studies should aim to record the activity profiles and injuries suffered by YSP occupying different playing positions to determine whether these variables are related. Additionally, the study of injury epidemiology in YSP would benefit from research using a within-subjects design to investigate how the method used to group outfield playing positions (i.e as defenders, midfielders and attackers *or* central, lateral and forward players) affects the detection of positional differences. Such a study may explain whether the equivocal findings of current literature are due to authors adopting different classification systems, and may provide consensus for future investigations.

Suggestions arising from Chapter Six

Chapter Six investigated the individual and combined association of genetic variants with injury in YSP. It is acknowledged that in a relatively large cohort, not all players were of the same geographic ancestry, and there is evidence that genetic variation can differ between ancestral groups (Mills et al., 2001).

Accordingly, confining analysis to the most represented ancestral group may increase the likelihood of detecting genetic associations by increasing the 'signal to noise ratio'. On the other hand, excluding players on the basis of their ancestry reduces sample size, thus reducing statistical power and the potential for finding genetic links with injury. Moreover, isolating a single geographical ancestry would not usually provide an accurate representation of the total YSP population in that country (or group of countries), and on that basis it was decided to include all participants, regardless of ethnic background. Future studies should aim to replicate these findings in similar, independent populations to increase confidence in the results of Chapter Six, and should increase the number of variants investigated in order to identify other SNPs associated with injury. Such studies may wish to employ a candidate gene approach, i.e. hypothesising which particular SNPs might be associated with specific injuries, as used in Chapter Six, though the use of genome wide association studies may aid the discovery of a greater number of individual variants (Evangelou and Ioannidis, 2013), albeit at a greater financial cost and with the requirement for considerably more participants. From a mechanistic standpoint, the use of *in vivo* and *in vitro* studies could help explain the association of SNPs with injury observed in Chapter Six and previous studies. For example, *in vivo* measurements could be used to determine whether the SNPs associated with injury also affect muscular strength or joint range of motion, whilst *in vitro* measurements could be used to investigate the effect of altered gene expression on tissue regeneration. In the first instance, future studies should aim to increase the number of

participating players, clubs and nationalities to improve statistical power and external validity.

7.5 CONCLUSION

There are relatively few studies that have explored the influence of maturation or playing position, and no previous investigations concerning the influence of genetic variation, on injury in YSP. The findings of this thesis are the first to demonstrate that post-PHV high-level YSP are at greater risk of injury than pre- and mid-PHV (Chapter Four), and that genetic variants (both individually and in combination) are associated with injury in YSP (Chapter Six). Furthermore, the work in this thesis has led to the new hypothesis that, in high-level YSP, the interaction of genetic variation with extrinsic risk factors is greater in post-PHV players than pre- and mid-PHV players, thus contributing to the greater prevalence and severity of common injuries in post-PHV. It can also be concluded that the influence of playing position on injury prevalence and severity in YSP is minimal (Chapter Five), although the classification of playing position remains a challenging task. In addition, this thesis describes the homogeneity in the rate of common injuries in YSP from different nations in different continents (Chapter Three), suggesting that the implementation of injury prevention strategies should be universal in YSP. Future research should seek to quantify the type and duration of player activity to provide greater context regarding the influence of exposure volume and injury-inciting events on the injuries sustained by YSP, and specifically, how these variables interact with the risk factors identified in this thesis. By identifying and managing ‘at risk' YSPs appropriately, it is hoped that the novel information

from this thesis could be used to help reduce injury incidence and optimise development time, thus increasing the chance of high-level YSPs reaching their full potential as professional soccer players.

CHAPTER EIGHT

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8.1 REFERENCES

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CHAPTER 9

APPENDICES

9.1 APPENDICES

APPENDIX 1 – Elite Soccer Club Gatekeeper Information Sheet

GATEKEEPER INFORMATION SHEET

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

The elite youth players from _____ are being invited to take part in a research study. Before you decide if you wish to give these players the option of participating in this research project, it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the project?
The purpose of this project is to increase our understanding of the genetic mechanisms underpinning injury within elite football players, at both youth and senior levels. Not only will this information provide the medical and coaching staff with a better understanding of their players, but it has the potential to help coaching staff tailor the training, nutrition and recovery to optimise performance, while reducing the risk of injury in players that may be more predisposed to certain types of injury.

The aims of the project are three-fold. We aim to:
a) Investigate if there is an age-associated risk of certain injuries among elite soccer players?
b) Investigate if there is a player position-specific risk of certain injuries in elite soccer players?
c) Investigate if variations of certain genes are associated with the incidence of soccer-specific injuries in elite soccer players?

What will happen to the players if they take part?
To participate in this project, the players must have been signed to the club for at least six months. If you grant permission for the players to have the option of participating in this research project, and they provide written informed consent to participate, they will be asked to provide a 2 mL saliva or 5 mL blood sample, from which we will isolate DNA and analyse the genotype of specific gene variants thought to be linked to soft tissue injuries. This test lasts about 5 minutes, is completely non-invasive, and will be conducted by either the research team or the club doctor/physiotherapist.

Anthropometry
Participants will have their height, sitting height and body mass measured by members of the research team or the club sport science department. This information, together with the participant's age, will be used to calculate the level of maturation, and is necessary to answer two of the three research questions in this project.
Questionnaire
Participants will be asked (with help from the parents if under 16 years of age) to complete a questionnaire, which will include questions about his ethnic origin, which position he plays, and what standard he has achieved so far in football. The reason we ask about ethnicity is because the differences in genetic make-up can vary between ethnic groups, so it is important to account for this.
Saliva sample
All participants under 18 years of age will be asked to provide a 2 mL saliva sample, which they will dribble into a specialised collection tube. The only restrictions placed on the participants for saliva sampling is that they do not eat or drink anything in the 30 minutes prior to giving the sample. Otherwise, there are no restrictions regarding physical activity or diet.

Blood sample
For participants aged 18 years or over, there is the possibility of providing a 5 mL venous blood sample, which will be taken by a trained phlebotomist, either a member of the research team or the club doctor. There are absolutely no restrictions placed on the participant for this test, regarding either diet or exercise. The participant will be asked to sit or lie down while the

phlebotomist draws the blood into a 5 mL EDTA blood collection tube. The blood sample will only be taken if there is the possibility to transport the blood safely and securely to Liverpool John Moores University for immediate storage, or if the club has the means to store the blood temporarily, e.g. it holds a (UK) Human Tissue Act license.

Injury records
The research team will ask the participants/parents/guardians to provide their consent for the club to send the research team the injury data recorded for that participant. It will be explained that the information will be anonymised, so that the information will not be identifiable. This will not cost the participant any time but it will, of course, require the cooperation of the club's medical team, and we are asking for your agreement to do this.

Are there any risks / benefits involved?
Blood sample
There is a small risk of infection or haemorrhage when taking venous blood samples but every precaution will be taken to minimise this risk with the use of sterile equipment and an experienced phlebotomist. The saliva collection does not come with any risks.

Taking part in this study will involve minimal time and effort from the participants. They will be contributing to the broader benefits of the study to elite footballers of different ages, i.e. by providing their DNA and information on their injury history, they will be enabling us to further our understanding of the cause of soccer specific injuries. This will potentially benefit footballers across the world, and could enable us to increase the likelihood of young talented footballers reaching their full elite potential and completing a full career in football.

Will the information from the players taking part in the study be kept confidential?
- Data remains strictly confidential between the participant and the researcher. As this is a research project, no personal genetic data will be shared with any other party, including the football club.
- Personal information will be treated in the strictest confidence with no association been made between the subjects identities and the data observed.

Data storage
- Each participant will be assigned a unique study ID code, which will be identifiable only to the principle investigator (PI) in a separate file stored securely on a Liverpool John Moores University secure server (password-controlled).
- Player injury data will be provided to the PI by the football club in confidence using a project specific, password protected data spreadsheet, and these data will be stored together with genetic information by the PI using the unique study ID codes NOT identifiable information.
- The handling and storage of all samples, e.g. saliva and blood samples, will comply with the UK Human Tissue (HT) Act. The genetic material will be stored so that it can be re-analysed for different gene variants at a later date. This form of generic consent has become the norm in UK research and is in fact recommended as good practice by international research councils and the HT Authority.
- Any identifiable information regarding the football club, yourself and the players in your club will be kept strictly confidential.

What is expected of me?
- You must decide if you wish to give the players at your football club/academy the option of participating in this research project.
- If you decide to proceed, we ask you to verbally inform the players (and the parents of the players, who are under 16 years of age) of the project and what is involved. We then ask you to distribute the invitation letter to the players (and their parents if they under 16 years old), the study information sheets and assent/consent forms for participants and for parents/guardians. There will also be a questionnaire for the participant to complete (with help from his parent if under 16). This questionnaire will ask the participant to define his

ethnic origin and his level of football achievement. If you would like the research team to send this information, please provide the relevant contact details of the players/parents.

- We then ask you to collate the returned hard copies of assent/consent forms and to collect 2 mL saliva (in the collection tubes provided by us) from the players, who have provided written informed consent (from the players and parents if players are less than 16 years old).
- We will then arrange the transportation of the collection tubes from your club/academy to Liverpool John Moores University.
- Your final task will be to collate the injury data from each of the players, who have provided written informed consent to take part in this project, and send that data (anonymised) to the investigators in Liverpool John Moores University.

What do I do now?

- If you are happy for the players in your club to participate in this project, please sign and return the **Gatekeeper Consent Form** provided
- **For participants who are aged under 16 only**, please make sure **Signed Parental Consent Forms** are collected back **BEFORE the players participate in the study.**

Contact Details of Researcher
Principle Investigator:
Dr Robert Erskine
R.M.Erskine@ljmu.ac.uk

This study has received ethical approval from LJMU's Research Ethics Committee (16/SPS/0009)

If you have any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

APPENDIX 2 – Carer (Parent/Guardian) Information Sheet

CARER (PARENT/GUARDIAN) INFORMATION SHEET

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

The elite youth players from _____ are being invited to take part in a research study led by Liverpool John Moores University. Before you decide if you wish to give your son the option of participating in this research project, it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you wish to give your son the option to take part or not.

What is the purpose of the study?
We want to see if injury risk in elite youth soccer players is greater at different stages of maturity, and if different playing positions are at greater risk of certain injuries. We also want to find out if DNA markers from a saliva sample can tell us if someone is more likely to get a certain type of soccer injury. If we find that this is the case, it could be possible for football clubs in the future to use this information to find out which of their players need extra help in preventing injuries by changing their training and recovery patterns. This should help young players remain injury free for longer and give them a greater chance of reaching their full potential as an elite senior player.

The aims of the project are:
a) To see if there is a risk of certain injuries in elite football players of different levels of maturity.
b) To see if there is a greater risk of injury due to the position of the player.
c) To see if different DNA markers from a saliva sample are linked to a player's risk of injury.

Does the player have to take part?
No. It is up to you and the player to decide whether or not to take part. If you both agree you will be given this information sheet and asked to sign a consent form. The player is still free to withdraw at any time and without giving a reason. A decision to withdraw will not affect their rights/any future treatment/service they receive.

What will happen to the players if they take part?
If you and your son give your consent for your son to participate in this research project, they will be asked to provide a 2 mL saliva, from which we will isolate DNA and analyse the genotype of specific gene variants thought to be linked to soft tissue injuries. This test lasts about 5 minutes, is completely non-invasive, and will be conducted by either the research team or the club doctor/physiotherapist.

Body measurements
Participants will have their height, sitting height and body weight measured by members of the research team or the club sport science department. This information, together with the participant's age, will be used to calculate the level of maturation, and is necessary to answer two of the three research questions in this project.

Questionnaire
We ask you to help your son complete a questionnaire, which will include questions about his ethnic origin, which position he plays, and what standard he has achieved so far in football. The reason we ask about ethnicity is because the differences in genetic make-up can vary between ethnic groups, so it is important to account for this.

Saliva sample
All participants under 18 years of age will be asked to provide a 2 mL saliva sample, which they will dribble into a specialised collection tube. The only restrictions placed on the participants for saliva sampling is that they do not eat or drink anything in the 30 minutes prior to giving the sample. Otherwise, there are no restrictions regarding physical activity or diet before or after the sample has been taken.

Injury records
You are asked to provide your consent for the football club to send the research team the injury data recorded for that participant. The data will be anonymised, so that the information will not be identifiable. This will not cost the participant any time.

Are there any risks / benefits involved?
The saliva collection and anthropometric measurements do not come with any risks. Taking part in this study will involve very little time and effort from the participants, who will be contributing to the broader benefits of the study to elite footballers of different ages, i.e. by providing their DNA and consenting for us to access information on their injury history, they will be enabling us to further our understanding of the cause of soccer specific injuries. Once we have identified robust genetic associations with specific football injuries, this information could be translated into talent development programmes. Thus, talented players could be identified at a young age as having a predisposition to certain injuries, and their training and recovery could be adapted to minimise injury risk and give them the greatest possible chance of remaining injury-free and reaching their full potential.

Will the information from the players taking part in the study be kept confidential?
- Data remains strictly confidential between the participant and the researcher. As this is a research project, no personal genetic data will be shared with any other party, including the football club.
- Personal information will be treated in the strictest confidence with no association been made between the subjects identities and the data observed.

Data storage
- Each participant will be assigned a unique study ID code, which will be identifiable only to the principle investigator (PI) in a separate file stored securely on a Liverpool John Moores University secure server (password-controlled).
- Player injury data will be provided to the PI by the football club in confidence using a project specific, password protected data spreadsheet, and these data will be stored together with genetic information by the PI using the unique study ID codes NOT identifiable information.
- The handling and storage of all samples, e.g. saliva samples, and extracted DNA, will comply with the UK Human Tissue Act legislation. The genetic material will be stored so that it can be re-analysed for different gene variants at a later date. This form of generic consent has become the norm in UK research and is in fact recommended as good practice by international research councils and the Human Tissue Authority.

What should I do now?
- If you are happy for your child to take part in this study, please ask him if he would like to take part.
- The study will have been verbally explained to your son by a member of the medical/sport science team at his football club.
- Please give the participant information sheet to your son and reiterate what the study involves, and why it is important for us to perform this research.
- Please complete the parent/carer informed consent form.
- Please help your son to complete the participant assent form.
- Please help your son complete the study questionnaire.
- Please send both assent/consent forms to the club liaison (doctor/physiotherapist/sport scientist).

Contact Details of Researcher
Further information may be obtained from the following:
Principle Investigator: Dr Robert Erskine
R.M.Erskine@ljmu.ac.uk

This study has received ethical approval from LJMU's Research Ethics Committee (16/SPS/0009)

If you have any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

APPENDIX 3 – Participant Information Sheet (Elite Soccer Player Under 16 Years old)

PARTICIPANT INFORMATION SHEET (ELITE SOCCER PLAYERS UNDER 16 YEARS OLD)

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

You are being invited to take part in a research study. Before you decide, it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part or not.

Why do we want to do this study?
We want to see if injuries in talented football players are related to the age, playing position or the genes that are passed onto us from our parents. Our genes are in almost every cell in our body and control how we look (such as eye colour and hair colour) and also how we adapt to exercise. We think that our genes can also influence the risk of getting injured. The information from this study could be used to help football clubs look after their young players, to help them stay injury free for longer, thereby giving them a greater chance of playing football at the highest level.

The study will check:
a) To see if the age of a football player increases the risk of getting an injury.
b) To see if the player's position increases the risk of a football player getting an injury.
c) To see if our genes can increase the risk of a football player getting an injury.

Do I have to take part?
No. It is up to you and your parents to decide if you take part. If you both agree, you will be and asked to read a form and write your name to say that you want to take part. One of your parents or guardians will also be asked to sign a form to say that they are happy for you to take part. You are still free to pull out at any time and without giving a reason.

What will happen to me if I take part?
Saliva sample
You will be given a special plastic tube, and someone from your football club or the research team will show you how to dribble a very small amount of saliva into this tube. We will use this saliva sample to see if our genes can increase the risk of getting a football injury. This test lasts about 5 minutes and will be supervised by someone from the football club or the research team. Before doing this, you will not be allowed to eat or drink anything for 30 minutes.

Body measurements
Someone from the football club or the research team will measure your height and weight, and record your birthday. This information will be used to see if age can increase the risk of getting an injury in football.

Questionnaire
We will ask you (with help from your parents) to answer some questions about where your family comes from, which position you play, and what the highest standard of football you have played so far.

Injury records
Your football club will send the research team information on all of the injuries you have experienced while at the club. This information will be kept secure.

Are there any risks / benefits involved?
The saliva collection and body measurements do not have any risks. Taking part in this study will involve very little time or effort. You will be helping us to understand what causes different injuries in football. This could be used to help young talented footballers stay injury-free and reach their full potential.

Will the information from the players taking part in the study be kept confidential?

- As this is a research study, no information from the saliva sample will be shared with anyone else, including the football club without the player and parent agreeing to this.
- All personal information will be kept safe and it will not be possible for somebody outside of the research project to identify the participant from the personal identification code that we will give each player.

Storing information

- All information will be stored safely and only read by members of the research team. At the end of the study, all personal identifiable information will be deleted.
- Information linking the participant with their personal study code will be stored in a safe place (on a University password-controlled computer), and read only by the lead researcher at Liverpool John Moores University.
- The handling and storage of saliva samples will be in line with UK law. The saliva samples will be re-analysed at a later date, which is normal in scientific research.

Contact Details of Researcher

Further information may be obtained from the following:

Principle Investigator: Dr Robert Erskine
R.M.Erskine@ljmu.ac.uk

This study has received ethical approval from LJMU's Research Ethics Committee (16/SPS/0009)

If you have any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

APPENDIX 4 – Participant Information Sheet (Elite Soccer Players 16 Years or Older)

PARTICIPANT INFORMATION SHEET (ELITE SOCCER PLAYERS 16 YEARS OR OLDER)

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

You are being invited to take part in a research study. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part or not.

What is the purpose of the study?
We want to see if injury risk in elite youth soccer players is greater at different stages of maturity, and if different playing positions are at greater risk of certain injuries. We also want to find out if DNA markers from a saliva sample can tell us if someone is more likely to get a certain type of soccer injury. If we find that this is the case, it could be possible for football clubs in the future to use this information to find out which of their players need extra help in preventing injuries by changing their training and recovery patterns. This should help young players remain injury free for longer and give them a greater chance of reaching their full potential as an elite senior player.

The aims of the project are:
a) To see if there is a risk of certain injuries in elite football players of different levels of maturity.
b) To see if there is a greater risk of injury due to the position of the player.
c) To see if different DNA markers from a saliva sample are linked to a player's risk of injury.

Do I have to take part?
No. It is up to you to decide whether or not to take part. If you agree you will be given this information sheet and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw will not affect your rights/any future treatment/service you receive.

What will happen to me if I take part?
To participate in this project you must have been a signed player for this club for at least 6 months. If you give your consent to participate in this research project, you will be asked to provide a 2 mL saliva, from which we will isolate DNA and analyse the genotype of specific gene variants thought to be linked to soft tissue injuries. This test lasts about 5 minutes, is completely non-invasive, and will be conducted by either the research team or the club doctor/physiotherapist.

Anthropometry
Participants will have their height, sitting height and body mass measured by members of the research team or the club sport science department. This information, together with the participant's age, will be used to calculate the level of maturation, and is necessary to answer two of the three research questions in this project.

Saliva sample
All participants under 18 years of age will be asked to provide a 2 mL saliva sample, which they will dribble into a specialised collection tube. The only restrictions placed on the participants for saliva sampling is that they do not eat or drink anything in the 30 minutes prior to giving the sample. Otherwise, there are no restrictions regarding physical activity or diet before or after the sample has been taken.

Blood sample
For participants aged 18 years or over, there is the possibility of providing a 5 mL venous blood sample, which will be taken by a trained phlebotomist, either a member of the research team or the club doctor. There are absolutely no restrictions placed on the participant for this

test, regarding either diet or exercise. The participant will be asked to sit or lie down while the phlebotomist draws the blood into a 5 mL EDTA blood collection tube. The blood sample will only be taken if there is the possibility to transport the blood safely and securely to Liverpool John Moores University for immediate storage, or if the club has the means to store the blood temporarily, e.g. it holds a (UK) Human Tissue Act license.

Questionnaire
We ask you to complete a short questionnaire, which will include questions about his ethnic origin, which position you play, and what standard he has achieved so far in football. The reason we ask about ethnicity is because differences in genetic make-up can vary between ethnic groups, so it is important to account for this.

Injury records
You are asked to provide your consent for the football club to send the research team the injury data recorded for that participant. The data will be anonymised, so that the information will not be identifiable. This will not cost the participant any time.

Are there any risks / benefits involved?
The saliva collection and anthropometric measurements do not come with any risks. There is a small risk of infection or haemorrhage (profuse bleeding) when taking venous blood samples but every precaution will be taken to minimise this risk with the use of sterile equipment and an experienced phlebotomist.

Taking part in this study will involve very little time and effort from the participants. They will be contributing to the broader benefits of the study to elite footballers of different ages, i.e. by providing their DNA and information on their injury history, they will be enabling us to further our understanding of the cause of soccer specific injuries. This will potentially benefit footballers across the world, and could enable us to increase the likelihood of young talented footballers reaching their full elite potential and completing a full career in football.

Will the information from the players taking part in the study be kept confidential?
- Data remains strictly confidential between the participant and the researcher. As this is a research project, no personal genetic data will be shared with any other party, including the football club.
- Personal information will be treated in the strictest confidence with no association been made between the subjects identities and the data observed.

Data storage
- Each participant will be assigned a unique study ID code, which will be identifiable only to the principle investigator (PI) in a separate file stored securely on a Liverpool John Moores University secure server (password-controlled).
- Player injury data will be provided to the PI by the football club in confidence using a project specific, password protected data spreadsheet, and these data will be stored together with genetic information by the PI using the unique study ID codes NOT identifiable information.
- The handling and storage of all samples, e.g. saliva and blood samples, and extracted DNA, will comply with the UK Human Tissue Act legislation. The genetic material will be stored so that it can be re-analysed for different gene variants at a later date. This form of generic consent has become the norm in UK research and is in fact recommended as good practice by international research councils and the Human Tissue Authority.

Contact Details of Researcher
Further information may be obtained from the following:
Principle Investigator: Dr Robert Erskine
R.M.Erskine@ljmu.ac.uk

This study has received ethical approval from LJMU's Research Ethics Committee (15/SPS/009)

If you have any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

APPENDIX 5 – Consent Form For Elite Football Club Gatekeeper

LIVERPOOL JOHN MOORES UNIVERSITY

ELITE FOOTBALL CLUB GATEKEEPER

CONSENT FORM

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

Principle Investigator: Dr Robert Erskine

PLEASE TICK IN THE BOXES ON THE RIGHT IF YOU AGREE WITH EACH STATEMENT

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily

2. I understand that the participation of all players is voluntary and that they are free to withdraw at any time, without giving a reason and that this will not affect their legal rights.

3. I understand that any personal information collected during the study will be anonymised and remain confidential

4. I agree to give the participants/participant carer the option to provide consent for the removal, storage and use of the anonymised saliva/blood samples to allow the DNA analyses to be carried out.

5. I agree to give the participants/participant carer the option to provide consent for the football club to provide the research team with information on the participant's injury history.

6. I give consent to allow the participant/participant carer the option to provide consent to the investigation of unlimited DNA analyses from the anonymised participant tissue samples

7. I agree to give consent to allow the participant/participant carer the option to take part in the above study

Name of Participant	Date	Signature

Name of Researcher	Date	Signature

Name of Person taking consent *(if different from researcher)*	Date	Signature

APPENDIX 6 – Consent Form for Carer of Elite Soccer Player Younger Than 16 Years Old

LIVERPOOL JOHN MOORES UNIVERSITY

CONSENT FORM

Carer of elite soccer player younger than 16 years

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

Participant ID number: #

Principle Investigator: Dr Robert Erskine

PLEASE TICK IN THE BOXES ON THE RIGHT IF YOU AGREE WITH EACH STATEMENT

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my son's participation is voluntary and that he is free to withdraw at any time, without giving a reason and that this will not affect my legal rights.

3. I understand that any personal information collected during the study will be anonymised and remain confidential.

4. I give consent for the storage and use of my sons' saliva sample to allow the investigation of unlimited DNA analyses to be carried out.

5. I give consent to allow my son's saliva sample to be used for future genetics projects.

6. I give consent for my football club to pass on my injury information to the Principle Researcher in an anonymised format.

7. I agree to take part in the above study

Name of Parent/Guardian of Participant	Date	Signature

Name of Researcher	Date	Signature

Name of Person taking consent *(if different from researcher)*	Date	Signature

APPENDIX 7 – Assent form for Elite Soccer Player Under 16 Years Old

LIVERPOOL JOHN MOORES UNIVERSITY

ASSENT FORM FOR CHILDREN

(to be completed by the child and their parent/guardian)

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

Participant ID number: #

Principle Investigator: Dr Robert Erskine

Child (or if unable, parent/guardian on their behalf) / young person to circle all they agree with:

Have you read (or had read to you) information about this project?	Yes/No
Has somebody else explained this project to you?	Yes/No
Do you understand what this project is about?	Yes/No
Have you asked all the questions you want?	Yes/No
Have you had your questions answered in a way you understand?	Yes/No
Do you understand it's OK to stop taking part at any time?	Yes/No
Are you happy to take part?	Yes/No

If any answers are 'no' or you **don't** want to take part, don't sign your name!

If you **do** want to take part, you can write your name below

Your name _____

Date _____

Your parent or guardian must write their name here if they are happy for you to do the project.

Print Name _____

Sign _____

Date _____

The researcher who explained this project to you needs to sign too.

Print Name _____

Sign _____

Date _____

APPENDIX 8 – Consent Form for Elite Soccer Player Over 16 Years Old

LIVERPOOL JOHN MOORES UNIVERSITY

CONSENT FORM

Elite soccer player over 16 years old

RESEARCH PROJECT TITLE: The genetic association with soccer injuries

Liverpool John Moores University Research Ethics Committee approval number: 16/SPS/0009

Participant ID number: #

Principle Investigator: Dr Robert Erskine

PLEASE TICK IN THE BOXES ON THE RIGHT IF YOU AGREE WITH EACH STATEMENT

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights.

3. I understand that any personal information collected during the study will be anonymised and remain confidential

4. I give consent for the storage and use of my saliva or blood sample to allow the investigation of unlimited DNA analyses to be carried out.

5. I give consent to allow my saliva or blood sample to be used for future genetics projects.

6. I give consent for my football club to pass on my injury information to the Principle Researcher in an anonymised format.

7. I agree to take part in the above study

Name of Participant	Date	Signature

Name of Researcher	Date	Signature

Name of Person taking consent *(if different from researcher)*	Date	Signature

Questionnaire: Ethnic origin and soccer achievement

PROJECT TITLE: The genetic association with soccer injuries (16/SPS/0009)

Thank you for your interest in this research study. Prior to participation, we would like you to answer a few questions concerning your general health and physical activity level. Please answer the following questions as honestly as you can.

Participant ID code: Date of birth: _____

_____

Gender (please circle): Male / Female Height:

_____

Nationality: Body weight:

_____ _____

What is your ethnic group? Please circle ONE section from **A** to **F**, then tick the appropriate box to indicate your background.

A) White: English ☐ Scottish ☐ Welsh ☐ N. Irish ☐ Other ☐

If other, please state here: _____

B) Mixed: White and Black Caribbean ☐ White and Black African ☐ White and Asian ☐ Other ☐

If other, please state here: _____

C) Asian: Indian ☐ Pakistani ☐ Chinese ☐ Japanese ☐ Other ☐

If other, please state here: _____

D) Black: Caribbean ☐ African ☐ Other ☐

If other, please state here: _____

E) Other ethnic background: ☐ Please state here:

_____

F) I do not wish to state my ethnic origin ☐

Questions concerning your footballing achievement

1. What is/was your main playing position (If multiple, please state preferred)?

   _____

   _____

2. What is the highest level you have played football?:

   _____

   _____

Thank you for completing this questionnaire. All information will be kept strictly confidential.