The Physiological and Genetic Factors Underpinning the Response to Muscle Damaging Exercise

PHILIPP BAUMERT

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

December 2018

Authors Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of Liverpool John Moores University. Apart from the help and advice acknowledged, the work within was solely completed and carried out by the author.

Any views expressed in this thesis are those of the author and in no way represent those of Liverpool John Moores University and the School of Sport and Exercise Sciences. This thesis has not been presented to any other University for examination either in the United Kingdom or overseas. No portion of the work referred to in this research project has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

Signed

Date

Abstract

It is often observed that there is a high individual variability in the response to exercise-induced muscle damage (EIMD), even when tested in a homogeneous cohort accounting for age, sex, ethnicity and physical activity. The response to EIMD is very complex as several tissues, including skeletal muscle fibres, the extra-cellular matrix (ECM), and tendon, play a potential role in the damage response. Therefore, the overall aim of this PhD thesis was to investigate the physiological and genetic factors underpinning the response to muscle damaging exercise. For that, the following objectives were (i) to comprehensively assess the physiological mechanisms and recovery pattern of neuromuscular fatigue of the hamstring muscle group following an intermittent sprint (IS) intervention; (ii) to investigate inter-individual differences in skeletal muscle repair/recovery after an artificial wounding (scratch) assay using of primary human skeletal muscle cells *in vitro*; (iii) to ascertain whether multiple genetic variations, which are linked to varying tissues, forming a polygenic profile could distinguish between high and low responders following muscle damage *in vivo* and *in vitro*; and (iv) to assess whether a genetic profile is linked with the response to both EIMD and chronic resistance exercise. The methodological and analytical approaches utilised in this thesis identified a number of important, novel and impactful findings. Following IS, the impaired hamstring muscle function and delayed recovery is probably caused primarily by damage to the contractile tissue, and participants with a greater force generating capacity (larger physiological cross-sectional area) of the biceps femoris long head were less susceptible to hamstring strength loss immediately after IS, providing evidence that the structure of the muscle protects it against peripheral fatigue/damage. The *in vitro* study showed that skeletal muscles with an increased number of stem cells of the connective tissue (fibroblasts) might have a better capacity to reorganise the complex ECM, which results in a faster muscle strength recovery after muscle damaging exercise. However, a larger number of active muscle stem cells (myoblasts) seems to be important for the latter stage of muscle regeneration. Individuals possessing a non-preferential genetic profile demonstrated increased rate of muscle damage biomarkers than individuals

with a preferential genetic profile. Lastly, we calculated a second polygenic profile which was linked with both the EIMD and the chronic resistance exercise response. These polygenic profiles may be used to anticipate an individual's response/adaptation to EIMD and to chronic resistance exercise, thus enabling resistance exercise to be prescribed on a personalised level to improve muscle health and function.

Acknowledgements

A person learns only in two ways. By acquiring knowledge and through association with quality people. Therefore, I would like to acknowledge and thank my brilliant supervisory team for their continuous support and mentorship throughout my PhD, both on a personal and professional level. Dr Mark Lake, who cheered my up during tough times and taught me not to take everything too seriously; Prof Barry Drust, who has shown me to see the bigger picture of my studies; Prof Claire Stewart, who has helped me with the long term vision of my projects, and, of course, my Director of Studies, Dr Robert Erskine. Alongside all the techniques, the most important thing Rob taught me is to have confidence in myself. Rob, I am grateful for the way in which you gave me support and space in perfect balance so that I was able to develop personally and as a scientist under your great supervision in a positive work environment at LJMU.

I would also like to thank the participants for donating their time and effort, and the technical & office staff members for doing a great job of supporting us students. A special mention should go to my family, and in particular to my Mum and Dad. I knew I could always rely on you for help and advice. Further thanks go to all friends and colleagues (especially of the old post graduate room 1.47 and Sam Temple, who became a close friend and explained me all the details of UK football during our data collection) that have supported me both academically and socially throughout my PhD. Without you all, these last four years would not have been as enjoyable as they have proven to be. No road is long with good company!

I would like to give special thanks to the biomechanics research group, as all of the biomechanical researchers (e.g. Raihana Sharir, Niels Nedergaard and Jasper Verheul) have been unbelievable supportive. Finally, I would like to thank Marie-Isabel, who is still by my side after my PhD, including a long-distance relationship between two different countries for more than two years. There are not enough words or ways to express how grateful I am, and I am looking forward to the next chapter of our life, which we will go through together.

Table of Contents

I List of Figures

II List of Tables

III Abbreviations

1-RM	One-repetition maximum
ACE	Angiotensin-I converting enzyme
ACTN3	Gene that encodes the α-actinin-3 protein
AGT	Gene that encodes Angiotensinogen
ANOVA	Analysis of variance
CCL2	Chemokine (C-C motif) ligand-2
CCR2	Chemokine (C-C motif) receptor type -2
CE	Resistance exercise
CK	Creatine kinase
COL	Gene that encodes the collagen protein
DES	Gene that encodes the desmin protein
DNA	Deoxyribonucleic acid
d_{PT}	Patellar tendon moment arm
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EMG	Electromyography
FDR	False discovery rate
G-REX	Genetics of Recovery after EXercise cohort
G-ResisT	Genetics of RESIStance Training cohort
IGF	Insulin-like growth factor
IKD	Isokinetic dynamometer
IL	Interleukin
IS	Intermittent sprint
LD	Linkage disequilibrium
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid

MVC	Maximal voluntary contraction
MG	Moderate genotypes group
MTU	Muscle-tendon unit
MYLK	Gene that encodes the myosin light chain kinase
MyoD	Myogenic differentiation factor
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NOS	Gene that encodes the nitric oxide synthase
NPG	Non-preferential genotype group
Pax7	Paired box protein-7
PCSA	Physiological cross-sectional area
PG	Preferential genotype group
RE	Resistance Exercise
ROM	Range of motion
ROS	Reactive oxygen species
SEM	Standard error of mean
SD	Standard deviation
SLC30A8	Gene that encodes the solute carrier family 30 (zinc transporter) member eight protein
SNP	Single nucleotide polymorphism
TGS	Total genotype score
TNF	Tumour necrosis factor
TRIM63	Tripartite Motif Containing 63 gene
TTN	Gene that encodes the titin protein
VDR	Vitamin D receptor
W_{peak}	*In vivo* peak power output

1 General Introduction

Sporting success is the result of the relative contributions of nature (heritability) and nurture (environmental) factors, and the diverse environments of different sports are likely to act as agents of natural selection (Kujala et al., 2000). The current understanding suggests above 50% of the variance for an elite athlete status can be accounted for by genetic variation (De Moor et al., 2007, Georgiades et al., 2017). An athlete's potential to succeed at the highest level of sport may be determined in part by their physical resilience, such as the capability to recover, and their responsiveness to training stimuli (Durand-Bush and Salmela, 2002, Reilly and Williams, 2003). For instance, highly dynamic skills, such as high-speed running and landing in soccer, require eccentric (muscle lengthening) contractions, in order to control the deceleration of the joint (Stanton and Purdam, 1989, Padulo et al., 2013). This can result in ultrastructural muscle damage manifested in Z-line disturbance, as well as disruption of the extracellular matrix (ECM), which provides structural scaffolding for muscle remodelling and has an integral role in force transmission (Tidball, 1991, Garg and Boppart, 2016). However, some individuals experience stiff or sore muscles, which typically occurs 24-72 hours after exercise, whilst others do not feel any muscle soreness despite performing the same relative intensity of exercise. Several factors have been reported, which contribute to this individual differences, including age (Fielding et al., 1991), ethnic origin (Sherwood et al., 1996), sex (Sewright et al., 2008), training level (Balnave and Thompson, 1993), and nutrition (Howatson and Van Someren, 2008). Nonetheless, when all these factors are taken into account, it still cannot entirely explain the large variation in the response to exercise-induced muscle damage (EIMD), and a number of investigations suggest that multiple genetic variations may play a (key) role in influencing a person's susceptibility to EIMD (Baumert et al., 2016a). Insufficient recovery of previously fatigued and damaged muscles, e.g. caused by an exposure to a number of maximal sprints, is thought to increase muscle injury risk (Malone et al., 2016, Malone et al., 2018, Chumanov

et al., 2011, Duhig et al., 2016). Given the impact of such injuries, it is important to understand their manifestation, in order to minimise their occurrence.

Skeletal muscle regeneration is a complex process that is mediated by muscle stem cells (i.e. satellite cells). Furthermore, recent investigations have reported an important role for muscle connective tissue stem cells (known as fibroblasts) during skeletal muscle regeneration following EIMD (Mackey et al., 2017, Murphy et al., 2011) and muscle injuries (Joe et al., 2010). However, little is known about the interplay between satellite cells and muscle fibroblasts regarding skeletal muscle regeneration following *physiological* EIMD within the first days, and whether specific genetic polymorphisms influence their dynamics and impact on skeletal muscle regeneration.

Chronic resistance exercise (RE), i.e. resistance training, is a potent stimulus for increasing muscle size and strength, which are both important for athletic performance (Harries et al., 2012, Granacher et al., 2016), and are also increasingly recognised as a cornerstone for the prevention (e.g. Nordic hamstrings) of muscle (Petersen et al., 2011) and ACL injuries (Petushek et al., 2018, Webster and Hewett, 2018). However, as the rate of non-contact injuries is only reduced but still occur in some athletes, it could be suggested that prevention training has a variable effect on athletes. It is possible that individuals who show a detrimental response to acute RE (e.g. a high inflammatory response and elevated muscle soreness), might possess a non-preferential genetic profile, which also leads to an attenuated adaptation with respect to gains in muscle strength and hypertrophy following chronic RE, potentially leading to a higher risk of non-contact injuries.

Gene–environment interactions likely account for a large portion of the “missing heritability” of genetic associations with traits and diseases. In concert with large-scale genome-wide association studies (GWAS) (Willems et al., 2017, Zillikens et al., 2017), additional studies with homogenous cohorts and controlling for lifestyle factors and comprehensive phenotype assessments are necessary to understand the complex interplay between multiple genetic polymorphisms (i.e. a polygenic profile) and environmental variables affecting athletic performance (Munafò and Smith, 2018).

The experiments, on which this thesis is based, used the strategy of multiple approaches (triangulation). By using a combination of assessments from different research disciplines, including genetics, cell biology, muscle physiology and biomechanics, the overarching aim of this PhD thesis was to investigate the physiological and genetic factors underpinning the response to EIMD. In the first two experimental chapters, the causes and consequences of EIMD were comprehensively assessed, whilst the last two experimental chapters investigated the association between genetic variations and the acute and chronic response following EIMD. More precisely, the objectives of this PhD thesis were:

1. To characterise the physiological mechanisms underpinning neuromuscular fatigue and the recovery pattern following intermittent maximal sprints, to acquire a better understanding of EIMD in an applied setting (Chapter 3).
2. To determine the effect that inter-individual differences in the ratio of skeletal muscle myoblast to fibroblast composition has on skeletal muscle repair/recovery after an artificial wounding (scratch) assay using primary human skeletal muscle cells *in vitro*, and the association of this ratio with recovery after intermittent maximal sprints (Chapter 4).
3. To ascertain whether a polygenic profile could distinguish between high and low responders following a controlled *in vivo* eccentric exercise (acute resistance exercise, RE) intervention in previously untrained individuals, and whether those genetic variations were also associated with the artificial wounding (scratch) assay *in vitro* (Chapter 5). This would, therefore, provide a genetic link between *in vivo* and *in vitro* muscle damage, thus potentially shedding new light on the aetiology of EIMD.
4. To determine whether the polygenic profile associated with *acute* RE from Chapter 5 could anticipate individual adaptations to *chronic* RE, in terms of gains in muscle size and strength (Chapter 6) and

5. To ascertain whether a new polygenic profile associated with the adaptations to chronic RE (gains in muscle size and strength) (Chapter 6) could estimate the individual responses to acute RE in Chapter 5 (e.g. loss of strength, increase in soreness, inflammation, etc.). Objectives 4 and 5 have implications for furthering our understanding of the genetic and physiological mechanisms underpinning and linking the *acute* and *chronic* responses to RE.

2 Literature Review: Genetic Variation and Exercise-induced Muscle Damage: Implications for Athletic Performance and Injury

*Publications resulted from this chapter**:***

1. *Baumert, P., Lake, M. J., Stewart, C. E., Drust, B., & Erskine, R. M. (2016). Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. European journal of applied physiology, 116(9), 1595-1625.*
2. *Baumert, P., Lake, M. J., Stewart, C. E., Drust, B., & Erskine, R. M. (2016). Inter-individual variability in the response to maximal eccentric exercise. European journal of applied physiology, 116(10), 2055.*

2.1 ABSTRACT

Prolonged unaccustomed exercise involving muscle lengthening (eccentric) actions can result in ultrastructural muscle disruption, impaired excitation–contraction coupling, inflammation and muscle protein degradation. This process is associated with delayed onset muscle soreness and is referred to as exercise-induced muscle damage. Although a certain amount of muscle damage may be necessary for adaptation to occur, excessive damage or inadequate recovery from exercise-induced muscle damage can increase injury risk. Furthermore, it is apparent that inter-individual variation exists in the response to exercise-induced muscle damage, and there is evidence that genetic variability may play a key role. Although this area of research is in its infancy, certain gene variations, or polymorphisms have been associated with exercise-induced muscle damage (i.e. individuals with certain genotypes experience greater muscle damage, and require longer recovery, following strenuous exercise). These polymorphisms include *ACTN3* (R577X, rs1815739), *TNF* (-308 G>A, rs1800629), *IL6* (-174 G>C, rs1800795), and *IGF2* (ApaI, 17200 G>A, rs680). Knowing how someone is likely to respond to a particular type of exercise could help coaches/practitioners individualise the exercise training of their athletes/patients, thus maximising recovery and adaptation, while reducing overload-associated injury risk. The purpose of this review is to provide a critical analysis of the literature concerning gene polymorphisms associated with exercise-induced muscle damage, and to highlight the potential mechanisms underlying these associations, thus providing a better understanding of exercise-induced muscle damage.

2.2 INTRODUCTION

People who engage in unaccustomed, strenuous physical exercise can experience stiff or sore muscles, a feeling that is usually apparent for 24-72 hours after exercise. This phenomenon is known as delayed-onset muscle soreness. Several investigations have revealed that these unaccustomed eccentric actions, during which the muscle is lengthened while it is active, provoke stiffer and more tender muscles compared to concentric or isometric contractions (Armstrong, 1984, Armstrong et al., 1991). These contractions are strongly associated with damage to skeletal muscle consisting of structural disruption of sarcomeres, disturbed excitation-contraction coupling and calcium signalling, leading to an inflammatory response and the activation of several muscle protein degradation pathways. This process has been referred to as exercise-induced muscle damage (Hyldahl and Hubal, 2014, Peake et al., 2005) and is normally accompanied by swelling, a temporary reduction in both maximum strength and range of motion (Baird et al., 2012, Brown et al., 1999, Clarkson et al., 1992). Circulating muscle-specific proteins [e.g. creatine kinase (CK) myoglobin and α-actin] are commonly used to indicate exercise-induced muscle damage (Huerta-Alardín et al., 2005, Martinez Amat et al., 2007), whereas tenascin-C is thought to be an indicator for disruption of the overlying connective tissue and the extracellular matrix (Flück et al., 2003).

Exercise-induced muscle damage can be divided into the initial damage phase, which occurs during the exercise bout, and the secondary damage phase, which is linked with the delayed inflammatory response (Kuipers, 1994, Howatson and Van Someren, 2008). These phases are eventually followed by muscle remodelling (Flann et al., 2011, Thiebaud, 2012, Tidball, 2005). Although there is evidence to suggest that a certain amount of muscle damage is a positive stimulus for muscle restructuring, hypertrophy and strength gains (Roig et al., 2008), in rare cases, strenuous unaccustomed exercise can lead to exertional rhabdomyolysis, which is characterized by muscle fibre necrosis (Warren et al., 2002b). Intracellular muscle contents leak into the circulation and extracellular fluid, which can lead to kidney failure or even to death (Knochel, 1990, Clarkson et al., 2005b).

From the plethora of studies that have investigated exercise-induced muscle damage, it is apparent that variability in the response to muscle damaging exercise exists between (Vincent et al., 2010, Clarkson et al., 2005b) and within studies (Nosaka and Clarkson, 1996). Variations between studies can occur due to different study population, age, gender and a small sample size (Eynon et al., 2013, Toft et al., 2002). However, intra-study variation within a homogenous cohort warrants further consideration, with evidence to suggest that genetic variability may play a role. Some genes have common variations in sequence, known as polymorphisms, which, depending on where this polymorphism occurs within the gene, can directly affect gene expression and ultimately the amount of protein produced. The most common type of sequence variation is a single nucleotide polymorphism (SNP), where one nucleotide substitutes another. Another type of common sequence variation is the insertion/deletion (indel) polymorphism, in which a specific nucleotide sequence is present (insertion) or absent (deletion) from the allele. Some polymorphisms can modify the protein product, thus potentially altering function. It follows, therefore, that polymorphisms of genes encoding key proteins in the muscle-tendon unit (such as the *ACTN3* R577X SNP) have implications for the ability to recover from strenuous exercise, thus influencing the risk of injury. This may be particularly relevant in elite athlete groups, who are known to have different genetic profiles compared to the general population (Yang et al., 2003, Myerson et al., 1999). Moreover, specific gene polymorphisms (e.g. *COL1A1* rs1800012, *COL5A1* rs12722, rs3196378, *MMP3* rs679620, rs591058 and rs650108) have been associated with tendon/ligament injury prevalence (e.g. Achilles tendinopathy/rupture and anterior cruciate ligament rupture) (Bell et al., 2012, Laguette et al., 2011, Collins and Raleigh, 2009). However, very little is known about the potential genetic association with muscle damage and muscle regeneration in response to muscle damaging exercise or the mechanisms that underpin that association.

Knowing who requires longer to recover from a bout of strenuous exercise, may help practitioners prescribe personalised exercise medicine to their patients, thus optimising health and reducing the risk of injury and further muscle wasting. One of the greatest

challenges facing exercise genetic research is the investigation of functionally relevant genetic variation and of their mechanisms of action. The aims of this review are to (i) provide a critical review of the current literature on exercise-induced muscle damage and, therefore, to improve our understanding of the different phases of the responses to muscle damaging exercise; (ii) emphasize those studies that have investigated the association between genetic variation and muscle damage; and (iii) propose mechanistic explanations that may underpin these associations.

2.3 GENETIC VARIATION AND THE INITIAL PHASE OF EXERCISE-INDUCED MUSCLE DAMAGE

Exercise-induced muscle damage can result in damage to the ultrastructure of the muscle fibre (including Z-line streaming), to the extracellular matrix, and to overextended sarcomeres and t-tubules of skeletal muscle tissue (Brown et al., 1997b, Kjær, 2004, Friden and Lieber, 1992, Friden et al., 1981, Friden and Lieber, 2001). Structural disruption of sarcomeres is thought to be caused by the heterogeneity of sarcomere length (Morgan, 1990) and, consequently, some sarcomeres resist eccentric actions more than others (Allen et al., 2005, Friden et al., 1981). Prolonged strain causes weaker sarcomeres to be stretched beyond the optimum overlap of actin and myosin filaments (Figure 2-1). This results in popped sarcomeres and appears as a broadening, smearing or even disruption of the Z-lines. Interestingly, the thinnest Z-lines are detected in type II muscle fibres, which generate the highest shortening velocities, while the widest Z-lines are found in slow-twitch muscle fibres (Knoll et al., 2011). Consequently, fast-twitch fibres are more sensitive than slow twitch fibres to Z-disk streaming (Proske and Morgan, 2001, Appell et al., 1992). This mechanical damage is one mechanism by which a prolonged loss of strength occurs immediately after excessive strain (Cheung et al., 2003, Hyldahl and Hubal, 2014, Friden and Lieber, 1992).

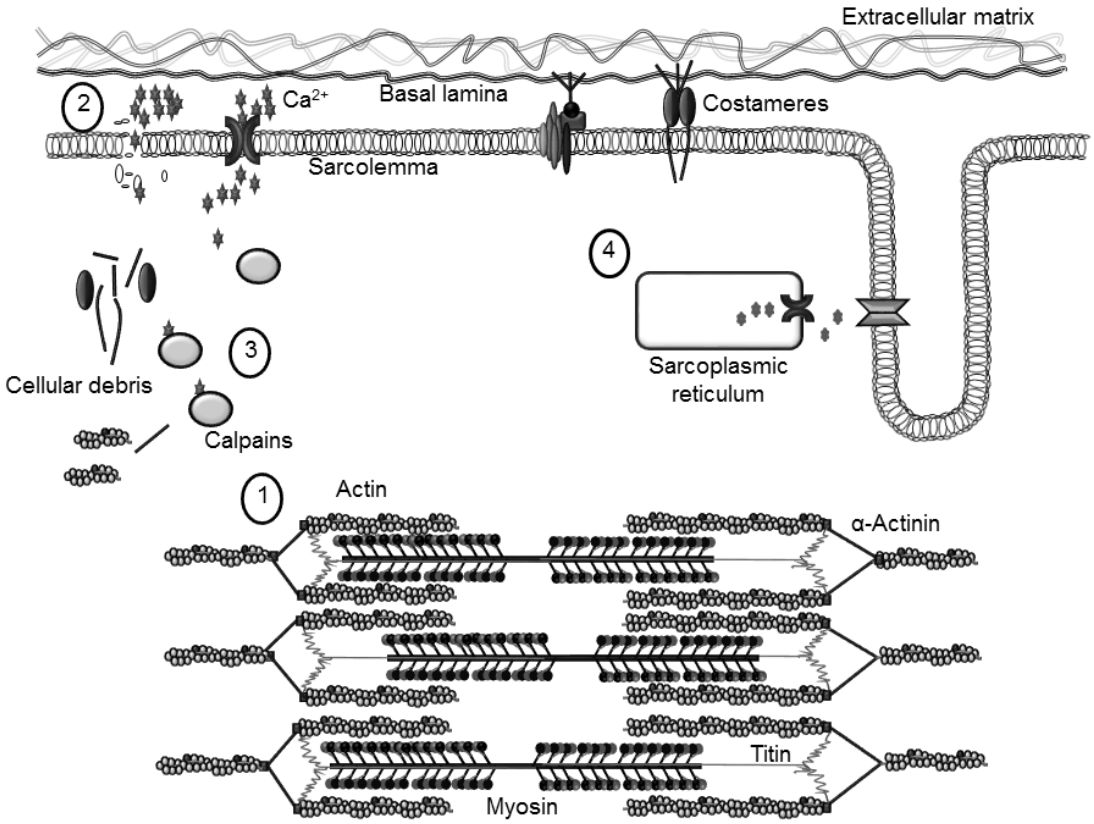

Figure 2-1 Initial phase of exercise-induced muscle damage. *Due to different abilities of each sarcomere to resist eccentric actions, some of the sarcomeres will be stretched beyond the optimum overlap of actin and myosin filaments, resulting in Z-line streaming (Morgan, 1990) (1). This is accompanied by increased permeability of the sarcolemma (2). Extracellular Ca^{2+} influx into the muscle fibre activates different Ca^{2+}-sensitive proteases (calpains). Calpain activation leads to proteolysis of cytoskeletal and costameric proteins (Thiebaud, 2012) (3). However, a failure of excitation-contraction coupling also seems to play an important role in strength loss following strenuous exercise, as murine muscle exposed to caffeine revealed an attenuated loss of muscle strength (Warren, Lowe et al. 1993) (4). Figure adapted from Hyldahl and Hubal (2014).*

The transmission of muscle fibre force to the tendon (leading to joint movement) not only occurs in the longitudinal direction in line with the direction of pull of the tendon, but also in the lateral direction (between adjacent fibres to the overlying connective tissue and extracellular matrix) (Kjær, 2004, Hughes et al., 2015). The extracellular matrix in skeletal muscle provides structural and biochemical support to the contractile tissue, and is associated with the inflammatory response and satellite cell activation (see section 2.5) (Hyldahl and Hubal, 2014, Kjær, 2004). The relative proportion of different collagen subtypes in the extracellular matrix of skeletal muscle and tendon varies depending on the position and function of the connecting tissues (Kjær, 2004, Duance et al., 1977, Davis et

al., 2013). The contractile apparatus is connected to the extracellular matrix by costameres (structural complexes comprising proteins such as dystrophin, focal adhesion kinase and integrins) and by intermediate filament proteins, such as desmin (Hughes et al., 2015). According to Ramaswamy et al. (2011), more than 80% of muscle force is transferred via this lateral pathway. Thus, costameres, intermediate filament proteins and the extracellular matrix are considered essential for the integrity of skeletal muscle and the maintenance of lateral force transmission. Furthermore, they are thought to play an important role in injury prevention by stabilizing the myofilaments (Lovering and De Deyne, 2004, Stauber et al., 1990, Hughes et al., 2015). The degradation of cytoskeletal, costameric and extracellular matrix proteins could negatively influence the lateral transmission of force between adjacent muscle fibres, which could, at least in part, be the source of the prolonged decrease of maximum strength seen following strenuous exercise (Raastad et al., 2010).

Activation of Ca^{2+} proteases (calpains) appears to play an important part in the muscle damage-repair process. Damage to the sarcolemma results in the accumulation of excess intracellular Ca^{2+}, which activates different calcium-sensitive proteases, which are mostly localized at the I band and Z disk regions of myofibrils (Belcastro et al., 1998). The activation results in proteolysis within minutes of cytoskeletal and costameric proteins (Thiebaud, 2012, Lovering and De Deyne, 2004, Boppart et al., 2008, Zhang et al., 2008, Allen et al., 2005), and calpain activity is still measurable three days after exercise-induced muscle damage (Raastad et al., 2010). This intra- and extracellular damage requires the removal and repair of the damaged proteins, and is therefore followed by an inflammatory response and by activation of the ubiquitin–proteasome pathway (see section 2.4) (Wei et al., 2005, Tidball, 2005). However, the loss of strength after eccentric muscle contractions was reversed by exposing mouse muscles to caffeine (Balnave and Allen, 1995, Warren et al., 1993). Caffeine facilitates the influx of free intracellular Ca^{2+} from sarcoplasmic reticulum into the cytosol of the muscle (Warren et al., 1993, Proske and Morgan, 2001). This phenomenon cannot be explained by damage to the sarcomere, so it can be concluded that sarcomere damage is not the only cause of strength loss, as impaired ECC also appears to

play a role (Cheung et al., 2003, Hyldahl and Hubal, 2014). Increased permeability of the sarcolemma, due to damaged muscle fibre structure, metabolic disturbance, and fibre remodelling, is likely to be the main reason for elevated plasma CK and myoglobin (Kjær, 2004, Baird et al., 2012).

A repeated bout of the same eccentric exercise causes significantly fewer symptoms, such as a lower sensation of pain and almost no increase in serum CK activity plus faster recovery of muscle function (Brown et al., 1997a). This well-established phenomenon is referred to as the repeated bout effect and can last up to six months (Nosaka et al., 2001). A repeated bout of strength training results in a different expression of genes, which are involved in pro- and anti-inflammatory responses, leading to reduced inflammation (Gordon et al., 2012). There is also evidence that the repeated bout effect, at least in part, is based on restructuring of the muscle after damage (McHugh, 2003). Likewise, extracellular matrix remodelling is believed to be associated with protection of skeletal muscle against future damage which is indicated by an increase in gene expression of collagen types I and III and laminin-β2 (Mackey et al., 2011). This is thought to occur in line with muscle remodelling of intermediate filaments and the addition of sarcomeres in series (leading to longer fibres) (Friden et al., 1984, Armstrong, 1990, Hyldahl and Hubal, 2014).

Considering all of the above, candidate SNPs influencing the initial phase of contraction-induced damage are likely to be functional SNPs of genes encoding key structural proteins within the sarcomere, the extracellular matrix and the costameric protein complexes linking the two. The following sections will highlight the evidence to support this hypothesis. Table 2-1 summarises every candidate SNP, which has been discussed in this review.

Table 2-1 *Gene polymorphisms associated with exercise-induced muscle damage.*

Gene polymorphism	Subjects	Exercise performed	'Protective' allele	Reference
ACE (I/D) (rs4646994)	Moderately active young men and women	50 unilateral eccentric elbow flexion contractions	D	Yamin et al. (2007)

	Physically active young men and women	Step up exercise for 5 min followed by 15 knee bends with a backpack weighted at 30% of their body weight	-	Heled et al. 2007
ACTN3 R577X (rs1815739)	Untrained healthy young men and women	50 unilateral eccentric elbow flexion contractions	-	Clarkson et al. (2005b)
	Untrained healthy young men	4 series of 20 bilateral maximal eccentric knee extensions	R	Vincent et al. (2010)
	Wild type & *Actn3* knockout mice	Eccentric contractions on isolated extensor digitorum longus muscles at 30% stretch	R	Seto et al. (2011)
	Professional male soccer athletes	Plyometric leg exercise	R	Pimenta et al. (2012)
	Moderately active young men	Two bouts of 50 drop jumps separated by two weeks	X	Venkunas et al. (2012)
	Male and female patients	Retrospective cohort study for risk of exertional rhabdomyolysis	R	Deuster et al. (2013)
	Female athletes	Retrospective cohort study for risk of muscle injury	X	Iwao-Koizumi et al. (2014)
CCL2 -3441(C>T) (rs3917878)	Untrained healthy young males & females	50 unilateral eccentric elbow flexion contractions	C	Hubal et al. (2010)
CCL2 -289 (G>C) (rs2857656)	Elite soccer players	Retrospective cohort study for risk of non-contact musculoskeletal soft tissue injuries	C	Pruna et al. (2013)
CCR2 -941(A>C) (rs3918358)	Healthy untrained men and women	50 unilateral eccentric elbow flexion contractions	A	Hubal et al. (2010)
CCR2 4439 (T>C) (rs1799865)	Healthy untrained men and women	50 unilateral eccentric elbow flexion contractions	T	Hubal et al. (2010)

CKM NcoI (A>G) (rs1803285)	Moderately active young men and women	Step up exercise for 5 min followed by 15 knee bends with a backpack weighted at 30% of their body weight	G	Heled et al. (2007)
	Moderately active young men and women	50 unilateral eccentric elbow flexion contractions	-	Yamin et al. (2010)
	Healthy men and women of different ages	4-21 km running race	-	Miranda-Vilela et al. (2012)
	Male and female patients	Retrospective cohort study for risk of exertional rhabdomyolysis	A	Deuster et al. (2013)
IGF2 13790 (C>G) (rs3213221)	Healthy untrained men and women	50 unilateral eccentric elbow flexion contractions	C	Devaney et al. (2007)
IGF2 17200 (G>A) (rs680)	Healthy untrained men and women	50 unilateral eccentric elbow flexion contractions	G	Devaney et al. (2007)
IGF2AS 1364 (A>C) (rs4244808)	Healthy untrained men and women	50 unilateral eccentric elbow flexion contractions	C	Devaney et al. (2007)
IGF2AS 11711 (G>T) (rs7924316)	Healthy untrained men and women	50 unilateral eccentric elbow flexion contractions	G	Devaney et al. (2007)
IL1B -3737 (C>T) (rs4848306)	Healthy untrained men	3 sets of 8 contractions at 80% of the subject's maximal voluntary contraction followed by a 4th set to voluntary failure for leg press, leg curl, and leg extension, respectively	C	Dennis et al. (2004)
IL1B -511 (C>T) (rs16944)	Healthy untrained men	3 sets of 8 contractions at 80% of the subject's maximal voluntary contraction followed by a 4th set to voluntary failure for	-	Dennis et al. (2004)

		leg press, leg curl, and leg extension, respectively		
	(Non-) professional athletes versus control	Cross-sectional study	-	Cauci et al. (2010)
IL1B 3954 (C>T) (rs1143634)	Healthy untrained men	3 sets of 8 contractions at 80% of the subject's maximal voluntary contraction followed by a 4th set to voluntary failure for leg press, leg curl, and leg extension, respectively	T	Dennis et al. (2004)
	(Non-) professional athletes versus control	Cross-sectional study	-	Cauci et al. (2010)
IL6 -174 (G>C) (rs1800795)	Moderately active young men and women	50 unilateral eccentric elbow flexion contractions	G	Yamin et al. (2008)
	Male and female patients	Retrospective cohort study for risk of exertional rhabdomyolysis	-	Deuster et al. (2013)
	Older obese women	7 sets of 10 bilateral eccentric knee extensions with a load corresponding to 110% of 10-repetitions maximum.	C	Funghetto et al. (2013)
INS 1045 (C>G) (rs3842748)	Healthy untrained men and women	50 unilateral eccentric elbow flexion contractions	C	Devaney et al. (2007)
MLCK 49 (C>T) (rs2700352)	Untrained healthy young men and women	50 unilateral eccentric elbow flexion contractions	C	Clarkson et al. (2005b)
MLCK 37885 (C>A) (rs28497577)	Untrained healthy young men and women	50 unilateral eccentric elbow flexion contractions	C	Clarkson et al. (2005b)

	Male and female patients	Retrospective cohort study for risk of exertional rhabdomyolysis	C	Deuster et al. (2013)
OPN -66 (T>G) (rs28357094)	Healthy untrained men and women	24 unilateral eccentric elbow flexion contractions	T	Barfield et al. (2014)
SLC30A8 (C>T) (rs13266634)	Untrained healthy young men and women	50 unilateral eccentric elbow flexion contractions	T	Sprouse et al. (2014)
SOD2 (C>T) (rs4880)	Healthy male and female volunteers of different ages	4-21 km running race	C	Akimoto et al. (2010)
TNF -308 (G>A) (rs1800629)	Moderately active young men and women	50 unilateral eccentric elbow flexion contractions	A	Yamin et al. (2008)

2.3.1 Alpha-Actinin-3 R577X Polymorphism and the Initial Phase of Exercise-Induced Muscle Damage

Of all the polymorphisms that have been associated with exercise-induced muscle damage, the most investigated is the *ACTN3* R577X SNP (Clarkson et al., 2005b, Deuster et al., 2013, Pimenta et al., 2012, Seto et al., 2011, Venckunas et al., 2012, Vincent et al., 2010) (Table 2-1). The protein isoforms α-actinin-2 and α-actinin-3 are crucial components of the Z-line in mammalian skeletal muscle and anchor actin filaments to the Z-lines, cross-linking the thin filaments to the adjacent sarcomeres (Mills et al., 2001, North et al., 1999, Blanchard et al., 1989). Whilst α-actinin-2 is ubiquitously expressed in skeletal muscle, α-actinin-3 is only expressed in fast twitch fibres of human skeletal muscle (North and Beggs, 1996, North et al., 1999). A functional SNP (rs1815739; substitution of a C with a T nucleotide) results in an abortive stop codon (X-allele) rather than the expression of the amino acid arginine (R-allele) at amino acid 577 of exon 16 on chromosome 11, resulting in an individual being either RR, RX or XX genotype. As a consequence, XX homozygotes are not able to express the protein α-actinin-3 (MacArthur and North, 2004, North and

Beggs, 1996, North et al., 1999). A sub-section of the population is XX homozygous, ranging from less than 1 % in African Bantus to 18 % in Europeans, to 25 % in Asian populations (Mills et al., 2001). Absence of α-actinin-3 does not result in a disease phenotype due to compensatory up-regulation of α-actinin-2 (North et al., 1999) but there is evidence that this nonsense SNP affects physical performance (Erskine et al., 2014b, Niemi and Majamaa, 2005, Clarkson et al., 2005a, Moran et al., 2007).

The *ACTN3* XX genotype has been associated with smaller muscle volume (Erskine et al., 2014b), slower baseline sprint times (Niemi and Majamaa, 2005, Moran et al., 2007), lower strength (Erskine et al., 2014b, Clarkson et al., 2005a), and lower muscle power (Clarkson et al., 2005a, Seto et al., 2011, Walsh et al., 2008, Moran et al., 2007, Erskine et al., 2014b). These findings are supported by *Actn3* knock-out mouse models, demonstrating a shift in the properties of fast muscle fibres towards a more oxidative fast fibre profile, lower muscle strength, reduced mass and decreased diameter of IIb fibres (Chan et al., 2011, MacArthur et al., 2008, MacArthur et al., 2007). Strong evidence has been presented that, as a consequence of the up-regulation of α-actinin-2 in XX homozygotes, more calsarcin-2 is bound to α-actinin-2 and less to calcineurin (Seto et al., 2013). The binding affinity of calsarcin-2, which functions as an inhibitor of calcineurin activation, is greater for α-actinin-2 compared to α-actinin-3. Consequently, a higher level of free calcineurin is able to activate the downstream signalling of the slow myogenic programme. Given the larger size, higher force and power generating capacity, and lower fatigue resistance of type II fibres compared to type I fibres (Bottinelli et al., 1996), the evidence presented by Seto et al. (2013) provides a mechanistic explanation for the associations between *ACTN3* genotype and muscle size, strength, power, and endurance phenotypes.

Recent investigations have suggested that α-actinin-3 may be evolutionarily optimized for the minimization of muscle damage (Yang et al., 2003). The majority of the human studies support the hypothesis that XX homozygotes are more susceptible to strenuous exercise compared to their RR or RX counterparts (Pimenta et al., 2012, Vincent et al., 2010, Deuster et al., 2013). For instance, *ACTN3* XX homozygotes are approximately three times more

likely to develop exertional rhabdomyolysis compared to people of RR or RX genotypes (Deuster et al., 2013). However, other studies have revealed no differences between *ACTN3* genotypes regarding markers of muscle damage (Clarkson et al., 2005b), or have shown contrary effects post-exercise (Venckunas et al., 2012) or in muscle injury risk (Iwao-Koizumi et al., 2014). The cross-sectional study of Clarkson et al. (2005b) revealed no differences in strength loss but a lower baseline CK activity in the blood in *ACTN3* XX homozygotes compared to carriers of the *ACTN3* R-allele. These baseline differences in CK activity may have been due to *ACTN3* genotype-dependent differences in muscle mass (i.e. smaller muscle volume in XX homozygotes versus R-allele carriers) (Erskine et al., 2014b).

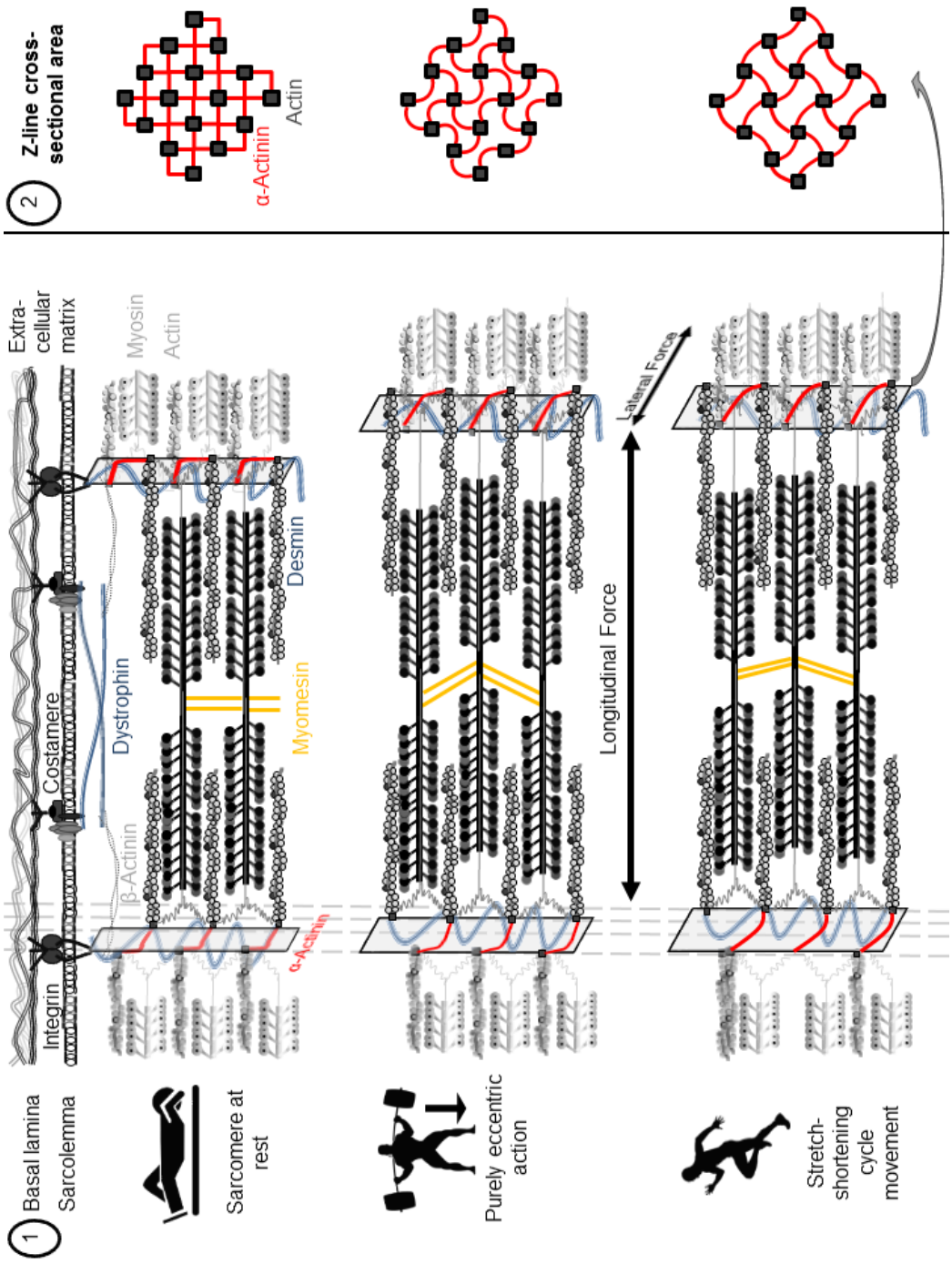

Figure 2-2 Proposed changes in sarcomere structure during stretch-shortening cycle movements and purely eccentric actions, focussing on α-actinin (highlighted in red). *The left-hand side shows the sarcomere longitudinally in a quasi-3D model at rest, and the α-actinin elongation during purely eccentric actions, and stretch-shortening cycle movements (1). The right-hand side illustrates the sarcomere cross-sectional area at the level of the Z-line (2). At rest, α-actinin is set to roughly 90° between the antiparallel actin filaments, while under active tension, the space between the antiparallel actin filaments increases and α-actinin is stretched to a basket-weave lattice (Gautel, 2011b). Alpha-actinin is thought to play a key role in the longitudinal (via the anchoring of actin filaments to the Z-line) and lateral (via costamere fibre-to-fibre interaction) transmission of muscle fibre force (Hughes et al. 2015; Yang and Xu 2012). Moreover, human type II muscle fibres from* ACTN3 *XX homozygotes (where α-actinin-3 deficiency is compensated by the presence of α-actinin-2) are less stiff than type II muscle fibres from* ACTN3 *R-allele carriers (Broos et al., 2012). Thus, it is likely that α-actinin-2 is able to store more energy than α-actinin-3 during the active stretch phase of the stretch-shortening cycle, which is released during the shortening phase (Kjær, 2004; Yang and Xu, 2012). We propose that stretch-shortening cycle movements increase the actin*

*filament spacing to a greater extent compared to purely eccentric actions, thus elongating α-actinin to become almost completely straight at peak eccentric force. Individuals with α-actinin-3 deficiency (*ACTN3 *XX homozygotes) might, therefore, benefit from having a more elastic Z-line during stretch-shortening cycle movements compared to R-allele carriers (Broos et al., 2012), resulting in a reduced damage response to stretch-shortening movements (Venckunas et al., 2012). Figure adapted from Gautel (2011b).*

Movements with repeated stretch-shortening cycles, eccentric followed by immediate concentric muscle contraction) (Venckunas et al., 2012) seem to have a different demand profile for the muscle-tendon unit compared to purely eccentric actions (Figure 2-2) (Seto et al., 2011, Vincent et al., 2010). Due to the fact that α-actinin is linked to both the longitudinal and lateral transmission of force (Hughes et al., 2015, Yang and Xu, 2012), we propose that α-actinin-3 deficiency (XX genotype) with a more elastic Z-line (Broos et al., 2012) might result in benefits to stretch-shortening cycle movements compared to R-allele carriers. Although the stretch-shortening cycle includes an eccentric element, contrary to the type of maximal eccentric contractions typically used in exercise-induced muscle damage studies, the force and the eccentric phase involved in the active braking phase of stretch-shortening cycles are generally fast and of short duration (Nicol et al., 2006). Interestingly, muscle activation decreases with increasing velocity in the eccentric phase under stretch-shortening cycle conditions (Benoit and Dowling, 2006), which indicates that other non-contractile (elastic) structures, such as the extracellular matrix/tendon, might provide an important contribution to the power output by storing energy (Kjær, 2004, Yang and Xu, 2012). Indeed, a highly compliant elastic musculotendinous system is thought to elevate the use of elastic strain energy in stretch-shortening cycle movements (Wilson et al., 1991). Thus, individually performed eccentric actions with greater longitudinal force transmission might damage the link between the contractile structure and the Z-line, which might activate the calpain system to a greater extent.

The transmission of muscle fibre force to the tendon may occur faster by the stiffer Z-line including α-actinin-3 in the longitudinal direction (Hughes et al., 2015, Broos et al., 2012) and, also, might reduce muscle damage in eccentric actions performed without a stretch-shortening cycle compared to the α-actinin-3 deficient fibres (Seto et al., 2011, Vincent et

al., 2010). Head et al. (2015) revealed a significantly increased sarcoplasmic reticulum Ca^{2+} pumping and leakage in *ACTN3* XX homozygotes, which was probably due to a higher expression of the specific Ca^{2+} channel sarco(endo)plasmic reticulum calcium-adenosine-triphosphatase-1 gene, and of the Ca^{2+} binding proteins, calsequestrin and sarcalumenin, in the sarcoplasmic reticulum (Head et al., 2015). Increased dynamics with elevated intracellular Ca^{2+} levels during and after exertional muscle damage may lead to increased cytoskeletal damage and membrane disruption (Zhang et al., 2008, Head et al., 2015, Quinlan et al., 2010). Muscle damage induced by exclusively performed eccentric actions might lead to increased desmin degradation (Yu, 2013), which results in fewer connections with the extracellular matrix and adjacent myofibrils, and could be an explanation for the higher susceptibility of XX homozygotes in this mode of exercise. Taken together, the different effect of the *ACTN3* R577X SNP in diverse mode of exercises could explain the fact that studies show mixed results. This may be why there are differences in *ACTN3* genotype frequency in short and long distance athletes of stretch-shortening cycle-related sports (e.g. running) (Yang et al., 2003), whereas both short and long distance athletes in power sports, commonly carried out without stretch-shortening cycles (e.g. swimming), show no difference in genotype/allele frequency distribution (Ben-Zaken et al., 2015). This demonstrates why future studies should not only distinguish between power and endurance athletes, but should focus on sport-specific movements when investigating the association with genetic variation.

2.3.2 Myosin Light Chain Kinase Polymorphisms and the Initial Phase of Exercise-Induced Muscle Damage

Every myosin head is connected with two light chains on the long lever arm, which are known as the essential and the regulatory light chains. In skeletal and cardiac muscles of mammals, troponin and tropomyosin have the role of triggering the contraction following the increase in free cytosolic Ca^{2+}, while the regulatory light chain modulates Ca^{2+} activation (Sweeney et al., 1993, Cheung et al., 2003, Lossie et al., 2014). Repeated Ca^{2+} influx due

to muscular contraction activates myosin light chain kinase, and this enzyme phosphorylates the regulatory light chains. It has been shown that regulatory light chain phosphorylation results in increased Ca^{2+} sensitivity (Szczesna et al., 2002), which increases the rate of force development predominantly in type II muscle fibres (Childers and McDonald, 2004). This might be the result of an increased number of force-generating cross-bridges. However, the increased force output by light chain phosphorylation might also result in elevated muscle damage, which has been shown in skinned fast-twitch fibres (Childers and McDonald, 2004).

Two different SNPs of the myosin light chain kinase gene [49 (C>T) (rs2700352) and 37885 (C>A) (rs28497577)] have been investigated concerning exercise-induced muscle damage (Clarkson et al., 2005b). T-allele carriers of the 49 (C>T) SNP have shown increased baseline strength in comparison to CC carriers but TT homozygotes revealed increased circulatory levels of the muscle damage biomarkers (CK and myoglobin) following eccentric exercise. Furthermore, A-allele carriers of the 37885 (C>A) SNP have revealed greater muscle strength loss and increased plasma CK following strenuous exercise. This is in line with the findings of Deuster et al. (2013), who showed that exertional rhabdomyolysis cases are about 5 times more likely for the A-allele of the 37885 (C>A) SNP of the myosin light chain kinase gene compared to carriers of the C-allele. The mechanisms, however, are unclear. Clarkson et al. (2005b) suggested that these SNPs may alter regulatory light chain phosphorylation, thus leading to higher muscle strain and subsequently greater muscle damage following strenuous exercise.

2.3.3 Muscle-Specific Creatine Kinase Polymorphisms and the Initial Phase of Exercise-Induced Muscle Damage

The creatine kinase enzyme is expressed in the cytosol and mitochondria of tissues with high energy consumption (e.g. skeletal muscle fibres). The cytosolic enzyme is composed of the two subunits muscle type (M) and brain type (B), which provide three different combination possibilities: CK-BB (predominantly in brain), CK-MB (in cardiac muscle) and

CK-MM (in skeletal muscle). Skeletal muscle-specific CK is bound to the M-Line structure and to the sarcoplasmic reticulum of myofibrils (Wallimann et al., 1992, Brancaccio et al., 2007). In healthy individuals, most serum CK consists of skeletal muscle CK (Brancaccio et al., 2007). Creatine kinase can leak from muscle fibres into the circulation following the mechanical tearing of the sarcolemma and opening of stretch-activated channels following contraction-induced damage, although the exact mechanism is still unclear (Allen et al., 2005).

The skeletal muscle CK-encoding gene is located at the 19q13.2–13.3 region of the chromosome 19 (Nigro et al., 1987). The NcoI (A>G) SNP (rs1803285) of the muscle creatine kinase gene, is mapped to the 3' untranslated region, which means it could affect the localization, translation efficiency and stability of the mRNA, which might mediate the location and function of the protein (Wilson et al., 1995). Interestingly, the genes for the ryanodine receptor 1 (Robinson et al., 2006) and myotonic dystrophy protein kinase (Brunner et al., 1989), which are associated with muscle function and specific myopathies, are mapped to the same area of chromosome 19. According to Deuster et al. (2013), NcoI GG homozygotes are present in 28.1 % of African Americans, in 14.2 % of Caucasians, 0 % of Hispanic and 8.3 % of Asian individuals. Investigations of the NcoI SNP of the muscle creatine kinase gene have revealed different outcomes. In the study of Deuster et al. (2013), GG homozygotes were reportedly 3.1 times more likely to experience exertional rhabdomyolysis than carriers of the A-allele. However, Heled et al. (2007) revealed that *NcoI* AA homozygotes had a six-fold higher risk of being a high responder of circulating CK to eccentric exercise than GG or AG genotypes. Other studies do not support a role for the NcoI SNP of the muscle creatine kinase gene in explaining the CK variability between individuals (Miranda-Vilela et al., 2012, Yamin et al., 2010). However, the mechanism remains poorly understood and is confounded by the different methodological designs implemented by researchers. Furthermore, Heled et al. (2007) and Yamin et al. (2010) have only investigated CK-response as a marker for muscle damage. Further studies with several other muscle damage markers such as muscle strength loss and soreness could provide a

better physiological/systems-based understanding of the influence of this NcoI SNP on exertional muscle damage. An additional restriction fragment length polymorphism, the TaqI SNP of the muscle creatine kinase gene, has been shown to be in strong linkage disequilibrium with the NcoI SNP (Miranda-Vilela et al., 2012). The TaqI 1-2 genotype has indicated a lower risk for inflammation after a track event between 4 and 21 km, whereby the participants could choose their preferential distance. However, no further studies have been undertaken towards understanding a potential role for this SNP in association with muscle damage. It is possible that these SNPs change the half-life of the CK enzyme and the intracellular concentration of CK (Heled et al., 2007). Elevated intracellular CK concentration might increase calpain activation, thus resulting in greater protein degradation.

2.4 GENETIC VARIATION AND THE SECONDARY PHASE OF EXERCISE-INDUCED MUSCLE DAMAGE

The secondary phase of muscle damage is a complex event that has been linked to inflammation (Schoenfeld, 2010), where leucocytes infiltrate muscles with damaged fibres and remain there for days or even weeks (Tidball, 2005). Although the results of published studies are inconsistent (Schneider and Tiidus, 2007), *in vitro* (Kanda et al., 2013, Suzuki and Ford, 1999) and *in vivo* studies (Paulsen et al., 2010) support a role for neutrophils in muscle damage. It is assumed that neutrophils (Suzuki et al., 1996) migrate to the region of injury in the early stage of muscle damage (Figure 2-3). Neutrophils contribute to the degradation of damaged muscle tissue by producing reactive oxygen species (ROS), which are thought to attract macrophages to the area of trauma (McGinley et al., 2009, Nguyen and Tidball, 2003).

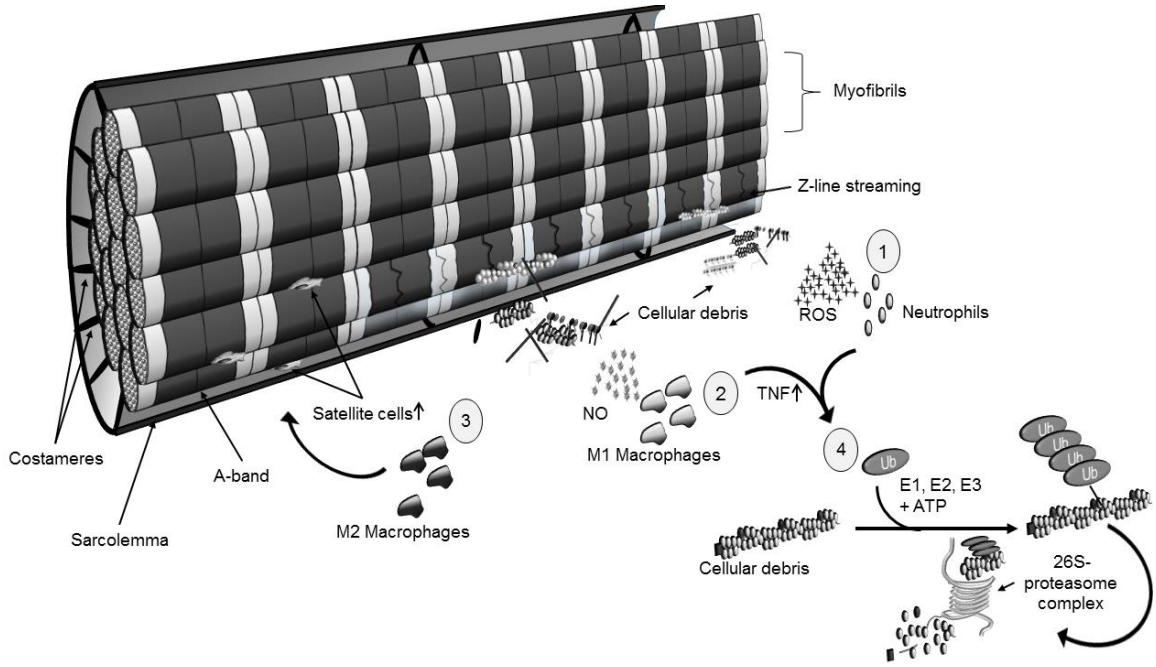

Figure 2-3 The secondary phase of muscle damage. *Leucocytes infiltrate the site of myotrauma (Tidball 2005). Firstly, neutrophils migrate to damaged muscle fibres and produce reactive oxygen species (ROS) to degrade cellular debris (Suzuki et al. 1996) (1). Neutrophils are substituted by macrophages within 24 hours (Malm et al. 2000), with M1 macrophages removing cellular debris by producing cytotoxic levels of nitric oxide (NO) (2). In the latter stage of muscle damage, a shift from M1 to M2 macrophages is associated with the activation of satellite cells and the subsequent regeneration of muscle fibres (Tidball 2011) (3). Neutrophils and macrophages also express tumour necrosis factor (TNF), which activates the ubiquitin–proteasome pathway (Tidball and Villalta 2010) (4). This pathway regulates proteolysis by attaching ubiquitin polymers (Ub) to cellular debris via three different types of enzymes (E1 - E3 ligases). As a result, these ubiquitin-marked proteins will be degraded by the 26S-proteasome complex (Reid 2005).*

Reactive oxygen species can directly and indirectly modulate muscle damage through several mechanisms (Toumi et al., 2006). A potential mechanism to link oxidative stress with calpain-mediated proteases is via ROS decreasing plasma membrane Ca^{2+}-adenosine-triphosphatase activity (Siems et al., 2003), which might encourage Ca^{2+} accumulation within the cell (Powers and Jackson, 2008). Although ROS is toxic, it may also play an important role as a secondary messenger in cell signalling and in the regulation of gene expression resulting in ROS-mediated adaptation to exercise (Schoenfeld, 2012, Hornberger et al., 2003, Crane et al., 2013).

In contrast to neutrophils, there is strong evidence that macrophages and monocytes infiltrate the endomysium and especially the perimysium of the injured area of the muscle (Hubal et al., 2008, Paulsen et al., 2010). Macrophages replace neutrophils within 24 hours

and remain present for up to 14 days after exercise (Malm et al., 2000). During the early stages of muscle damage, there is an increase of M1 macrophages (which express CD68 surface marker but not CD163), supporting the removal of cellular debris by producing cytotoxic levels of nitric oxide. This is followed by a shift from M1 to M2 macrophages ($CD68^{-}$/ $CD163^{+}$), which promote the activation of satellite cells and the subsequent regeneration of muscle fibres (see section 2.5) (Mahoney et al., 2008, Kanda et al., 2013, Tidball and Villalta, 2010, Philippou et al., 2012, Zanou and Gailly, 2013).

Leucocyte accumulation and the following remodelling appear to be gradual processes regulated by the extent of damage (Paulsen et al., 2010, Paulsen et al., 2012). In an extreme case of muscle damage, remodelling may become maladaptive characterized by necrosis, incomplete healing, and fibrotic scar tissue formation (Butterfield, 2010). Cytokines play particularly well-characterized roles in an orchestrated regulated fashion of the activation and modulation of the inflammatory response (Paulsen et al., 2012). Recent investigations revealed that some cytokines are also expressed by skeletal muscle, and are therefore named myokines (Pedersen et al., 2003). The role of cytokines in the phase of inflammation following exercise-induced muscle damage is explained in the comprehensive review of Paulsen et al. (2012). Cytokines are classified as (i) pro-inflammatory cytokines [promoting inflammation, e.g. interleukin (IL)-1α, IL-1β and tumour necrosis factor (TNF)]; (ii) anti-inflammatory cytokines (inhibiting inflammation, e.g. IL-10, IL-4 and IL-13) and chemokines (abbreviated from chemotactic cytokines), which attract leucocytes and other cells to migrate from the blood to the region of injury [e.g. chemokine (C-C motif) ligand 2 (CCL2)] (Paulsen et al., 2012, Peake et al., 2005, Suzuki et al., 2002). Muscle cytokine expression after strenuous exercise is predominantly pro-inflammatory (Peake et al., 2005).

In addition, some cytokines such as IL-6 can act either as a pro- or an anti-inflammatory agent, depending on the environment (Pedersen and Febbraio, 2008). The majority of cytokines are released from several cell types including muscle fibres, fibroblasts, neutrophils, and macrophages, and the expression of cytokines is determined by the mode, intensity and duration of exercise (Peake et al., 2015). Furthermore, the action patterns of

some of these cytokines change during the inflammatory response. These findings make it difficult to identify the specific roles of each cytokine after exercise-induced muscle damage (Smith et al., 2008). However, the invading neutrophils and macrophages express TNF at the early phase of the inflammatory response (Philippou et al., 2012, Tidball and Villalta, 2010, Warren et al., 2002a). Tumour necrosis factor is able to activate the ubiquitin–proteasome pathway, which is one of the main mechanisms for the cellular protein degradation in eukaryotic cells (Murton et al., 2008, Li et al., 2005). The ubiquitin–proteasome pathway regulates proteolysis by attaching ubiquitin polymers to damaged proteins via three distinct types of enzymes (known as E1 - E3 ligases). Subsequently, the 26S-proteasome complex degrades the ubiquitin-marked protein (Reid, 2005). Tumour necrosis factor increases the gene expression of the E3 ligases, muscle ring finger 1 (MuRF1) and muscle atrophy F-box (MAFbx; also referred to as Atrogin1) (Li et al., 2005, Li et al., 2003, Murton et al., 2008, Bodine et al., 2001b). Thus, it is thought that TNF is an important factor in the instigation of the remodelling process after exertional muscle damage (Murton et al., 2008).

2.4.1 Interleukin-1 Polymorphisms and the Secondary Phase of Exercise-Induced Muscle Damage

The interleukin-1 *(IL1)* family of cytokine genes is located together on chromosome 2, and includes IL-1α *(IL1A)*, IL-1β *(IL1B)* and IL-1 receptor antagonist (IL-1Ra; *IL1RN*) (Dennis et al., 2004). Interleukin-1α and IL-1β are agonists of the IL-1 receptor type I (IL-1R1) and promote inflammation. In general, IL-1β acts synergistically with TNF and induces the expression of several other pro-inflammatory genes (Dinarello, 2009). Following eccentric exercise in humans, systemic levels of IL-1β increase marginally (Peake et al., 2005), but there is an increase of local IL-1β levels within skeletal muscle up to 5 days post exercise (Fielding et al., 1993). In contrast, IL-1Ra acts as an antagonist of IL-1R1, preventing the binding of IL-1α and IL-1β with IL-1R1, respectively. Instead of IL-1β, IL-1Ra is highly concentrated in plasma following intense physical exercise (Paulsen et al., 2012). In the

absence of IL-1Ra, the activity of IL-1 is unrestricted and leads to increased inflammatory response (Dinarello, 2009).

Different SNPs of the *IL1B gene* have been investigated in relation to the response to exercise and exercise-induced muscle damage: (i) at position -511 (C>T) (rs16944) in the promoter region (di Giovine et al., 1992); (ii) at position +3954 (C>T) (rs1143634) in exon 5 (TaqI restriction site polymorphism) (Bioque et al., 1995); (iii) at position -3737 (C>T) (Dennis et al., 2004, Vangsted et al., 2011). Dennis et al. (2004) investigated the associations of selected *IL1* SNPs with the inflammatory response following a single bout of resistance exercise. Twenty-four sedentary Caucasian males were recruited based on specific clusters of *IL1* SNPs (haplotypes) (+4845 *IL1A*, +3954 *IL1B*, -511 *IL1B*, and -3737 *IL1B* polymorphisms). Only participants with the *IL1B* C/C (+3954) or with the T/T (-3737) genotype showed an increased inflammatory response (changes in inflammatory associated cytokines and M1 macrophages number) in skeletal muscle. However, the concentration of macrophages did not change. This leads to the assumption that the cytokine release by each macrophage is elevated or local production by the skeletal muscle itself is increased. Individuals with the above-mentioned genotypes, who also carried the C-allele of the *IL1RN* +2018 (T>C) SNP, demonstrated a further increase of inflammatory response following resistance exercise.

Cauci et al. (2010) found that the *IL1B* +3954 (C>T) SNP, together with the -511 (C>T), have no influence on athletic phenotype, which is in accordance with the findings that neither plasma IL-1β nor *IL1B* mRNA is influenced by physical activity (Petersen and Pedersen, 2005, Mahoney et al., 2008). In addition, a multi-allelic insertion polymorphism in intron 2 of the *IL-1RN* gene (rs380092) contains a variable number tandem repeat of an 86-bp length of DNA (Mansfield et al., 1994). Allele 2 (two repeats of the 86 bp region) of the *IL1RN intron 2* variable number tandem repeat was significantly more frequent in athletes compared to non-athletes. In addition, there was a higher frequency distribution of the 1/2 (allele 1 with four repeats and allele 2 with two repeats of the 86 bp region) genotype variable number tandem repeat *IL1RN* in high-grade professional athletes than in non-

professional athletes. In contrast, the frequency of *IL1RN* allele 2 homozygotes did not differ between athletes and non-athletes. Unfortunately, this study has only distinguished between professional (high-grade), non-professional (medium-grade) athletes, and non-athletes. Athlete status was not discriminated within the different types of sport, which is necessary, as different mode of exercises require different physical traits. However, *in vitro* investigations showed that the *IL1RN* allele 2 has been associated with a lower expression of IL-1Ra (Dewberry et al., 2000), but increased production of the pro-inflammatory cytokine IL-1β (Santtila et al., 1998). Cauci et al. (2010) suggested that carriers of *IL1RN* allele-2 displayed a moderate increase of IL-1-dependent inflammation, which results in benefits to athletic performance. *IL1RN* allele 2 might support the removal of cellular debris, promoting a faster recovery. However, *IL1RN* allele 2 homozygotes may lead to a sharp increase of inflammation, which negatively influences the recovery or remodelling. Further investigation is necessary to confirm these findings.

2.4.2 Tumour Necrosis Factor -308 G>A Polymorphism and the Secondary Phase of Exercise Induced Muscle Damage

Tumour necrosis factor (formerly known as tumour necrosis factor-α) is a pro-inflammatory cytokine with short half-life and low circulating levels (Reid and Li, 2001, Pedersen, 2011) and is associated with the occurrence of metabolic disorders (Borst, 2004). Plomgaard et al. (2005) have shown that TNF infusion in healthy individuals alters insulin signalling transduction and subsequently induces insulin resistance in skeletal muscle. Like IL-1β, systemic TNF concentration does not change or is only slightly increased after intense exercise (Peake et al., 2015). However, local expression of TNF within the skeletal muscle is significantly elevated after exercise (Peake et al., 2015). Tumour necrosis factor is associated with up-regulation of catabolic pathways and suppression of protein synthesis in skeletal muscle (Ling et al., 1997), mediated by NF-κB, which stimulates the ubiquitin-proteasome pathway (Reid and Li, 2001). This is in line with Tiainen et al. (2012), who have shown that high plasma levels of TNF are associated with reduced physical performance in

men. Furthermore, intravenous infusion of TNF in rats led to a significant drop in systemic IGF-I and IGF-binding proteins 3 levels, suggesting a negative influence of TNF on the IGF-system (Llovera et al., 1998).

The minor A-allele of the rare *TNF* -308 (rs1800629) SNP is associated with increased plasma TNF concentration (Karimi et al., 2009) and with impaired improvement of physical performance in older women following physical activity (Pereira et al., 2013). Presumably, the A-allele is a stronger activator of TNF transcription than the G-allele (Wilson et al., 1997). To the best of our knowledge, only one study has investigated an association between the *TNF* -308 (G>A) SNP and its association with exercise-induced muscle damage. Interestingly, carriers of the A-allele showed a non-significant (P = 0.06) blunting of elevated plasma CK following eccentric exercise (Yamin, 2009, Yamin et al., 2008). However, no AA homozygotes were included in this investigation. The *TNF* -308 A-allele was associated with higher plasma TNF concentration and impaired improvements in physical fitness following chronic exercise in older populations, while in young, healthy individuals, A-allele carriers demonstrated blunted CK activity in the blood after eccentric exercise. However, CK activity was measured at the peak activity 96 hours post-exercise in Yamin et al. (2008). The blunted CK activity of *TNF* -308 A-allele carriers in the study by Yamin et al. (2008) might not be attributed to the muscle damage itself but may be caused by attenuated remodelling, such as myoblast fusion which is accompanied by CK activity (Zalin, 1972). Due to the fact that membrane damage might be repaired in a short time (Bansal et al., 2003), other mechanisms should be considered for the prolonged leakage of CK. Elevated TNF attenuates myoblast fusion and differentiation which might impair the regeneration of the muscle (Stewart et al., 2004). Subsequently, carriers of the *TNF* -308 A-allele might have a higher susceptibility to muscle atrophy and sarcopaenia due to the impaired ability of muscle remodelling. However, Lappalainen (2009) has indicated some technical limitations of the assay which might have influenced the data interpretation of Yamin et al. (2008). Further studies are needed, which investigate a potential association between the *TNF* -308 SNP and other muscle damage markers.

2.4.3 Interleukin-6 –174 G>C Polymorphism and the Secondary Phase of Exercise-Induced Muscle Damage

Interleukin-6 (IL-6) modulates the release of different cytokines, such as of TNF and IL-1Ra (Steensberg et al., 2003, Starkie et al., 2003). The human *IL6* gene is mapped to chromosome 7p21–24 with a 303 bp upstream promoter (Fishman et al., 1998). Interleukin-6 plasma concentration is affected by exercise duration and intensity (Fischer et al., 2004), and the amount of muscle mass involved (Ostrowski et al., 2000), particularly during weight-bearing exercise (Catoire and Kersten, 2015). Eccentric exercise induces a delayed peak and a slower decrease of plasma IL-6 after exercise in comparison to other modes of exercise, such as running (Fischer, 2006, Pedersen and Fischer, 2007). According to McKay et al. (2009), IL-6 may play a role as an important signalling molecule associated with satellite cell proliferation after strenuous exercise. Furthermore, damaged extracellular matrix might have an effect on IL-6 expression, as IL-6 is involved in collagen synthesis (Andersen et al., 2011). These findings suggest that the different circulating IL-6 timescale of prolonged but non-damaging exercise and of eccentric exercise occurs due to a different source and function of IL-6 expression. Whilst muscle fibres, peritendinous connective tissue (Langberg et al., 2002) and adipose tissue (Holmes et al., 2004) all express and release IL-6 into the circulation without activating pro-inflammatory pathways (Pedersen, 2011), eccentric exercise might induce more local IL-6 expression within the skeletal muscle with pro-inflammatory properties (Nieman et al., 1998, Nieman et al., 2000). The delayed peak of plasma IL-6 concentration after strenuous eccentric exercise might occur due to release into the circulation following the mechanical tearing of the sarcolemma and opening of stretch activated channels due to exertional muscle damage.

A functional −174 G>C SNP (rs1800795) has been detected in the promoter region of the *IL6* gene. The frequency distribution of the G-allele ranges between 45 to 100% in the worldwide population (Borinskaya et al., 2013) and it is associated with an increased plasma IL-6 response in healthy people (Bennermo et al., 2004, Fishman et al., 1998, Pereira et

al., 2011). The −174 G allele might affect the glucocorticoid receptor and elevate the transcriptional activation due to its close positioning with the receptor (Yamin et al., 2008, Rein et al., 1995). This *IL6* SNP shows a somewhat ambiguous picture: according to Ruiz et al. (2010b), both GG and GC genotypes are more frequent in elite power athletes compared to endurance athletes and to non-athletes. There was no difference between endurance athletes and the control group, which is in the line with the findings of Yamin et al. (2008). In young individuals, C-allele carriers of the *IL6* SNP presented higher CK values following eccentric exercise compared with GG homozygotes (Yamin et al., 2008, Yamin, 2009). In power-orientated sports, which are associated with muscle damage during training or competition, GG homozygotes might have benefits with faster recovery and elevated satellite cell proliferation in the long term. However, Deuster et al. (2013), who did not observe any association between this *IL6* SNP and exertional rhabdomyolysis, challenge this conclusion.

Aging-related declines in physical function are associated with chronically elevated systemic IL-6 concentration (Ershler and Keller, 2000, da Cunha Nascimento et al., 2015). However, Walston et al. (2005) could not confirm any association between *IL6* genotypes and serum IL-6 in older women. Furthermore, in the study of Funghetto et al. (2013), in older obese women, plasma CK integral (area under the curve of CK between the different time points) values were lower and IL-6 integral values were higher for carriers of the C-allele after eccentric exercise. However, there was only a moderate increase in plasma CK concentration and no change in IL-6 concentration, probably resulting from the relatively low intensity of the eccentric exercise protocol used. Of note, the interaction between the −174 G>C SNP and obesity seems to be a complex one (Joffe et al., 2013). Linkage disequilibrium of this −174 G/C SNP with several other SNPs on the *IL6* gene cannot be excluded (Qi et al., 2007). In diseased, obese and older populations with chronically elevated circulating IL-6, an increased IL-6 response might be harmful after eccentric exercise (Funghetto et al., 2013, Bennermo et al., 2004).

In summary, the pattern of circulatory IL-6 and CK levels in association with the *IL6* −174 G>C SNP appears to be diametrically opposed. It might be that an elevated IL-6 response and lower CK levels associated with the G-allele are beneficial due to increased IL-6 production of macrophages (Patel et al., 2010) and satellite cell proliferation (McKay et al., 2009) in a healthy population following eccentric exercise (Yamin et al., 2008). However, the G-allele might have a negative effect in those presenting with chronic low-grade systemic inflammation. Without knowing the actual source of IL-6 expression and its subsequent pro- or anti-inflammatory effect, cumulative plasma IL-6 concentration is probably an inaccurate biomarker of muscle damage (Pedersen and Febbraio, 2008). The influence of the *IL6* −174 G>C SNP is not fully clear and needs further investigation, particularly in conjunction with both local and circulatory measures of IL-6 expression/concentration.

2.4.4 Chemokine Ligand 2 and Chemokine Receptor Type 2 Polymorphisms and the Secondary Phase of Exercise-Induced Muscle Damage

Like interleukin-6, the chemokine (C-C motif) ligand-2 (CCL2), also known as monocyte chemoattractant protein 1 (MCP1), can be classified as an exercise factor, as it mediates systemic changes induced by chronic exercise training (Catoire and Kersten, 2015). Monocyte chemoattractant protein 1 receptor (*CCR2*) is one of the major receptors, which binds CCL2, beside CCL7 and CCL13 (Harmon et al., 2010). CCL2 is mainly expressed within the interstitial space between myofibres following muscle damaging exercise, and is co-localized with macrophages and satellite cells in the muscle (Hubal et al., 2008). Concentric exercise does not influence local *CCL2* expression (Hubal et al., 2008). However, in line with the findings of Warren et al. (2005), that *Ccr2*-knockout mice have shown impaired regeneration, inflammation, and fibrotic response following freeze injury, a strong interaction between *CCL2/CCR2* and the immune response after muscle damage is suggested (Hubal et al., 2008, Yahiaoui et al., 2008). Interestingly, whilst local *CCL2* mRNA expression further increased after a second bout of eccentric exercise in comparison to the

first bout (Hubal et al., 2008), systemic response of CCL2 decreased after repeated downhill running (Smith et al., 2007).

Hubal et al. (2010) investigated several *CCL2/CCR2* SNPs in association with exercise-induced muscle damage in the elbow flexor muscles. Following strenuous exercise, the T-allele of the *CCL2* rs3917878 (C>T) SNP was associated with a delayed recovery of maximum strength in men and a higher CK response in women (Hubal et al., 2010). C-allele carriers of the *CCR2* (rs3918358) SNP showed a delayed recovery of strength in females, and the C-allele of the *CCR2* (rs1799865) SNP increased soreness in both genders (Hubal et al., 2010). The significant differences between the alleles of these three SNPs occurred 4–10 days following exertional muscle damage, confirming the action pattern of CCL2/CCR2 in muscle repair/regeneration. Furthermore, the GG genotype of the *CCL2* gene variant (rs2857656), for which significant differences were found in pre-exercise maximum strength compared to the major C-allele (Harmon et al., 2010), was associated with the magnitude of muscle injury in professional soccer players (Pruna et al., 2013). According to Hubal et al. (2010), there were moderate associations between *CCL2/CCR2* genotypes and baseline CCL2 activity (as a product of CCL2 expression and the availability of CCR2). Higher CCL2 activity might be an advantage in the recovery period following muscle damage in healthy individuals due to its ability to serve as a chemoattractant to macrophages and its possible activation of satellite cell proliferation (Yahiaoui et al., 2008). However, further investigation is needed to identify the potential molecular mechanisms underpinning the influence of each of these SNPs in changing CCL2 activity in response to muscle damaging exercise in elderly and obese people, in whom chronic systemic inflammation is already an issue.

2.4.5 Osteopontin -66 T>G Polymorphism and the Secondary Phase of Exercise-Induced Muscle Damage

The extracellular matrix protein and pro-inflammatory cytokine osteopontin (also known as secreted phosphoprotein 1) is expressed in numerous cell types including skeletal muscle

(Kahles et al., 2014, Zanotti et al., 2011, Giachelli et al., 1998). Whereas the earliest studies suggested that it had a central role in bone remodelling (Rodan, 1995), subsequent studies suggest that osteopontin has also a role as a chemoattractant for macrophages (Hirata et al., 2003), and possibly neutrophils (Yang et al., 2014). Osteopontin is virtually undetectable in resting skeletal muscle but, after induced muscle damage in mice, osteopontin expression is elevated 100-times compared to baseline transcription levels (Hoffman et al., 2013, Hirata et al., 2003).

A common SNP in the transcriptional promoter of the osteopontin gene (-66 T>G, rs28357094), which overlaps a specificity protein-1 transcription factor-binding site, results in different phenotypic characteristics (Barfield et al., 2014). The minor G-allele is associated with an 80% reduction in osteopontin gene expression *in vitro* (Giacopelli et al., 2004, Barfield et al., 2014) and with a 17% increase in baseline upper arm muscle volume in women (Hoffman et al., 2013). Surprisingly, this increased muscle volume did not influence muscle strength (Hoffman et al., 2013). After exercise-induced muscle damage, women carrying the G-allele revealed significantly elevated muscle swelling, increased loss of muscle strength (Barfield et al., 2014) and CK values were elevated in two women with the rare GG genotype (Hoffman et al., 2013). In contrast, the G-allele was linked with less grip strength and with more rapid progression in patients with Duchenne muscular dystrophy (Pegoraro et al., 2011). Further investigations of Barfield et al. (2014) revealed several enhancer sequences on the osteopontin gene promoter for multiple steroid hormone-binding sites (i.e. oestrogen receptor, glucocorticoid receptor, vitamin D receptor and a potential NF-κB binding site). Oestrogen hormone treatment of modified human myoblasts with the allele-specific osteopontin promoters has shown that the human myoblasts with the transfected G-allele promoter revealed a threefold increase in osteopontin gene expression, whereas the T-allele construct was unaffected by oestrogen treatment. From this, we can infer that there may be an allele-specific interaction between the oestrogen enhancer and the more proximal specificity protein-1 transcription factor site leading to a hypothetical model for sexual dimorphism (Barfield et al., 2014). Thus, women

with the G-allele seem to be more susceptible to muscle damage. Likewise, a similar allele-specific interaction between the NF-κB or glucocorticoid binding site and the specificity protein-1 transcription factor site might explain the association between the G-allele and Duchenne muscular dystrophy. Barfield et al. (2014) suggest that chronic inflammation might lead to an augmentation of the pro-inflammatory response, which accelerates the progress of the disease. However, the study of Barfield et al. (2014) has several limitations. TT genotype has shown a similar loss of force over time in both the exercised and non-exercised arm following exertional muscle damage. In addition, due to the low number of volunteers (n = 6) who completed the eccentric exercise intervention, further investigations are needed to replicate and verify these findings.

2.5 SKELETAL MUSCLE REMODELLING FOLLOWING EXERCISE-INDUCED MUSCLE DAMAGE

Skeletal muscle regeneration is a complex process that is mediated by satellite cells, and in which several factors are activated to regulate muscle remodelling (Kurosaka and Machida, 2012). Satellite cells are mononucleated muscle stem cells and are located on the outer surface of the muscle fibre, between the basal lamina and sarcolemma (Hawke and Garry, 2001). Usually, satellite cells remain quiescent but are activated following damage (Figure 2-4) (Chambers and McDermott, 1996, Grobler et al., 2004). They proliferate 24-48 hours later and then do one of three things: (i) return to quiescence and restore the population of satellite cells; (ii) migrate to the site of injury and support the repair process by increasing the nuclei-to-cytoplasm ratio; (iii) fuse with other myogenic cells to form myotubes, thus generating new fibres to replace damaged myofibres (Hawke and Garry, 2001, Grobler et al., 2004, Tidball and Villalta, 2010, Sharples and Stewart, 2011).

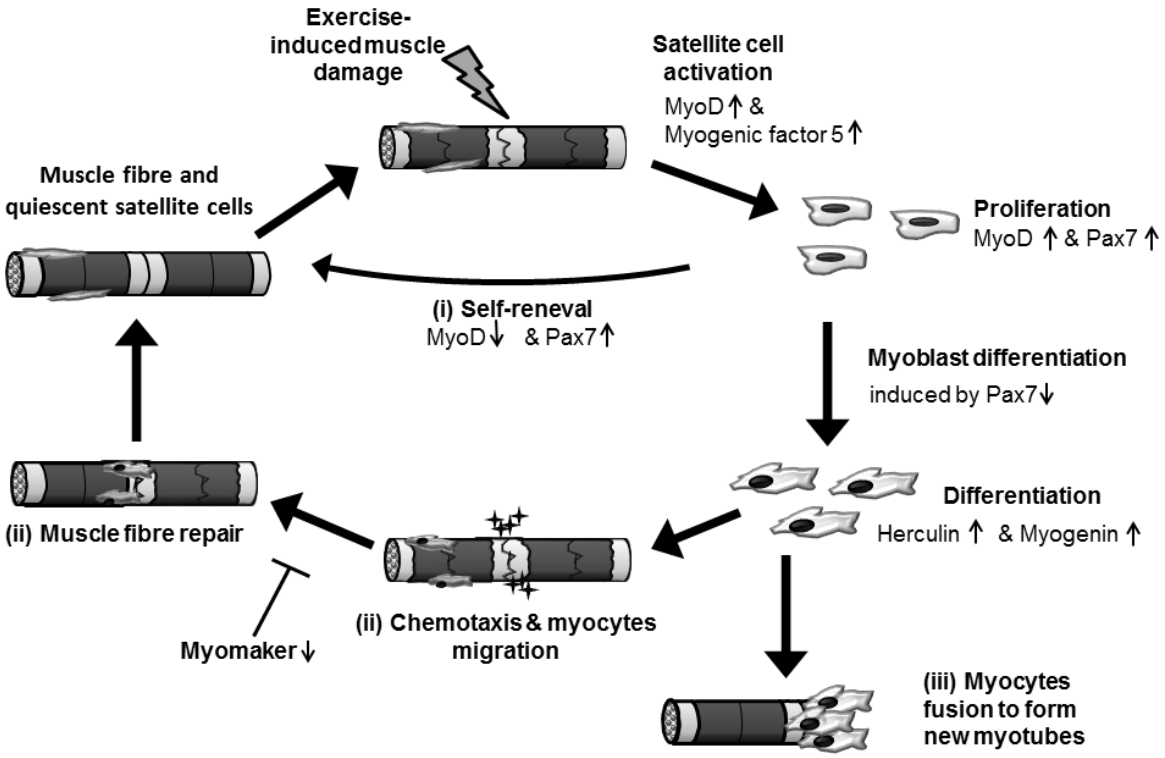

Figure 2-4 The cycle of skeletal muscle fibre regeneration following exercise-induced muscle damage. *This cycle is mediated by satellite cells, which are activated following stressful physiological conditions such as exercise-induced muscle damage (Grobler et al. 2004). Activated satellite cells initially up-regulate two different myogenic regulatory factors, MyoD and myogenic factor-5 and, during the proliferation, paired box protein 7 (Pax7). If satellite cells return to quiescence and restore the population of satellite cells, MyoD will be down-regulated (i). However, subsequent cell differentiation is accompanied with down-regulation of Pax7/3. During this early differentiation stage, herculin and myogenin are up-regulated. Myoblasts differentiate into myocytes and then eventually migrate to the site of injury and support the repair process by increasing the nuclei-to-cytoplasm ratio (ii). Different chemotactic gradients, including a large number of chemokines, support the migration to the region of injury. A recent investigation in mice revealed that the absence of myomaker, which is expressed on the cell surface of myoblasts, leads to inhibition of myoblast fusion (Millay et al. 2013). Alternatively, the myocytes fuse with other myogenic cells to form myotubes, thus generating new fibres to replace damaged myofibres (iii). Figure adapted from Tidball (2011) and Al-Shanti and Stewart (2009).*

Macrophages are essential, not only for removing tissue debris, but also in the activation of satellite cells. M1 macrophages provoke myoblast proliferation (Arnold et al., 2007, Cantini et al., 2002) and, together with neutrophils, they attract satellite cells to the site of injury by releasing TNF (Torrente et al., 2003). M2 macrophages stimulate the differentiation of satellite cells into mature myofibres (Arnold et al., 2007), and *in vitro* studies indicate that macrophages support differentiation through ultimate increases in myogenin expression

(Cantini et al., 2002). Activated satellite cells initially up-regulate two different myogenic regulatory factors, MyoD and myogenic factor-5 (Smith et al., 1994). In the period of proliferation, the satellite cells express paired box protein 7 (Pax7) and MyoD but those that return to quiescence to maintain the satellite cell pool only express Pax7 (Tedesco et al., 2010, Al-Shanti and Stewart, 2009). However, subsequent down-regulation of Pax7/3 induces cell differentiation. The satellite cells exit the cell cycle and enter the early differentiation stage where myogenic factor 6 (herculin) and myogenin are up-regulated (Zammit, 2008, Wahl et al., 2008, Le Grand and Rudnicki, 2007). Myoblasts differentiate into myocytes and then eventually fuse and form multinucleated myofibres (Le Grand and Rudnicki, 2007). Recent investigations revealed that MyoD and myogenin induce myomaker gene transcription (Millay et al., 2014, Millay et al., 2013). The absence of myomaker, which is expressed on the cell surface of myoblasts, leads to inhibition of myoblast fusion in mice (Millay et al., 2013). However, more information is required to explain the roles of myomaker in muscle regeneration and recovery following muscle damaging exercise.

The extracellular matrix provides structural and biochemical support to contractile tissue and it is associated with the inflammatory response and satellite cell activation (Hyldahl and Hubal, 2014, Kjær, 2004). Activated satellite cells migrate to the site of injury along the basal lamina (Hughes and Blau, 1990), a process that is facilitated by the basal lamina components (i.e. collagen IV, laminin-2 and nidogens) (Goetsch and Niesler, 2011). Components of the extracellular matrix (collagen I and III, fibronectin and other extracellular matrix molecules) provide a temporary scaffold to support the migration of the activated progenitor cells (Goetsch et al., 2013). Different chemotactic gradients, including a large number of chemokines, also support the migration from the niche towards the site of myotrauma, and some of these chemokines are released from the extracellular matrix itself (Griffin et al., 2010, Goetsch et al., 2013). Furthermore, there is evidence that synthesis of type I, III and probably IV collagen within the endomysium and the perimysium increase after contraction-induced damage (Mackey et al., 2004, Koskinen et al., 2001).

2.5.1 Gene Polymorphisms of the Insulin-like Growth Factor Family and the Remodelling Following Exercise-Induced Muscle Damage

The complex process of remodelling is influenced by growth factors including insulin-like growth factor-I (IGF-I) and IGF-II (Duan et al., 2010). In addition to IGF-I (*IGF1*) and IGF-II (*IGF2*), the IGF-system consists of several IGF-binding proteins, the insulin receptor, and cell surface receptors such as the IGF-I receptor and the IGF-II receptor (Wang et al., 2015). This system promotes satellite cell differentiation and proliferation (O'Dell and Day, 1998, Florini et al., 1996, Stewart and Rotwein, 1996b, Stewart et al., 1996, Stewart and Rotwein, 1996a) and is thought to play an important role during exercise-induced muscle hypertrophy (Sharples and Stewart, 2011, Matheny et al., 2009). For example, transgenic mice overexpressing Igf-I in skeletal muscle revealed a significant gain in muscle cross-sectional area vin comparison with wild type mice following chronic muscle overload (Paul and Rosenthal, 2002). Inactivation of the type 1 Igf receptor inhibits the presence of newly formed nuclei in exercised transgenic mice (Fernández et al., 2002, Jiao et al., 2013, Wilson et al., 2003), while maintaining local IGF-I concentration is considered crucial for maintaining muscle mass and strength with advancing age (Barton-Davis et al., 1998, Musarò et al., 2001).

Besides their role in hypertrophy, IGFs are crucial in muscle regeneration following exercise or muscle injury (Jiao et al., 2013, Mackey et al., 2011). Insulin-like growth factor-I acts mainly in an autocrine and paracrine manner to stimulate satellite cells to proliferate and differentiate. Different isoforms [IGF-IEa, IGF-IEb (in rat) and IGF-IEc (in human)] of IGF-I are associated with muscle damage and regeneration. Insulin-like growth factor-IEb and IGF-IEc are also known as mechano-growth factor, because the mRNA is expressed in response to overload or damage in skeletal muscle. The expression of mechano-growth factor is enhanced shortly after muscle damage, which subsequently promotes satellite cell activation (Hill and Goldspink, 2003). Afterwards, increased expression of IGF-IEa elevates

myoblast fusion (Yang and Goldspink, 2002, Jiao et al., 2013). Mechano-growth factor also promotes the activity of cytoplasmic superoxide dismutase, thus protecting against ROS during the inflammatory response to muscle damaging exercise (Dobrowolny et al., 2005). Both IGF-I and IGF-II mRNA increase during myoblast differentiation, but presumably autocrine IGF-II is the predominant myogenic factor during differentiation due to its enhanced expression, whilst IGF-II is probably elevated to suppress IGF-I gene expression via the mTOR pathway (Jiao et al., 2013, Wilson et al., 2003). Marsh et al. (1997) have also shown an age-dependent decline of *IGF2* gene expression following muscle damage in rats.

As far as we are aware, only Devaney et al. (2007b) have tested the association between IGF SNPs and exercise-induced muscle damage. Several different SNPs were investigated, as the *IGF2* gene region consists of three genes: *IGF2, IGF2* anti sense (*IGF2AS)*, and the insulin gene (Lee et al., 2005). The following SNPs: *IGF2* (17200 G>A, rs680); *IGF2* (13790 C>G, rs3213221); *IGF2AS* (1364 A>C, rs4244808); *IGF2AS* (11711 G>T, rs7924316), were significantly associated with exercise-induced muscle damage. Besides an association between the *IGF2* 17200 (G>A, rs680) and *IGF2* 13790 (C>G, rs3213221) SNPs and soreness (after 3 and 4 days), and CK activity in the blood (both after 7 days) following muscle damaging exercise, every *IGF2* SNP investigated was associated with strenuous exercise-induced muscle strength loss in men. Only the *IGF2AS* 1364 (A>C, rs4244808) SNP was associated with strength loss immediately after exertional muscle damage in both men and women. In contrast*,* carriers of the insulin gene 1045 (C>G, rs3842748) SNP have shown an increased CK activity 10 days after exercise-induced muscle damage only in women.

Varying IGF-I or IGF-II levels potentially caused by these SNPs could modulate satellite cell activation and differentiation. For instance, the *IGF1* cytosine adenine-repeat SNP located in the promoter region of the IGF-I gene is believed to change circulating IGF-I levels but the evidence is equivocal (Vaessen et al., 2001, Rosen et al., 1998, DeLellis et al., 2003, Allen et al., 2002). While Vaessen et al. (2001) suggest IGF-I levels are increased by these

SNPs, other investigations found a decrease (Rosen et al., 1998) or no difference in IGF-I levels (Allen et al., 2002, DeLellis et al., 2003).

It is remarkable that several SNPs of *IGF2* were associated with a loss of muscle strength directly after exertional muscle damage, in particular in men. It seems there must be another process, whereby the *IGF2* gene is involved in the response to muscle damaging exercise separately from regeneration and differentiation. Here, we would like to highlight a new hypothesis. Insulin-like growth factor I also plays an important role in the regulation of protein synthesis, including collagen and myofibrillar protein. Local IGF-IEa and IGF-IEc mRNA expression is positively correlated with musculotendinous mRNA expression of *COL1A1/3A1* (Boesen et al., 2013, Doessing et al., 2010) and may subsequently increase collagen synthesis in the extracellular matrix (Hansen et al., 2013). Lower circulating IGF-I levels induced by IGF SNPs may negatively influence the stability of the extracellular matrix. Therefore, a subsequent loss in the lateral transmission of force between adjacent muscle fibres might occur, which could be the source of the decrease in maximum strength observed immediately after strenuous exercise. Although, to the best of our knowledge, no direct effect of IGF-II concentration on human extracellular matrix protein synthesis is known, Keller et al. (1999) has shown that local Igf-II expression increases after injury in murine muscle. It is therefore possible that IGF-II is linked with exercise-induced muscle damage in human muscle, and possibly with extracellular matrix integrity. A direct or indirect influence of IGF-II level on extracellular matrix integrity would, at least in part, explain the significant strength loss after muscle damaging exercise and the association of the *IGF2* 13790 (C>G, rs3213221) SNP with the degree of injury in soccer players (Pruna et al., 2013).

2.6 ADDITIONAL GENE POLYMORPHISMS ASSOCIATED WITH EXERCISE-INDUCED MUSCLE DAMAGE

The following gene polymorphisms have been associated with exercise-induced muscle damage. However, further investigation is necessary to attribute these polymorphisms to a specific phase of exercise-induced muscle damage.

2.6.1 Angiotensin-I Converting Enzyme Insertion/Deletion Polymorphism

Angiotensin-I converting enzyme (ACE) has a key role in the interaction between the kallikrein-kinin and the renin-angiotensin systems (Schmaier, 2003). Angiotensinogen, which is a precursor protein in the renin-angiotensin system, is produced constitutively and released into the circulation mainly by the liver (Deschepper, 1994), and can be cleaved by the protease renin, resulting in the decapeptide angiotensin-I. The dipeptidase ACE converts angiotensin-I to the octapeptide hormone angiotensin-II, which acts as a vasoconstrictor (Munzenmaier and Greene, 1996), and induces skeletal muscle hypertrophy in response to mechanical loading (Gordon et al., 2001). Angiotensin-I converting enzyme also cleaves the vasodilator bradykinin (Dendorfer et al., 2001), which supports the increase of arterial blood pressure (Murphey et al., 2000), as well as Substance P (SP), a protein from the tachykinin family that functions as a neurotransmitter (released by group III and IV afferent fibres) (Harrison and Geppetti, 2001, Inoue et al., 1998).

The *ACE* insertion/deletion (I/D) polymorphism (rs4646994) was the first gene variation to be investigated in the context of human physical performance-related traits, and is the most investigated in the renin-angiotensin system (Gayagay et al., 1998, Montgomery et al., 1998). The insertion (I) allele of a 287 bp Alu sequence within intron 16 on chromosome 17 is linked to lower ACE activity in serum (Rigat et al., 1990) and in cardiac muscle (Phillips et al., 1993, Danser et al., 1995), and reduced bradykinin degradation (Murphey et al., 2000)

compared to carriers of the D-allele. Carriers of the I-allele are associated with greater endurance capacity (Montgomery et al., 1998, Ma et al., 2013), whereas the D-allele is associated with greater muscular strength (Williams et al., 2005), and elite power athlete status (Costa et al., 2009, Nazarov et al., 2001, Woods et al., 2001). However, recent investigations have observed that this distinction is not considered sufficiently specific to detect all the phenotypic effects (Lucia et al., 2005, Rankinen et al., 2000, Thompson and Binder-Macleod, 2006).

The association between the *ACE* I/D polymorphism and elite athlete status might be explained by a genotype link with the susceptibility to exertional muscle damage and injury. To the best of our knowledge, only two studies have investigated the influence of the *ACE* I/D polymorphism on contraction-induced damage in humans (Heled et al., 2007, Yamin et al., 2007). Yamin et al. (2007) observed different concentrations of circulatory CK between *ACE* genotypes after eccentric exercise: II homozygotes elicited the highest CK response, whilst DD homozygotes elicited the lowest plasma CK activity after strenuous exercise. This suggests that the I-allele is associated with a greater susceptibility to muscle damage, and the potential mechanism is explained below. However, Heled et al. (2007) could not find any association between *ACE* I/D polymorphism and CK response. The different outcome is probably attributed to the moderate-intensity exercise test and higher activity level and different ethnicities of the participants in the study of Heled et al. (2007). It should be noted that only CK level was investigated in both studies, which is only one of several indirect biomarkers of exercise-induced muscle damage.

In rabbit studies, inhibition of ACE revealed [in combination with neutral endopeptidase (NEP) inhibitor] elevated muscle damage in a muscle overuse model induced by electrical stimulation every second day with four sessions in total (Song et al., 2014), which is in line with the human findings of Yamin et al. (2007). The muscle damage was accompanied by increased tachykinin, substance P and its preferred receptor neurokinin-1 receptor expression, which suggests that the tachykinin family may play a role in the inflammatory processes and pain (Song et al., 2014, Dousset et al., 2007). Substance P is widely

expressed in human cells and tissues of the peripheral and central nervous systems but it is also found in extra neuronal cells and innervated tissues. Substance P and neurokinin-1 receptor have been associated with the inflammatory response in smooth muscle cells and dermal tissues but not in skeletal muscle (Renzi et al., 2000, Luger, 2002). However, elevated substance P might result in improved remodelling, as demonstrated in the healing of a rat Achilles tendon (Bring et al., 2012, Steyaert et al., 2010).

In contrast, angiotensin-II is known to be involved in inflammatory process following muscle damage. Blocking of the angiotensin-II receptor type 1 improves regeneration of injured skeletal muscle (Bedair et al., 2008) and supresses ROS production following strenuous exercise in mice (Sim et al., 2014). Furthermore, nerve growth factor up-regulation through activation of B_2 bradykinin receptors is strongly associated with increased pain sensitivity (hyperalgesia) (Murase et al., 2010, Babenko et al., 1999). Angiotensin-I converting enzyme D-allele carriers, which have a decreased bradykinin half-life, might have attenuated nerve growth factor expression following exertional muscle damage and therefore a decreased pain sensitivity. Attenuated substance P and bradykinin in the inflammatory process may explain the high frequency of D-allele carriers among elite strength/power athletes (Costa et al., 2009). Athletes with the D-allele might feel less pain and therefore might be able to (i) sustain high-intensity training for longer, (ii) reach the limits of their capacity in power/strength related competition (ii) or enable them to practise more often due to a decreased sensitivity to pain. In other sport-specific movements, such as short distance swimming (<200 m), it is crucial to sustain a high level of intensity accompanied with exercise-induced muscle burning (Costa et al., 2009, Woods et al., 2001).

Another possibility might be that angiotensin-II indirectly mediates skeletal muscle damage by influencing angiogenesis in response to exercise (Vaughan et al., 2013). It is well known that, in a damaged muscle in the days following eccentric exercise, resting capillary blood-flow is elevated and vasodilatation occurs (Rubinstein et al., 1998). According to Vaughan et al. (2013), the capillary density of skeletal muscle is lower in untrained carriers of the *ACE* I-allele compared to DD homozygotes. Lower capillary density might impair the

migration of neutrophils and macrophages as well as of the removal of cellular debris, which could negatively affect the extent of muscle damage and possibly muscle remodelling.

2.6.2 Mitochondrial Superoxide Dismutase 2 Ala16Val Polymorphism

Strenuous exercise results in oxidative stress, which causes structural damage to muscle fibres and stimulates an inflammatory response (Gomez-Cabrera et al., 2008), as discussed in section 2.4. A higher intracellular concentration of antioxidants within a muscle fibre is thought to protect against the negative impact of ROS (Schoenfeld, 2012, Peake and Suzuki, 2004). Superoxide dismutase is an antioxidant that protects cells and mitochondria from free radical damage by converting the anion superoxide into hydrogen peroxide (Huang et al., 2000). Inhibition of superoxide dismutase causes the accumulation of superoxide radicals, and can lead to increased damage of mitochondrial membrane and cell apoptosis (Huang et al., 2000). The Ala16Val (rs4880, C>T) SNP of the mitochondrial superoxide dismutase gene, has been associated with muscle damage susceptibility. The T-allele is associated with reduced mitochondrial superoxide dismutase efficiency against oxidative stress (Shimoda-Matsubayashi et al., 1996). Akimoto et al. (2010) demonstrated that trained runners of TT genotype had an increased plasma CK concentration after racing 4-21 km. This is in line with Ahmetov et al. (2014), who revealed that TT carriers of the mitochondrial superoxide dismutase gene were under-represented in power and strength athletes compared to controls and athletes of low-intensity sports, such as curling players and shooters. Interestingly, in the study of Ben-Zaken et al. (2013), the frequency of the C-allele was significantly higher in both endurance and power athletes in comparison to the control group. At first glance, these studies seem to be inconsistent with one another. On closer inspection, both studies recruited different types of participants. In the study of Ahmetov et al. (2014) participants covered a wide range of different sports, whereas in Ben-Zaken et al. (2013), only track and field related athletes participated: 100 and 200 m sprinters and long jumpers (power athletes); 5000 m and marathon runners (endurance athletes). These track and field athletes perform sport-specific movements that is

accompanied by stress to the musculoskeletal system through repeated eccentric muscle contractions performed over long periods of time, which leads to muscle damage. The inflammation accompanying this damage potentially produces more oxidative stress than the endurance sports (e.g. swimming) in the study by Ahmetov et al. (2014). Therefore, the T-allele might impair the protection against oxidative stress due to the lower efficiency of the mitochondrial superoxide dismutase gene. This may indicate that there is a relationship between this SNP and level of athletic performance in sports with a potential risk of muscle damage. Unfortunately, no study has tested the effect of the mitochondrial superoxide dismutase SNP on exercise-induced muscle damage over the course of time. This could provide insight into the influence of the mitochondrial superoxide dismutase C>T SNP on the secondary phase of muscle damage and the subsequent remodelling.

2.6.3 Solute Carrier Family 30 Member Eight C>T Polymorphism

Type 2 diabetes mellitus is associated with disturbed zinc homeostasis and down-regulation of the solute carrier family 30 (zinc transporter) member eight, the product of the *SLC30A8* gene (Somboonwong et al., 2015). Solute carrier family 30 member eight is mainly expressed in pancreatic islet beta cells and it transports zinc from the cytoplasm into intracellular vesicles, which is crucial for insulin crystallization, storage, and secretion (Cheng et al., 2015, Lemaire et al., 2009, Chimienti et al., 2006). The C-allele of the nonsynonymous *SLC30A8* (C>T) SNP (rs13266634) is strongly associated with type 2 diabetes mellitus risk, in particular in European and Asian populations but not in African populations (Cheng et al., 2015). This *SLC30A8* R325W SNP is associated with, amongst others, decreased fasting systemic insulin and attenuated insulin secretion in response to glucose intake (Staiger et al., 2007, Sprouse et al., 2014, Kirchhoff et al., 2008).

In recent years, there has been an increase in the number of investigations regarding insulin resistance and muscle function in people without type 2 diabetes mellitus. Insulin resistance is not only associated with lower force and muscle mass in individuals with diabetes (Andreassen et al., 2009, Andersen et al., 2004), but also in healthy people (Gysel et al.,

2014). Insulin signalling increases blood flow and protein synthesis at rest, and suppresses the breakdown of proteins after resistance exercise, thus improving net muscle protein balance in particular with amino acid delivery and availability (Biolo et al., 1999, Fujita et al., 2006). Furthermore, exercise-induced muscle damage has been associated with impaired glycogen synthesis (Costill et al., 1990) and reduced glucose uptake (Nielsen et al., 2015, Asp et al., 1996), probably due to muscle damage reducing muscle insulin sensitivity (Kirwan et al., 1991). This could be due to increased TNF expression attenuating insulin signalling transduction, subsequently inducing insulin resistance in skeletal muscle (Plomgaard et al., 2005) and supressing the activation of glucose transporter type 4 in muscle fibres (Asp et al., 1995).

Sprouse et al. (2014) reported that the TT genotype of the *SLC30A8* SNP was associated with lower biomarkers of muscle damage (reduced soreness, strength loss and plasma CK and myoglobin levels) following eccentric contractions of the elbow flexor muscles in men. By increasing the catabolic pathway, lower insulin levels can lead to a negative net protein balance (Woolfson et al., 1979, Sacheck et al., 2007). Therefore, carriers of the *SLC30A8* C-allele might need longer times to recover from strenuous exercise. Further studies should investigate if *SLC30A8* genotype-dependent insulin production is associated with the acute and chronic adaptations to resistance exercise, with regard to muscle protein synthesis and muscle hypertrophy, respectively.

2.7 POLYGENIC PROFILES ASSOCIATED WITH EXERCISE-INDUCED MUSCLE DAMAGE

In 2008, (Williams and Folland, 2008) postulated a genetic algorithm, the total genotype score (TGS), that combine previously associated SNPs regarding a specific phenotypic trait to estimate the combined effect of SNPs on elite athlete status. Since then, several computed polygenic profiles have been published to estimate the association of multiple SNPs on the elite endurance (Ruiz et al., 2009, Grealy et al., 2015, Yoo et al., 2016), and strength/power (Ruiz et al., 2010a, Hughes et al., 2011, Miyamoto-Mikami et al., 2017)

athlete status. It is likely that the accumulation of non-preferential alleles of SNPs, which are associated with EIMD, accentuate the detrimental response to muscle damaging exercises. Indeed, recent investigations demonstrated that a TGS, based on seven SNPs (*ACE,* rs4340; *ACTN3*, rs1815739; *CKMM*, rs1803285; *IGF2*, rs3213221; *IL6*, rs1800795; *MLCK*, rs28497577; *TNF*, rs1800629) previously associated with EIMD, was able to distinguish between high and low responders in terms of CK activity following a race of amateur marathon runners and triathletes (Del Coso et al., 2017a, Del Coso et al., 2017b). However, the CK response was not linked with the performance of the athletes, which raises the questions if CK activity really assesses the magnitude of ultrastructural muscle damage, and if it does, if the individual muscle damage response is an important factor for an athlete status in marathon or ironman. The limitation of the majority of these polygenic profiles is the dependence on former research that investigated individual SNPs on the investigated phenotypic trait. Future research needs to adapt the genetic algorithm of Williams and Folland (2008) so that the improved TGS can assess so far unrelated SNPs to phenotypic traits, i.e. to EIMD, as seen by Yoo et al. (2016) investigating individual endurance training effects. Further, more research is needed that investigates multiple SNPs forming a polygenic profile to understand the complex mechanism underlying the individual response to EIMD-interventions.

2.8 DISCUSSION

Exercise-induced muscle damage provokes a prolonged loss of muscle strength, and both elevated soreness and circulating muscle-specific protein levels. The grade and actual time-course of strength loss, soreness and of the inflammation response after exercise is variable. Several factors that are well documented can influence the response to muscle damaging exercise, such as exercise mode, intensity or duration (Smith et al., 1989), micro nutrition (Owens et al., 2014, Bhat and Ismail, 2015, Barker et al., 2013) and muscle (group) intervention (Clarkson and Hubal, 2002). Nevertheless, within-study variability is often seen in response to strenuous exercise (Nosaka and Clarkson, 1996).

Several studies have reported differences in SNP-specific gene activity resulting in different expression of the coding proteins, which may influence the susceptibly to exercise-induced muscle damage (Seto et al., 2011). Individuals, who are high responders to exercise-induced muscle damage (i.e. demonstrate a greater loss of muscle strength and higher circulating levels of CK or myoglobin) might have a higher predisposition to injury (Kibler et al., 1992, Clansey et al., 2012). This is in line with the observation that history of one type of muscle injury increases the risk of developing other types of muscle injuries (Orchard, 2001, Freckleton and Pizzari, 2013). The same principle may apply to high responders to exercise-induced muscle damage in a squad of athletes performing the same exercise training together. High responders, who might need a longer recovery time after a strength training intervention in comparison to others in the same squad, might have a higher potential for musculotendinous injuries due to overtraining. Both presumptions may result in an increased dropout rate of athletes with specific genotype profiles due to higher rates of (overtraining) injury extending over several years (Kibler et al., 1992). It would be interesting to investigate if a high responder to exercise-induced muscle damage is a low/high responder to chronic resistance training.

Association studies can potentially reveal new mechanisms of genes. For instance, several *IGF2* SNPs have been associated with strength loss immediately after muscle damaging exercise, which cannot exclusively be explained by satellite cell differentiation (Devaney et al., 2007b). It is interesting that (i) certain genotypes of several *IL6* gene SNPs appear to be beneficial in healthy individuals regarding muscle damage response, but are disadvantageous in chronic disease and ageing; (ii) sex-specific genotype associations with exercise-induced muscle damage have been reported (Devaney et al., 2007b, Sprouse et al., 2014). Further investigations are necessary to uncover genotype–phenotype interactions and, in particular, the interaction of specific polymorphisms. A specific polygenic profile might help explain the inter-individual variance in the response to both acute eccentric damaging exercise and chronic strength training.

Moreover, the *ACTN3* R577X SNP has been associated with different responses to muscle damaging exercise, according to the mode of exercise. It is likely that stretch-shortening cycle-related movements place different demands on the musculotendinous system compared to exercises, which are performed without stretch-shortening cycles, thus explaining the equivocal findings concerning the association between this SNP and exercise-induced muscle damage (see section 2.3.1). Consequently, we recommend that future studies distinguish between exercise-induced muscle damage caused by eccentric contractions with or without stretch-shortening cycles. Furthermore, real-world modes of exercise should be incorporated into studies investigating the genetic association with exertional muscle damage. Not only will this improve our understanding of the mechanisms underpinning the deteriorated response of ageing muscle to exercise, but also it will help in prescribing more practical exercise therapies to poor exercise responders.

2.9 CONCLUSIONS

In summary, whilst several genetic association studies have been performed with individual SNPs, the current literature does not provide enough detailed information about the mechanisms underpinning the effect of genetic variation in the context of exercise and exercise-induced muscle damage. Further, additional genetic exercise studies including the assessment of multiple genetic polymorphisms (polygenic profile) are necessary to understand the complex interplay between nature and nurture affecting the response to exercise-induced muscle damage. This may eventually allow the identification of individuals, who are at high-risk of developing specific injuries. For instance, those who are genetically more predisposed to muscle damage, and who require longer to recover from strenuous exercise, are at greater risk of developing over-use injuries. Knowing how someone is likely to respond to a particular type of exercise would help coaches tailor the training and nutrition of their athletes (moving from a one size fits all to an individualised approach), thus maximising recovery and positive adaptation, and reducing the risk of injury.

3 The Physiological and Biomechanical Factors Associated with Neuromuscular Fatigue of the Hamstrings Following Intermittent Sprints

3.1 ABSTRACT

Introduction We aimed to investigate the physiological and biomechanical factors associated with neuromuscular fatigue of the hamstrings following intermittent sprints (IS).

Methods Following ultrasound assessment of biceps femoris long head (BF_{LH}) architecture, 20 recreationally active young men completed 15 x 30 m sprints. Quadriceps and hamstring maximal voluntary contractions (MVC); central (voluntary activation, BF_{LH} EMG) and peripheral (torque-frequency-relationship) hamstring fatigue; serum CK activity; treadmill running and SLHL landing kinematics, were assessed before (PRE), immediately after (POST) and 48 h (POST48) after IS.

Results Quadriceps and hamstring MVC decreased POST with an additional decrease of hamstring MVC POST48, while leg muscle soreness and CK activity increased over time (all $P<0.05$). There was no change in voluntary activation over time ($P=0.099$) but BF_{LH} EMG decreased POST ($P=0.022$). There was an inverse relationship between BF_{LH} PCSA and % change in hamstring MVC at POST ($R^2=0.421$, $P=0.003$). Treadmill running demonstrated decreases in peak knee extension ($P=0.047$) during the late swing phase at POST. SLHL kinematics showed increased (i) peak hip flexion ($P=0.004$); (ii) hip range of motion in the transverse plane ($p=0.025$); and (iii) peak knee adduction ($P=0.035$) during the landing phase POST, and all kinematic values returned to baseline POST48.

Conclusions Hamstring neuromuscular activation decreases immediately POST IS (possibly linked to central fatigue). However, the sustained reduction in hamstring MVC might be caused by ultrastructural muscle damage. Changes in both running and SLHL kinematics appear to decrease the elongation stress on the hamstrings POST. Therefore, 48 h recovery following IS is insufficient and may increase injury risk. However, a larger BF_{LH} PCSA appears to protect against hamstring fatigue, thus highlighting the importance of resistance exercise in reducing hamstring injury risk.

3.2 INTRODUCTION

Hamstring muscle strain injury (HSI) is the most frequent injury type in sports (Crema et al., 2017), particularly those that involve high-speed running, such as soccer (Ekstrand et al., 2011, Opar et al., 2012, Brooks et al., 2006). Injury rate in professional soccer teams is positively correlated with financial costs (Ekstrand, 2013), and is inversely correlated with team success (Eirale et al., 2013, Hägglund et al., 2013). Furthermore, the likelihood to sustain a HSI during training has increased by 4% per year in soccer since 2001 (Ekstrand et al., 2016). Therefore, an understanding of HSI is critical to address risk factors in future and could have important implications for sports clubs and athletes.

Soccer players are required to perform intermittent sprints (IS), which are characterised by short-duration (<10 s) and relatively longer recovery times (>60 s) between maximal sprint bouts, which have a different physiological demand compared to repeated-sprint exercises with shorter recovery times (<60 s) (Bradley et al., 2010, Girard et al., 2011). The majority of HSIs occur during high-speed running in competition rather than in training and, during team sport competition, HSIs occur more often towards the end of each half (Ekstrand et al., 2011, Brooks et al., 2006, Woods et al., 2004). This suggests that fatigue may play a role in the development of HSI. Neuromuscular fatigue is responsible for acute as well as prolonged impairment of muscle function for days or even weeks and it can be classified as central fatigue when the origin is proximal to the neuromuscular junction (e.g. reduction in motivation), or as peripheral muscle fatigue when the origin is distal to the neuromuscular junction (e.g. disruption to excitation-contraction coupling, damage to the contractile proteins, impaired metabolism) (Byrne et al., 2004, Gandevia, 2001). Structurally damaged muscles exhibit prolonged strength loss and delayed-onset muscle soreness (DOMS) as well as the release of muscle-specific proteins [e.g. creatine kinase (CK)] into the circulation, which is referred to as exercise-induced muscle damage (EIMD) (Clarkson and Hubal, 2002).

Several investigations have shown that both central and peripheral fatigue contribute to impaired muscle function in the short term following simulated soccer matches in both quadriceps (Thomas et al., 2017) and hamstring (Timmins et al., 2014, Marshall et al., 2014) muscle groups, and that mainly peripheral fatigue explains the prolonged recovery of muscle function in the quadriceps muscle group (Thomas et al., 2017, Rampinini et al., 2011). Peripheral fatigue can be assessed *in vivo* via the torque-frequency relationship, as it gives an indication of reduced efficiency of the excitation-contraction coupling process, particularly at lower frequencies (i.e. 10-20 Hz) compared with higher frequencies (i.e. 50-100 Hz) (Jones, 1996). Intermittent sprints have been shown to induce EIMD in both quadriceps (Howatson and Milak, 2009) and hamstring muscle groups (Chen et al., 2017, Verma et al., 2015), resulting in CK release into the circulation over the following days (Wiewelhove et al., 2015). To the best of our knowledge, however, no studies have investigated the contribution of both central and peripheral fatigue towards impaired function of the hamstring muscle group over time following an IS-intervention, which is an essential part in many team sports, such as soccer.

Congested fixtures and substantial increases in typical match load during the last decade, including higher amounts of repeated high-speed running (Bush et al., 2015, Wallace and Norton, 2014), may not allow athletes to fully recover between matches, and poorly managed training workload together with insufficient recovery of previously fatigued and damaged muscles (probably caused by IS) is thought to increase HSI risk (Malone et al., 2016, Malone et al., 2018, Chumanov et al., 2011, Duhig et al., 2016). High-speed running induces both high muscle activation (Chumanov et al., 2007) and high distal tendon load (Martin et al., 2018) of the hamstrings in the late swing phase, when the hip is flexed and the knee extended, suggesting a high muscle lengthening (eccentric) contraction during the deceleration of the shaft before initial foot contact (Chumanov et al., 2007). Hamstring muscle activation increases with running speed, and computational models suggest that the biceps femoris long head (BF_{LH}) is relatively more eccentrically stretched during the late swing phase of sprinting compared to the other muscles within the hamstring muscle group

(Thelen et al., 2005, Chumanov et al., 2007), and this is presumed to be the mechanical mechanism underlying HSI in sprinting. However, direct measurements of the hamstring muscle-tendon-complex during (high-speed) running are difficult to perform and the underlying assumptions of the computational model regarding the contraction mode are under debate (Shield and Murphy, 2018, Van Hooren and Bosch, 2018). Further, a short BF_{LH} fascicle length has been suggested to increase HSI risk (Timmins et al., 2016), as a shorter fascicle length might be more susceptible to being eccentrically overstretched due to fewer in-series sarcomeres (Brockett et al., 2004). To our knowledge, no study has investigated the relationship between BF_{LH} architecture including physiological cross-sectional area (PCSA), which is calculated by the total area of muscle fibres perpendicular to their long axes (Alexander, 1975), and the hamstring muscle response to exercise-induced neuromuscular fatigue/damage over time.

Neuromuscular fatigue might cause a number of biomechanical alterations. Exercise-induced muscle damage within the quadriceps femoris together with the hamstring muscle group leads to changes in running kinematics (Dutto and Braun, 2004). More precisely, knee flexion range of motion (ROM) and hip tilt is decreased and hip rotation is increased in running 48 h after an EIMD-intervention (Paquette et al., 2017, Paschalis et al., 2007). However, it is not known whether IS influences kinematic patterns initially, and whether this can lead to prolonged changes in leg kinematics as changes in hip and knee joint motion (together) potentially reduce additional elongation stress on the hamstring muscles. Further, neuromuscular fatigue may increase the risk of non-contact anterior cruciate ligament (ACL) injury (Santamaria and Webster, 2010) as changes in movements (e.g. during landing) might result in less controlled cushioning knee/hip flexion during stop-tasks. It is debatable whether specific muscular or biomechanical parameters concerning knee (knee flexion angle, knee abduction angle and knee abduction moment), hip (hip adduction) and ground reaction forces might be associated with ACL injury, as the results of different prospective studies are inconclusive (Sharir et al., 2016, Rafeeuddin et al., 2016).

In light of the above considerations and limitations of previous studies, the purpose of the present study was to comprehensively investigate the physiological mechanisms and recovery pattern of neuromuscular fatigue to acquire the importance of EIMD in the context of the physiological response to IS. Using an interdisciplinary approach, the objectives were to quantify (i) the effect of IS on central and peripheral physiological fatigue parameters, particularly of the hamstring muscle group; (ii) the contribution of BF_{LH} architecture on fatigue parameters; and (iii) the consequential effects of IS on knee and hip joint kinematics during running and single-leg hop landing (SLHL) over time. We hypothesised that (i) central and peripheral fatigue would both contribute to the immediate loss of muscle function in both the quadriceps and hamstring muscle groups, but that peripheral factors would mainly contribute to the sustained loss of muscle function; (ii) BF_{LH} architecture (e.g. fascicle length and PCSA) would be associated with the response to hamstring fatigue; and that (iii) these changes would lead to reduced hip flexion and knee extension during the late swing phase of treadmill running as well as an increased knee flexion during landing (SLHL).

3.3 MATERIALS AND METHODS

3.3.1 Participants

Twenty recreationally active and healthy young male Caucasians (*mean* ± *SD*; age 20.3 ± 2.87 years; height 1.79 ± 0.05 m; body mass 75.0 ± 7.89 kg) participated in this study. Written informed consent was obtained from each participant prior to starting the study, which complied with the Declaration of Helsinki and was approved by the Research Ethics Committee of Liverpool John Moores University. Participant enrolment began on January 2016 and continued through September 2016, as there were two waves of recruitment. The month of the final test was April 2017. Participants were physically active but were excluded if they had performed strength training of the lower limbs within 6 months prior to participation in the study or if they performed more than three structured exercise sessions per week, as determined via physical activity questionnaire (Baecke et al., 1982b). Further

exclusion criteria were (i) any lower limb musculoskeletal injury in the past 12 months; and (ii) age under 18 or above 35 years; as assessed via interview and health questionnaire.

3.3.2 Experimental Design

Participants were required to visit the temperature-controlled laboratory (between 22 and 24°C) on three occasions: (i) familiarisation, (ii) testing day including assessments before (PRE) and after (POST); and (iii) assessments 48 h (POST48) after the IS-intervention. Prior to the testing day, participants were familiarised with the assessments as well as with the IS (by performing 2-3 submaximal sprints) and BF_{LH} architecture of the hamstring muscle group was assessed via ultrasound. On the test day, participants performed an IS-protocol of 15 x 30 m sprints to induce neuromuscular fatigue/damage in both the quadriceps femoris and hamstring muscle groups. All tests were performed at the same time of the day for each participant. Further, participants were instructed to maintain their normal routine (including eating habits), to refrain from drinking alcohol and to avoid any strenuous exercise 48 h prior to testing and throughout the study.

The test battery was always performed in the same order with the right leg of each participant and comprised (i) venous blood sampling [for analysing serum interleukin-6 (IL-6) concentration and CK activity]; (ii) hamstrings and quadriceps muscle soreness via visual analogue scale (VAS); (iii) isometric maximum voluntary contraction (MVC) torque of the knee-extensors, as well as both voluntary and involuntary [voluntary muscle activation (VA) and torque-frequency relationship via electrical stimulation] MVC torque of the knee-flexors together with normalised BF_{LH} EMG (see below); and (iv) single-leg horizontal hop landing (SLHL) and treadmill running (4.17 m s^{-1}) kinematics of the right leg (8-camera motion capture system) PRE and POST48 of the repeated maximal sprint protocol. At POST test, kinematic assessments were performed first followed by the aforementioned order of the assessment for practical reasons.

3.3.3 Maximal Repeated Sprint Protocol

The IS intervention consisted of 15 repetitions of 30 m maximal sprints with a deceleration zone of 12 m (IS-intervention). The 30 m distance was chosen as the upper average of both the total sprinting distance (346 ± 115 m) of wide-midfielders and the mean recovery time (70.2 ± 25.1 s) between sprint bouts in soccer (Bradley et al., 2010, Bradley et al., 2009), which allows the athlete to maintain the performance of the sprint bouts (Balsom et al., 1992). Similar protocols have been used elsewhere (Timmins et al., 2014, Chen et al., 2017, Verma et al., 2015). Prior to the IS-intervention, a five-minute warm-up was performed, comprising jogging, dynamic stretching and three self-paced 20 m runs at 60%, 80%, 100% of perceived top speed. During the IS-intervention, the participants were instructed to sprint maximally (verbal encouragement) and to stop within the deceleration zone. Further, they were instructed to move slowly back to the start line and to sit on a chair for the remaining time until the next sprint. The recovery comprised 90 s between repetitions and after every 5th repetition, the participants were allowed to rest for 3 min. Sprinting time during trials was measured and controlled with timing gates (Brower Timing Systems, Draper, UT, USA), which were placed on the start and finish line. Participants started 30 cm before the start line to avoid interfering with timing gates with the arms upon initial acceleration (Buchheit and Mendez-Villanueva, 2013). Further, heart rate (Polar Oy, Kempele, Finland) and rating of perceived exertion (RPE) (Borg, 1982) were recorded before and after each repetition. Water was available *ad libitum*. Participants were instructed to wear the same footwear for each testing day. As there was an upsurge in speed of the final sprint, fatigue was assessed with the performance decrement score using the following formula (Glaister et al., 2008, Fitzsimons et al., 1993):

$$Fatigue = \left(100\ X\ (total\ sprint\ time\ \div ideal\ sprint\ time)\right) - 100$$

Where total sprint time = sum of time from all 15 sprints; and ideal sprint time = total number of sprints (15) x fastest repetition sprint time. This decrement score was also used to quantify fatigue regarding sprinting time, heart rate and RPE.

3.3.4 Maximal Muscle Contraction (MVC)

Three test sessions were conducted with an isokinetic dynamometer (IKD; Humac Norm, CSMI Solutions, Massachusetts, USA). As *isokinetic* maximum voluntary contractions (MVC) torque tests are only weak predictors for HSI (van Dyk et al., 2016), we decided to focus on *isometric* MVC extension and flexion torque at optimal knee strength angles (optimal force-length relationship) to avoid further fatigue of the participants. Torque signals were interfaced with an acquisition system (AcqKnowledge, Biopac Systems, Santa Barbara, USA) for analogue-to-digital conversion and sampled at a frequency of 2 kHz. The participant was seated in an upright position and securely fastened with inextensible straps at the chest and waist while the arms were held crossed above the chest. The tibiofemoral epicondyle was set parallel to the axis of rotation of the lever arm, and a shin pad of the lever arm was strapped to the leg, 2 cm above the centre of the lateral malleolus. A Velcro strap secured the distal thigh just above the knee. The hip joint angle was set to 85° (180° = supine position) in order to analyse the knee flexor muscle group at a sprint specific angle associated with the late swing phase of sprinting (Guex et al., 2012). Participants were instructed to maximally extend and flex their leg to measure knee ROM. Knee extension angle was then set to 80º knee flexion (0º = full knee extension) based on results of previous findings which have shown that this is the optimal joint angle for peak knee extension moment in healthy young men (Erskine et al., 2009). Knee flexion (30º) angle was chosen for the optimal joint angle for peak knee flexion based on results from our pilot study. This was in line with sprinting kinematics that maximal hamstring muscle lengths occur during late swing phase when the knee is flexed between 30° and 45° (Thelen et al., 2005). Published studies during the time of data collection used a similar angle of MVC knee flexion torque (Nedelec et al., 2014, Kirk and Rice, 2016). Prior to isometric MVC assessments, participants underwent a standardised warm up consisting of 10 submaximal isokinetic leg extensions ($60^{º} \cdot s^{-1}$). Participants then performed three isometric MVCs of both quadriceps and hamstring muscle groups and with 60 s rests between each MVC for both knee flexion angles. The definition of MVC torque was the highest torque achieved during three maximal

contractions lasting about 3 s. Throughout the tests, participants received verbal encouragement and biofeedback (MVC outputs) were projected onto a screen in front of the participant (Erskine et al., 2009).

3.3.5 Hamstring Muscle Voluntary Activation

To measure hamstring muscle voluntary activation capacity via the interpolated twitch technique, stimulation electrodes (12.5 mm x 7.5 mm self-adhesive electrodes (DJO Global, California, USA) were used. The general procedure has been described elsewhere (Erskine et al., 2009, Erskine et al., 2010b, Marshall et al., 2014). Briefly, the anode was placed proximal to the popliteal fossa, and the cathode was placed beneath the gluteal fold and slightly medial to avoid activation of the VL. Protocols were completed with electrical stimulation pads carefully taped down during the sprinting protocol and were additionally marked on the skin with a permanent marker, to ensure a precise relocation for the POST and POST48 tests. Stimulation was delivered by a high-voltage stimulator (DS7AH; Digitimer Ltd., Welwyn Garden City, United Kingdom), and consisted of a doublet using two 200-V rectangular pulses (200 μs pulse width) with an inter-pulse duration of 10 ms (100 Hz stimulation). During each experimental session, relaxed hamstring muscles were stimulated while participants were fixed in the IKD with the same setting for MVC knee flexion (85º back rest, 30 º knee flexion). The amplitude started with 50 mA to familiarise the participants to the stimulation and was gradually increased in 20 mA increments until a plateau in doublet torque was achieved. We decided to use the individual maximal stimulation (100 %) intensity despite the fact that other publications used supramaximal stimulation (110-130 %) (Marshall et al., 2014) as we experienced lower MVC knee flexion torque output beyond 100 %. That setting was applied during all maximal contractions in the experimental session (average intensity 162.0 17.4 mA, range; 130–200 mA).

The maximal doublet stimulation was used two minutes later to elicit resting maximal doublet torque in the resting state (control doublet), followed 2.5 s later by a second (superimposed) doublet during an isometric knee flexion MVC. The superimposed doublet

torque was always calculated manually from careful selection and inspection of the respective time periods compared to a normal increase in voluntary torque. Voluntary activation was calculated according to the following equation:

$$VA\ (\%) = [1- (superimposed\ doublet\ torque / control\ doublet\ torque)]$$

3.3.6 Hamstring Muscle Group Maximal Compound Muscle Action Potential (M_{max})

The hamstring muscle group was stimulated with single square wave twitch pulses (200 µs duration) using an electrical stimulator. While the participant sat resting on the isokinetic dynamometer with the knee angle set at 30° (0° = full knee extension), compound muscle action potentials (*M*-waves) were evoked with 10 to 20 mA incremental amplitudes until a maximal *M*-wave (M_{max}) was achieved (average amplitude necessary to evoke M_{max} was 166.8 ± 19.8 mA, range; 130–210 mA). The M_{max} was defined as the mean peak-to-peak sEMG response from the three highest observed *M*-waves. M_{max} was used for normalisation of hamstring sEMG during knee flexion MVC (Lanza et al., 2018). Due to technical issues, BF_{LH} sEMG data normalised to M_{max} data was only available for n = 13.

3.3.7 Involuntary Peak Rate of Isometric Troque Development

The highest involuntary peak rate of isometric torque development (pRTD) was determined in the last three involuntary contractions of the maximum compound muscle action potential of the hamstrings (M_{max}) protocol (see above). Involuntary pRTD was calculated as peak ∆torque divided by ∆time, i.e as the steepest rise in torque between the manually determined torque onset (Erskine et al., 2014a) to peak involuntary torque.

3.3.8 Surface Electromyography and Antagonist Co-activation

Surface electromyographic (sEMG) activity was recorded from the vastus lateralis (VL) and BF_{LH} to determine the extent of antagonist muscle co-activation during MVCs of the respective muscle group. Previous reports have shown that VL and BF_{LH} were the representative muscles for the quadriceps femoris (Erskine et al., 2009) and for the hamstring muscle group (Kellis and Baltzopoulos, 1999), respectively. This procedure has been reported in detail elsewhere (Reeves et al., 2004b). Briefly, once the muscles were identified and the skin surface was shaved and cleaned with 70% ethanol, two bipolar Ag-AgCl surface electrodes with an inter-electrode distance of 2 cm (Noraxon duel sEMG electrode, Noraxon, Scottsdale, USA) were placed along the sagittal axis over the muscle belly at 33% of the respective muscle length from the distal end [according to SENIAM guidelines (Hermens et al., 2000, Watanabe et al., 2016)] and one reference electrode (Ambu Blue, Ambu, Copenhagen, Denmark) was positioned over the medial tibial condyle. The exact location of the electrodes were marked on the participant's skin with a permanent marker to ensure precise electrode repositioning for the following assessments.

Surface EMG signals were sampled at 2000 Hz (Biopac Systems, Santa Barbara, USA) and then band-pass filtered between 10–500 Hz (AcqKnowledge, Biopac Systems, Santa Barbara, USA). Surface EMG activity of both the agonist and antagonist muscles were analysed by calculating the root mean square of the sEMG signal of a 500-ms epoch around peak MVC. To compare BF_{LH} sEMG activity at all three time points, BF_{LH} sEMG of the hamstring MVC at 30° was normalised to the evoked compound muscle potential (M_{max}) of the BF_{LH} (see above) (Burden, 2010). Antagonist muscle co-activation (i.e. quadriceps activation during MVC knee flexion at 30° knee flexion, or hamstring activation during MVC knee extension at 80° knee flexion) was calculated with the following formula (where EMG_{max} is the maximum sEMG of the antagonist muscle when acting as an agonist at the same knee joint angle):

$$Antagonist\ muscle\ co-activation = \frac{EMGantagonist}{EMGmax} \times 100$$

Torque signals, electrical stimuli, and sEMG activity were displayed on a computer screen, interfaced with an acquisition system (AcqKnowledge, Biopac Systems, Santa Barbara, USA) used for analogue-to-digital conversion. Co-activation data were available for flexion n = 12; and extension n=10.

3.3.9 Torque-frequency Relationship

The torque-frequency relationship was determined by percutaneous electrical stimulation of the hamstring muscle group at 1 Hz and 10, 15, 20, 30, 50 and 100 Hz for 1 s each in a random order and with 15 s rest between each stimulation. The stimulus intensity for 100-Hz stimulation was the amplitude necessary to elicit ~20 % MVC knee flexion torque at PRE, and the same amplitude was used for the same test at POST and 48POST. The absolute torque at each frequency was normalised to the torque at 100 Hz for each time point (PRE, POST and POST48).

3.3.10 Delayed Onset Muscle Soreness (DOMS)

Using a visual analogue scale (VAS) that consisted of a 100 mm line (scale 0-10 cm; 0 cm=no soreness; 10 cm= unbearably painful), in conjunction with both a three-repetition bilateral squat (predominantly to determine quadriceps femoris muscle soreness) (Scott and Huskisson, 1979) and lunges (predominantly to determine hamstring muscle soreness), participants rated their perceived lower limb muscle soreness along the muscle length immediately after each movement. It has been reported that VAS is a valid and reliable measure of muscle soreness [intraclass correlation coefficient (ICC) > 0.96] (Bijur et al., 2001). Muscle soreness was also measured by recording the force required to elicit tenderness at nine fixed sites on the skin over the quadriceps (distal, central and proximal locations of the three superficial quadriceps heads, VL, vastus medialis and rectus femoris) and six sites on the hamstrings (distal, central and proximal locations of both BF_{LH} and the medial hamstrings), which were previously marked with a permanent marker to ensure the

same measuring position PRE, POST and POST48. At each site, a gradually increasing force was applied by the investigator with an algometer (FPK/FPN Mechanical Algometer, Wagner Instruments, Greenwich, USA) with a maximum of 10 kg. Lying in the prone position with the hip and knee fully extended and muscles relaxed, the participant was asked to indicate when the sensation of pressure changed to discomfort, and the force at the point was recorded (Newham et al., 1987).

3.3.11 Blood Samples

A 10 mL blood sample was drawn from an antecubital vein in the forearm and collected into a serum vacutainer (BD Vacutainer systems, Plymouth, UK). The blood samples were obtained at each time point and left at 22-24°C for 30 min to allow clotting, and then kept on ice when necessary. Serum samples were centrifuged at 1,300 g for 15 min at 4°C. All samples were then aliquoted into 1.5 mL microcentrifuge tubes [Axygen (Corning), New York, USA] and stored at -80°C until subsequent analysis (see below).

3.3.12 Serum Interleukin-6 (IL-6) Concentration

Serum samples were assayed for IL-6 concentration using commercially available human IL-6 enzyme linked immunosorbent assay (ELISA) kits (Quantikine®, R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Briefly, the serum samples were thawed and aliquots (200 µL) of each diluted sample, positive control or standard, with known concentrations of human IL-6 to establish standard values, were plated on a coated (monoclonal antibody specific for human IL-6) 96-well microtitre plate for 2 h. After washing, human IL-6 conjugate (200 µL) was added to each well and incubated for 2 h at room temperature (between 22 and 24°C). After the wells were washed, substrate solution (200 µL) was added to each well at room temperature (between 22 and 24°C) and protected from light. After adding 50 µL of stop solution to each well, the intensity of the colour produced after 20 min was measured with a Thermo Multiskan Spectrum microplate

reader (Thermo Fisher Scientific. Waltham, MA. USA) at 450 nm and values were calculated with Excel 365 (Microsoft, v. 365, USA) by generating a four-parameter logistic (4-PL) curve fit. The minimum detectable dose of human IL-6 was 0.70 pg/mL.

3.3.13 Serum Creatine Kinase Activity

Creatine kinase (CK) activity was assayed using a commercially available CK assay (Catachem Inc., Connecticut, NE, USA), as described in detail elsewhere (Sharples et al., 2011, Owens et al., 2014). Briefly, 10 µL blood serum were loaded onto a 96-well UV plate. The CK reaction reagent and diluent (Catachem) were prepared as per the manufacturer's instructions and heated for 2 min at 37°C. The reconstituted reagent contained the following active ingredients: 30 mmol/l PCr, 2 mmol/l ADP, 5 mmol/l AMP, 2 mmol/l NAD, 20 mmol/l N-acetyl-L-cystine, 3,000 U/l hexokinase, 2,000 U/l G-6-PDH, 10 mmol/l 1 Mg2, 20 mmol/l D-glucose, 10 mol/l di(adenosine 5=) pentaphosphate, and 2 mmol/l EDTA, buffered to pH 6.7. The reagent mixture was then added to the samples and the change in absorbance monitored continuously over 20 min in a Thermo Multiskan Spectrum plate reader at a wavelength of 340 nm.

3.3.14 Capillary Blood Lactate Concentration

All lactate probes were cleaned and calibrated in accordance with the manufacturer's instructions. Briefly, Capillary blood samples were taken from the finger-tip Safety-Lancet Extra 18G needle (Sarstedt; Nümbrecht, Germany) on testing day at rest before and immediately after the IS-intervention. Blood samples were analysed within 60 seconds of collection using a portable blood lactate analyser (Arkray Lactate Pro; Kyoto, Japan).

3.3.15 Ultrasound

Architectural parameters of the BF_{LH} were assessed using B-mode ultrasound imaging. Participants were in the prone position with the hip and knee fully extended and muscles

relaxed. The BF_{LH} was chosen, as this muscle is the most commonly injured hamstring muscle during team sports, particularly during sprinting (Slavotinek et al., 2002, Ekstrand et al., 2011). Longitudinal and cross-sectional panoramic ultrasound images of the right BF_{LH} were obtained (Philips EPIQ 7 Ultrasound System, Bothel, USA). The transducer (L18-5, 5-18 MHz wave frequency; aperture 38.9 mm) was carefully placed on the skin with transmission gel and BF_{LH} was scanned (i) longitudinally from its distal (=0%) to proximal (=100%) myotendinous junction along a line drawn with a permanent marker to mark the central pathway between the medial and lateral aspects of the muscle (incorporating the intra-muscular aponeurosis (Evangelidis et al., 2014) (Figure 3-1); and (ii) cross-sectionally at 20, 40, 60 and 80% along the total muscle length which was tape measured (Figure 3-2).

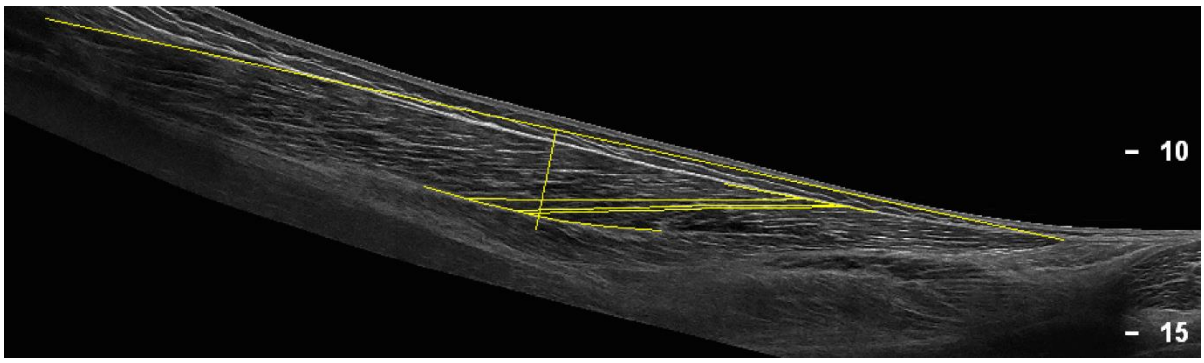

Figure 3-1 *Longitudinal image of biceps femoris long head, assessment of the biceps femoris long head is highlighted (total muscle length and fascicle length together with pennation angle at 50% of total muscle length).*

All images were analysed offline (ImageJ, version 1.51s, National Institutes of Health, Bethesda, USA). Two images for each of the four cross-sectional points were recorded for calculating BF_{LH} muscle volume. The volume of the muscular portion between every two consecutive scans was calculated with the following equation:

$$Volume = \frac{1}{3} * d * \left(a + \sqrt{(ab) + b}\right)$$

Where *a* and *b* are the anatomical cross-sectional areas of the muscle of two consecutive cross-sectional scans and *d* is the interval distance between the cross-sectional area measurements. The volume of the entire muscle was calculated by summing up all of the inter-scan muscular volumes (Esformes et al., 2002, Reeves et al., 2004a).

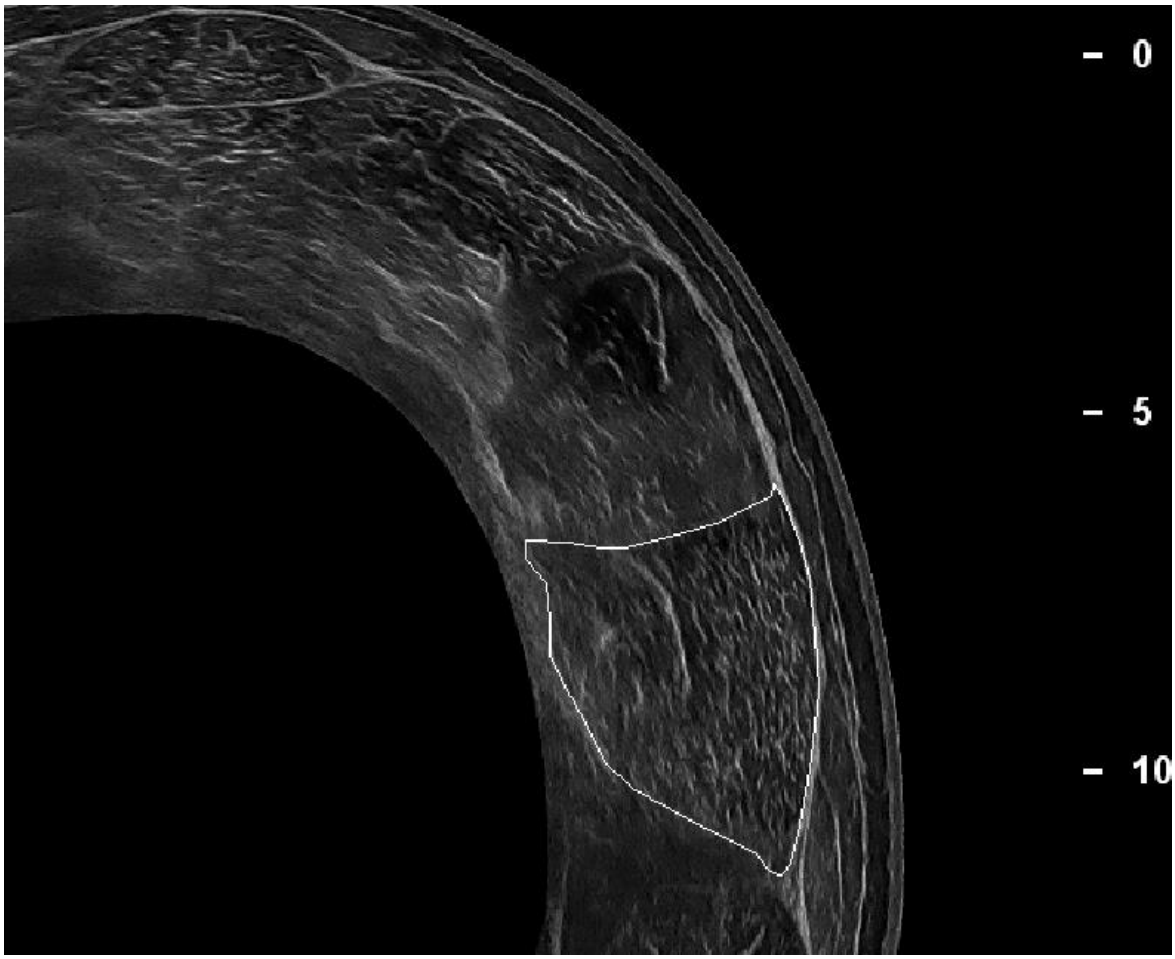

Figure 3-2 *Cross-sectional image at 60% muscle length (=100% proximal myotendinous junction), biceps femoris long head is highlighted.*

Two longitudinal images were then recorded to allow for the estimation of passive BF_{LH} muscle fascicle length and pennation angle, which were both assessed in 3 fascicles at 50 % of the total length of BF_{LH}. This point was measured offline (ImageJ). A comparison between offline and tape measurements of the total BF_{LH} length revealed a very high correlation (R^2=0.958, P<0.001). The calculation of the total fascicle length was measured by tracing the fascicle echo from the upper aponeurosis to the intra-muscular aponeurosis of the BF_{LH}. Muscle fascicle pennation angle was determined as the angle between the muscle fascicular paths and their insertion into the intra-muscular aponeurosis. The mean of the three measurements for each parameter were used to determine both fascicle length and pennation angle of the BF_{LH} muscle. PCSA was calculated by dividing BF_{LH} volume by its fascicle length. During the time of data collection, a similar methodological approach was published (Seymore et al., 2017). One longitudinal image of one participant in the present

study was not analysed due to low image quality. Ultrasound scans and image analysis was performed by the same investigator.

3.3.16 Kinematic and Kinetic Data

Three-dimensional kinematic and kinetic data were synchronously collected at 500 Hz using an eight-camera motion analysis system (Qqus 300+; Qualisys, Gothenburg, Sweden) together with a ground-embedded force plate (90 x 60 cm, 9287B; Kistler Holding, Winterthur, Switzerland) at 1,500 Hz. The data were filtered with a digital dual low-pass Butterworth filter at 20 Hz for motion and 60 Hz for force, as previously described (Verheul et al., 2017).

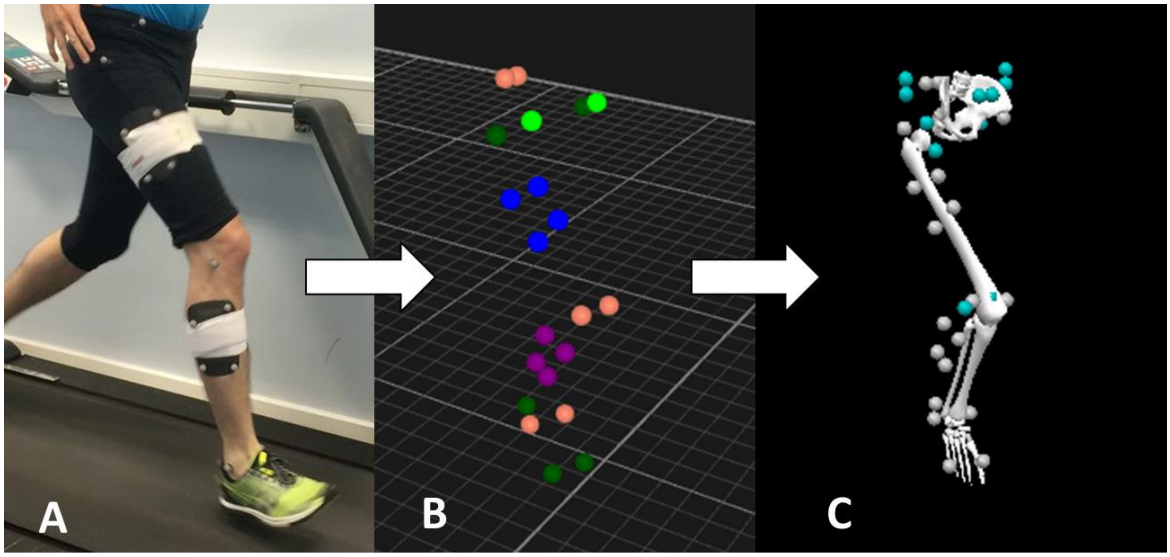

Figure 3-3 *Motion capture system was used to track the three-dimensional positions of (i) reflective markers placed on palpable anatomical landmarks (segment defining markers); and (ii) marker clusters on plates attached on the lateral side of the thigh and shank (both 4 markers) of the right leg (tracking markers) (A). Markers were tracked offline with Qualysis Track Manager Software (B) and hip and knee angle and angular velocities were calculated in visual3D software (C).*

Retroreflective markers (12mm diameter) were placed on anatomical landmarks on the right leg and pelvis, as previously described (McClay and Manal, 1999) (Figure 3-3). One standing static and two functional motion calibration trials were recorded of the participant PRE, POST and POST48. For the static trial, participants stood with their feet approximately shoulder width apart and knees fully extended. This static trial determined local coordinate systems, the location of joint centres, and the foot, shank, thigh, and pelvis segment lengths

of each participant. The functional trials defined functional hip joint centres (Schwartz and Rozumalski, 2005) and knee joint axes (Robinson and Vanrenterghem, 2012). Kinematic data were tracked using Qualisys Track Manager Software (Qualisys). Data processing and analysis were undertaken in Visual3D (C-Motion, Germantown, MD). To examine any changes between the time points, joint angles were normalised relative to the static trial of the accompanying time point for minimising the influence of potential slightly different marker positions between the trials. Lower extremity 3D joint angles and angular velocities were calculated using an X-Y-Z Cardan angle rotation sequence. Investigated variables included the knee and hip angles at the instant of landing, peak knee and hip angles, as well as ROM and time (i) from landing to the peak value (SLHL); and (ii) during stance and swing phase (treadmill run), for all three planes, were calculated as described in previous studies (Verheul et al., 2017, Apps et al., 2017).

3.3.17 Single-leg Horizontal Hop Landing

The distance of a maximum unilateral horizontal right leg hop was recorded at PRE with a measuring tape. Landing kinematics of a single-leg horizontal hop landing (SLHL) movement were subsequently analysed following an 80% maximal hop on the right leg onto a force plate. The same distance was used for the following two assessments (POST and POST48). The first three successful trials were digitised and averaged for each participant. Jamkrajang et al. (2017) have recently shown that a reduced lower limb model showed worse agreement for whole-body balance regarding jump tasks, when practised with swinging arms compared to a full-body model, compared to the full-body model (gold standard), for which many markers need to be placed on the body. Therefore, the participant was asked to perform SLHL by holding the arms akimbo and without crossing the knees when generating forward momentum (to eliminate a backswing). Knee and hip angles at the instant of landing, peak knee and hip angles, as well as ROM and time from landing to the peak value, for all three planes, were calculated between the initial contact with the

force plate and 200 ms after the landing. The initial contact was determined when the vertical force exceeded a threshold of 20 N.

3.3.18 Motorised Treadmill Run

Participants ran on a motorised treadmill (HP Cosmos Pulsar; Nussdorf, Germany) for 30 s at 4.17 m s^{-1}. The selected speed is based on pilot testing demonstrated that 15 km/h was the fastest speed on a motorized treadmill where the participants still felt comfortable. Motion analysis data were recorded for the last 10 s of the run and data were analysed for at least 6 consecutive strides. Peak knee and hip angle data, for all three planes, were calculated (i) between the initial contact and terminal stance of foot; and (ii) between initial and terminal swing phase. The touchdown of the foot during the treadmill run was determined from the kinematic data as occurring at the local minima and the toe-off during running as the local maxima of the vertical velocity of the head of the fifth metatarsal marker on the foot (Maiwald et al., 2009).

3.3.19 Reliability of the Current Methods

The test-retest reproducibility for isometric knee extensor MVC is high, with a coefficient of variation (CV) of 3.9% (Erskine et al., 2009). Likewise, the test-retest reproducibility for assessing voluntary activation via ITT is also high, with a CV of 2.4% (Marshall et al., 2014). The test-retest reproducibility for antagonist muscle (hamstring) co-activation (assessed via sEMG activity in the BFLH) during an isometric knee extension MVC, on the other hand, has been reported to be quite low, with a CV of 14.39% (Erskine et al., 2009). The test-retest reproducibility for ultrasonographic measurementis in the middle of the BF_{LH} is high for measuring muscle length (ICC=0.93; r=0.92), fascicle length (ICC=0.79; 0.55–0.91) and angle (ICC=0.80; range 0.56–0.91) (Freitas et al., 2018). Using panoramic ultrasound mode is associated with an excellent ICC 2, k (SEMs): fascicle length, 0.99 (0.11 cm); pennation

angle, 0.88 (1.1°); volume, 0.99 (2.07 cm^3), and PCSA, 0.99 (0.42 cm^2) (Seymore et al., 2017).

3.3.20 Data Analysis

One-way ANOVAs with repeated-measures were performed to determine whether there was a significant main effect for time (within subject factor) for the following dependent variables: MVC torque, voluntary muscle activation, antagonist muscle co-activation, muscle soreness (VAS-squat, VAS-lunge and algometer), RPE, CK activity, IL-6 concentration, and for kinematics data (hip and knee angle parameters). MVC torque data were analysed for interactions and main effects for muscle group and time using two-way mixed design ANOVAs, comparing differences between muscle groups across 3-time points; PRE, POST, and POST48. For within test comparisons, either, independent t-tests, or one-way ANOVAs were used where appropriate. For the torque-frequency relationship, normalised torque at each frequency was analysed using a two-way repeated measures ANOVA with stimulation frequency and time (PRE, POST and POST48) as the independent variables. Separate one-way repeated measures ANOVAs were used to determine if the change in normalised torque was similar across all stimulation frequencies at each time point. Linear regression analyses were used to analyse the relation between architectural parameters of the BF_{LH} (volume, fascicle length, fascicle pennation angle and PCSA) and fatigue biomarkers (relative MVC loss normalised to PRE MVC), serum CK activity, serum IL-6 concentration, muscle soreness, muscle ROM or changes in ROM during treadmill running or SLHL). Standard guidelines concerning violation of the sphericity assumption to adjust the degree of freedom of the F-test by the Huynh-Felt epsilon if epsilon is greater than 0.75 and to use the more stringent Greenhouse-Geisser adjustment if epsilon is less than 0.75 were followed (Girden, 1992). Results were expressed as mean ± SD, unless otherwise stated, with statistical significance set at $P<0.05$. All MVC data were analysed with AcqKnowledge software 4.4 (Biopac-Systems Inc., Goleta, USA) and SPSS 23

Software (IBM Inc., Armonk, NY: IBM Corp) was used for statistical analysis. Occasional missing data are reflected in the reported degrees of freedom.

3.4 RESULTS

3.4.1 Effect of IS-Intervention on Fatigue

There was a main effect of time for heart rate, sprinting time, RPE and lactate with all parameters increased POST IS-intervention (all $P < 0.001$), indicating fatigue had occurred. Lactate significantly increased from PRE (1.63 ± 0.45 mmol/L) to POST (9.82 ± 3.62 mmol/L). The average sprinting speed was 6.48 ± 0.33 m s^{-1}. The sprinting performance decrement was 3.98 ± 2.99 % during the IS-intervention but this varied between 1.49 % and 15.24 %. RPE increased by 96.5 ± 35.2 % during IS-intervention with a variation from 26.8 % to 134.4 % between participants.

There was no main effect for time regarding voluntary muscle activation or for any EMG activity assessments of the BF_{LH} or VL (all $P>0.05$; Table 3-1) except for BF_{LH} flexion EMG_{max}/M_{max} ($F_{F2,24}=4.35$, $P=0.022$). Post-hoc independent t-test revealed a significant decrease in normalised BF_{LH} EMG from PRE to POST ($P=0.019$), but this change was not evident at POST48 ($P=0.157$).

Table 3-1 *Effect of the intermitted sprint-intervention on fatigue Markers (mean ± SD).*

Assessment [unit]	n	PRE	POST	POST48	F-Test	P Value
Hamstring Muscle Voluntary Activation [%]	20	98.5 ± 2.64	94.1 ± 7.83	96.9 ± 5.96	F(1.4,38) = 2.75	0.099
BF_{LH} Flexion $sEMG_{max}/M_{max}$ [%]	14	3.32 ± 1.33	2.27 ± 0.72	2.85 ± 1.16	F(2,24) = 4.35	0.022*****
VL Extensions EMG_{max} [mV]	15	0.50 ± 0.29	0.47 ± 0.34	0.51 ± 0.34	F(2,24) = 0.17	0.772
QF CoA During 30° Flexion MVC [%]	12	5.74 ± 7.23	4.08 ± 6.86	4.33 ± 8.76	F(2,22) = 0.50	0.613
Hamstring CoA During 80° Extension MVC [%]	10	4.79 ± 3.37	6.62 ± 3.79	7.51 ± 4.16	F(2,18) = 1.64	0.223

BF_{LH} – Biceps femoris long head; CoA – Co-Activation; VL – Vastus lateralis; sEMG – surface Electromyography; QF – Quadriceps femoris; * Significant differences between PRE to POST.

There was an interaction between time x stimulation frequency regarding torque-frequency relationship (n=19; $F_{4.9,216}$=6.62, P<0.001). Post-hoc one-way ANOVA showed significant differences between PRE and POST, between POST and POST48 (both P<0.005), but not between PRE and POST48 (Figure 3-4). Post-hoc independent t-tests revealed significant differences between PRE and POST, and between POST and POST48 at frequency 10, 15 and 20 Hz (all P<0.05), all three time points at 30 Hz (P<0.05), and between PRE and POST, and between PRE and POST48 50 Hz (both, P<0.05).

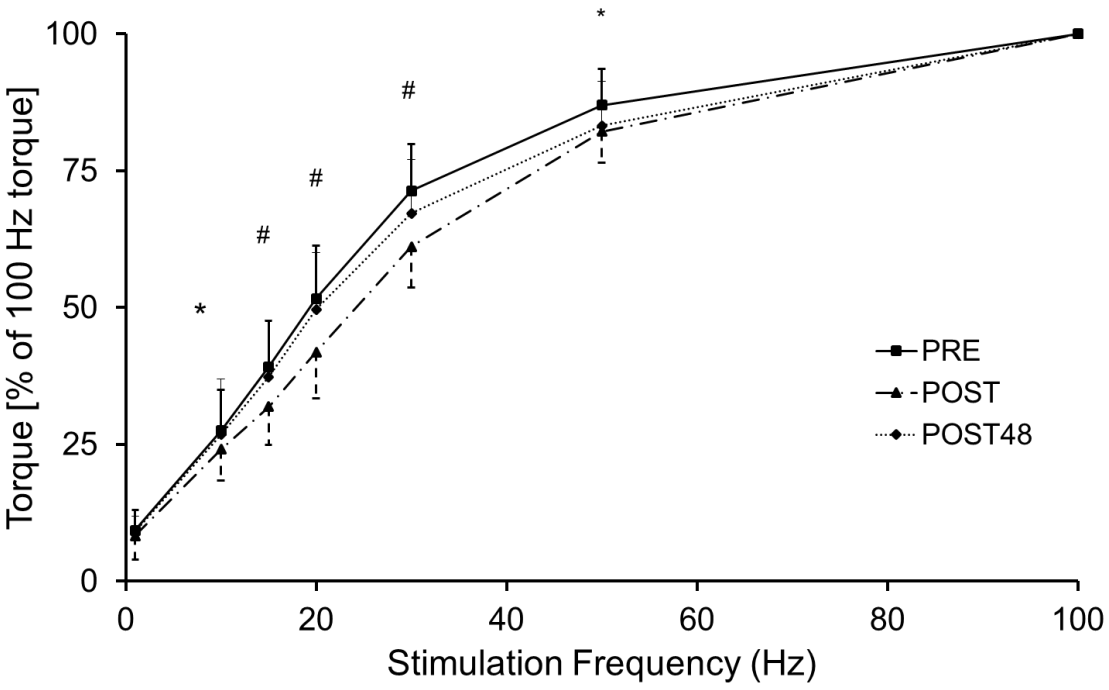

Figure 3-4 *Torque-frequency relationship, all frequencies normalised to 100 Hz. * significant differences between before (PRE) and immediadtely after (POST) IS-intervention, P<0.05; # significant differences between PRE and POST, and between POST and POST48, P<0.05. Results were expressed as mean ± standard error of mean.*

3.4.2 Effect of IS-Intervention on MVC, pRTD, Muscle Soreness and Serum Markers of EIMD

Isometric hamstring and quadriceps MVC, muscle soreness (all P<0.001) and serum CK activity ($F_{1.3,34=}$5.98, P=0.017) as well as IL-6 concentration ($F_{1.3,34=}$5.96, P=0.018) showed

a main effect of time, indicating EIMD had occurred following IS-intervention (Table 3-2). There was a correlation between quadriceps and hamstring relative MVC torque loss (% changes measured PRE to POST) (R^2=0.299, $F_{1,18}$=7.66, P=0.013). Involuntary pRTD decreased from PRE to POST (P<0.001) and remained decreased POST48 (P=0.007). Serum CK activity was significantly elevated POST (PRE: 27.9 ± 23.3 mU/mL; POST: 53.8 ± 45.3 mU/mL; P=0.027) and further increased POST48 (99.3 ± 104.5 mU/mL; P=0.012) compared to PRE. Serum IL-6 concentration was significantly elevated POST (PRE: 1.89 ± 3.10 pg/mL; POST: 7.68 ± 9.95 pg/mL; P=0.027) and reverted to baseline values POST48 (1.59 ± 3.46 pg/mL; P>0.05). There was a main effect of time regarding sensation of pressure for both quadriceps ($F_{2,28}$=8.27, P=0.002) and hamstring ($F_{2,28}$=4.37, P=0.022) muscle group. Sensation of pressure on the quadriceps was significantly different POST (8.01 ± 1.42 kg/cm^2; P=0.019) and POST48 (7.61 ± 1.36 kg/cm^2; P=0.005) compared to PRE (8.66 ± 1.63 kg/cm^2). For pressure sensation on the hamstrings, POST48 (7.59 ± 2.09 kg/cm^2) showed significant differences compared to PRE (8.58 ± 1.56 kg/cm^2; P=0.047) and POST (8.51 ± 1.969 kg/cm^2; P=0.011). However, the changes in sensation of pressure over time were smaller compared to both squat and lunge muscle soreness assessments (Table 3-2).

Table 3-2 *Effect of IS-Intervention on EIMD-Biomarkers (mean ± SD).*

Assessment [unit]	PRE	POST	POST48	F-Test	P Value
QUAD MVC [N·m]	270.5 ± 51.6	222.4 ± 52.5	243.0 ± 71.3	F(2,38) = 16.55	<0.001*
HAM MVC [N·m]	142.5 ± 25.0	124.8 ± 29.9	112.4 ± 30.1	F(2,38) = 25.12	<0.001*
Involuntary HAM pRTD (N·m·s^{-1})	214 ± 51.8	137 ± 43.6	178 ± 45.5	F(2,34) = 26.57	<0.001*
Squat Muscle soreness [cm]	0.20 ± 0.41	1.95 ± 1.61	2.87 ± 1.71	F(2,38) = 28.62	<0.001*
Lunge Muscle soreness [cm]	0.30 ± 0.57	2.30 ± 2.08	3.48 ± 2.07	F(2,38) = 17.02	<0.001†
Range of Motion [°]	120.3 ± 6.76	115.7 ± 6.77	116.0 ± 6.27	F(2,38) = 9.33	<0.001†

CK activity [mU/mL]	27.9 ± 23.3	53.8 ± 45.3	99.3 ± 104.5	F(1.3,34) = 5.98	0.017†
IL-6 concentration [pg/mL]	1.89 ± 3.10	7.68 ± 9.95	1.59 ± 3.46	F(1.3,34) = 5.96	0.018#

QUAD – Quadriceps muscle group; HAM – Hamstring muscle group; MVC – Maximal voluntary contraction; pRTD – Peak Rate of Torque Development; CK – Creatine Kinase; IL-6 – Interleukin-6; * Significant differences between all time points; † Significant differences between PRE to POST and POST48; # significant differences between POST to PRE and POST48.

There was an interaction between time and muscle groups concerning relative MVC (normalised to PRE MVC) torque loss ($F_{1.4,38}$=7.92, P=0.004). Percentage change in relative MVC decreased similarly in both quadriceps and hamstring muscle groups POST (Figure 3-5). However, quadriceps MVC recovered towards baseline values 48POST, whilst hamstring MVC continued to decrease from POST to POST48 (P=0.038).

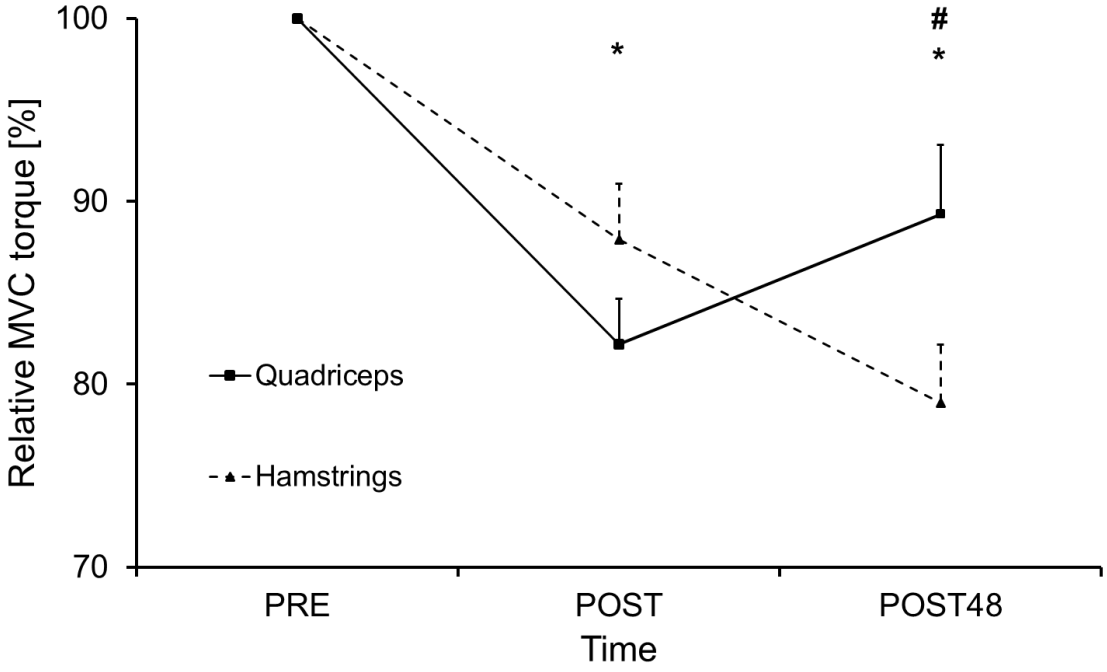

Figure 3-5 *Comparison of relative maximal voluntary contraction (MVC) loss between hamstring and quadriceps muscle group before (PRE), immediately after (POST) and 48h after (POST48) the intermittent sprint intervention. * signifcant differences compared to PRE, P< 0.001; # significant differences between quadriceps and hamstring MVC, P< 0.05. Results were expressed as mean ± standard error of mean.*

3.4.3 Architecture of the Biceps Femoris Long Head

The values of architectural parameters are presented in Table 3-3.

Table 3-3 *Architectural parameters of Biceps femoris long head (mean ± SD).*

Muscle length [cm]	Fascicle length [cm]	PCSA [cm^2]	Volume [cm^3]	Pennation Angle [°]
27.86 ± 2.13	7.94 ± 1.38	23.4 ± 4.62	182.2 ± 29.5	12.7 ± 2.77

Fascicle length at 50% of total BF_{LH} muscle length; PCSA – Physiological cross-sectional area.

Fascicle length and pennation angle of the BF_{LH} did not correlate with any outcome variable. There was no correlation between BF_{LH} volume and hamstring MVC torque PRE (R^2=0.188, $F_{1,18}$=4.16, P=0.056) and POST48 (R^2=0.134, $F_{1,18}$=2.78, P=0.113). However, there was an inverse correlation between BF_{LH} volume and hamstring MVC torque POST (R^2=0.281, $F_{1,18}$=7.04, P=0.016). BF_{LH} PCSA correlated inversely with relative hamstring MVC loss from PRE to POST (R^2=0.421, $F_{1,17}$=12.37, P=0.003, Figure 3-6), but not with relative hamstring MVC loss from PRE to POST48 (R^2=0.113, $F_{1,17}$=2.18, P=0.159, Figure 3-7).

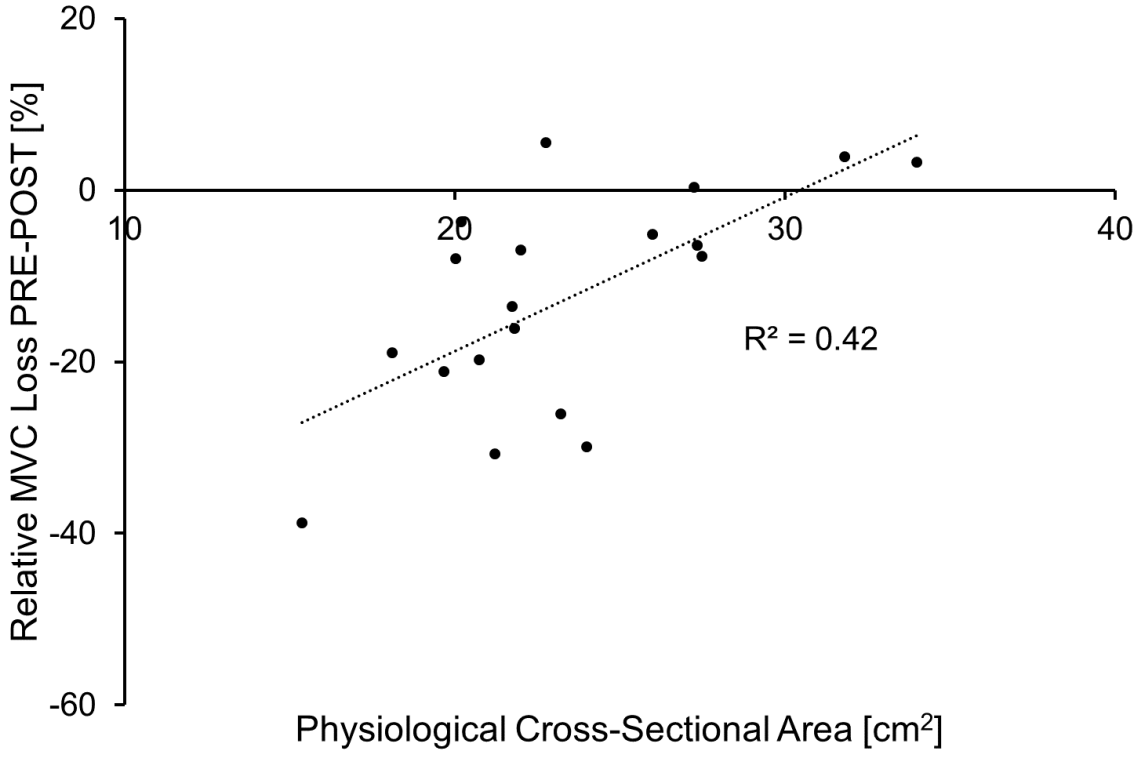

Figure 3-6 *Correlation between physiological cross-sectional area and relative maximum voluntary contraction (MVC) loss measured between before (PRE) and immediately after (POST) of the intermittent sprint intervention (P=0.003).*

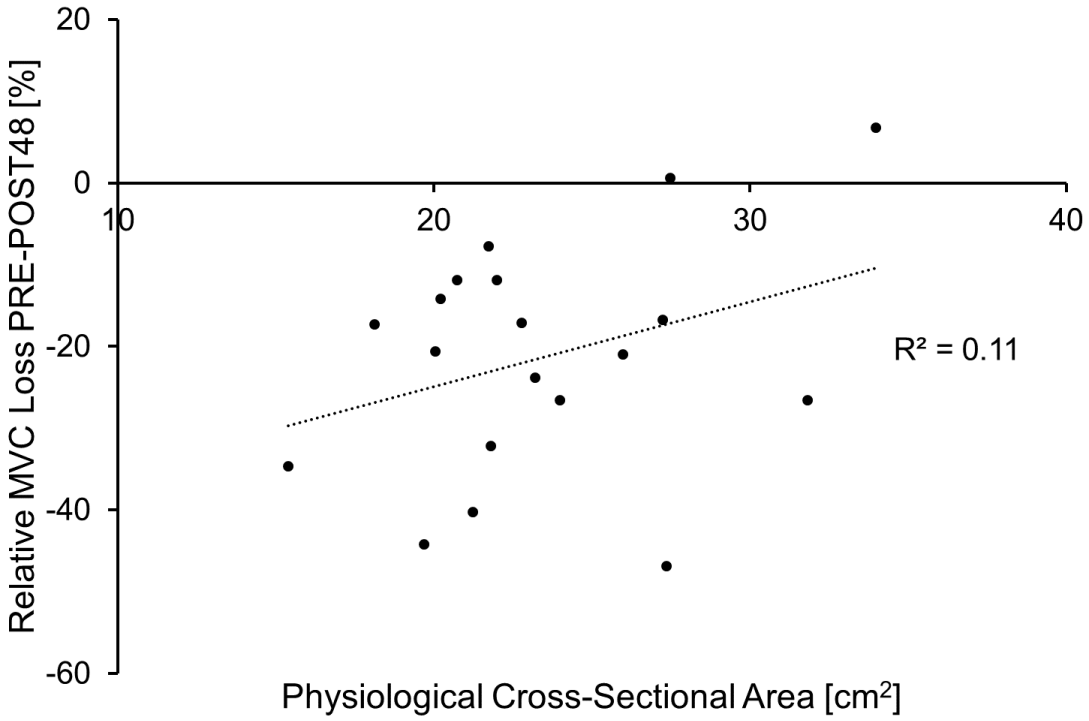

Figure 3-7 *No correlation between physiological cross-sectional area and relative maximum voluntary contraction (MVC) loss measured between before (PRE) and 48h after (POST48) of the intermittent sprint intervention (P=0.159).*

3.4.4 Effect of the IS-Intervention on Lower-Limb Kinematics

Three-dimensional motion analysis for SLHL demonstrated increased (i) hip flexion (p=0.004); (ii) hip ROM in the transverse plane (p=0.025); and (iii) knee adduction (p=0.035) during the landing phase of SLHL immediately after the IS-intervention, and the values reverted to baseline levels POST48. There was a main effect of time for the time-period between heel contact and maximal knee flexion (p=0.012), whereas the duration of the knee flexion took significantly longer POST48 compared to POST (Table 3-4).

Table 3-4 *Effect of the IS-Intervention on selected kinematics of the Single-Leg Hop Landing during the Landing Phase (mean ± SD).*

Kinematics [unit]	PRE	POST	POST48	F-Test	P Value
Peak hip flexion [°]	36.7 ± 9.61	41.7 ± 12.22	36.0 ± 11.08	F(2,34) = 6.61	0.004*
Hip peak internal rotation [°]	10.3 ± 6.87	6.97 ± 4.24	8.29 ± 6.22	F(2,34) = 3.11	0.058
Hip peak external rotation [°]	3.40 ± 4.22	9.01 ± 5.42	4.94 ± 5.17	F(2,34) = 9.61	<0.001*

Hip ROM in the transverse plane [°]	10.7 ± 4.48	13.67 ± 6.10	11.60 ± 5.75	F(2,34) = 4.13	0.025*
Peak knee adduction [°]	-3.81 ± 3.27	-6.53 ± 5.46	-3.96 ± 5.24	F(2,34) = 3.71	0.035*
Knee flexion at initial contact [°]	-12.4 ± 6.79	-13.1 ± 5.87	-11.6 ± 8.47	F(2,34) = 0.47	0.630
Duration of hip flexion [s]	0.073 ± 0.018	0.065 ± 0.021	0.084 ± 0.041	F(1.43,34) = 3.60	0.056
Duration of knee flexion [s]	0.16 ± 0.05	0.16 ± 0.04	0.18 ± 0.05	F(2,34) = 5.12	0.011*

ROM – Range of motion.

There was a non-significant effect of time for the duration of a running cycle (P=0.080; Table 3-5). The duration of the running cycle tended to take longer POST and POST48 compared to PRE. Treadmill running demonstrated decreases in peak knee extension (P=0.047) during the late swing phase at POST, which went back to baseline value POST48.

Table 3-5 *Effect of the IS-Intervention on Kinematics of Treadmill Running (4.17 m s^{-1}) (mean ± SD).*

Kinematics [unit]	**PRE**	**POST**	**POST48**	**F-Test**	**P Value**
Peak knee flexion (swing phase) [°]	-103 ± 13.9	-110 ± 12.4	-108 ± 12.6	F(2,20) = 2.84	0.082
Peak knee extension (swing phase) [°]	-3.66 ± 5.32	-7.08 ± 5.07	-4.29 ± 7.06	F(2,20) = 3.57	0.047*
Contact hip flexion (toe strike) [°]	25.2 ± 5.42	29.3 ± 7.62	17.6 ± 15.6	F(1.2,20) = 4.05	0.062
Contact knee flexion (toe strike) [°]	-15.0 ± 6.25	-17.6 ± 6.79	-13.3 ± 9.64	F(2,22) = 2.79	0.083
Duration Running cycle [s]	0.67 ± 0.03	0.68 ± 0.03	0.69 ± 0.02	F(2,20) = 2.88	0.080
Stance phase duration [s]	0.18 ± 0.02	0.19 ± 0.02	0.19 ± 0.03	F(2,20) = 1.01	0.356
Swing Phase [s]	0.50 ± 0.04	0.50 ± 0.05	0.50 ± 0.03	F(1.2,20) = 0.03	0.899

Knee fully extended=0°; negative number indicates a flexed knee.

3.5 DISCUSSION

The aim of the current study was to investigate the physiological and biomechanical factors underpinning impaired physical performance following an IS-intervention. The objectives were to comprehensively assess (i) the contribution of central and peripheral fatigue on acute and prolonged decrement in performance in response to a IS-intervention, (ii) whether BF_{LH} architecture correlated with fatigue indices; and (iii) the consequential effect of fatigue on lower-limb kinematics of landing (SLHL) and running (treadmill 4.17 m s^{-1}). In line with our hypotheses, immediate strength loss was associated with lower hamstring EMG activity (indicating impaired hamstring muscle recruitment) and markers of peripheral fatigue, but the magnitude and sustained changes in MVC torque over time was predominately associated with indicators of peripheral fatigue. Muscle damage biomarkers indicated that the peripheral fatigue might have been predominantly caused by ultrastructural damage within the hamstring muscle fibres. Further, both central and peripheral fatigue probably had an impact on the neuromuscular control of running and landing patterns immediately after the IS-intervention. Contrary to our hypothesis, BF_{LH} fascicle length was not associated with the extent of hamstring fatigue following the IS-intervention. However, BF_{LH} PCSA correlated with knee flexor MVC torque change immediately POST, indicating that the non-contractile structure of the muscle, e.g. the extracellular matrix, might contribute to the individual variability in the response to muscle damaging exercises.

3.5.1 Fatigue Following the Intermittent Sprint Intervention

The contribution of central and peripheral fatigue has previously been investigated in studies using a simulated soccer-match as an intervention for the quadriceps muscle group (Thomas et al., 2017) and hamstring muscle group (Marshall et al., 2014). The investigation of Thomas et al. (2017) showed that both central and peripheral fatigue contribute to acute neuromuscular fatigue of the quadriceps muscles but that mainly peripheral fatigue causes the delayed recovery. Marshall et al. (2014) concluded that impaired hamstring muscle

function was mainly caused by central fatigue, which was investigated via twitch interpolation technique and EMG (normalised to M_{max}). Presumably, the different results are based on varying methodological approaches and different muscle groups studied. Firstly, simulated soccer match protocols include additional (multidirectional) low and moderate-speed movements and this is accompanied with less time to rest during the intervention compared to the current IS-intervention. In line with the current study, Marshall et al. (2014) showed a decreased activity of normalised BF_{LH} sEMG over time, but no significant changes in parameters of fatigue detected using the twitch interpolation technique. This discrepancy might be explained by the fact that voluntary activation via twitch interpolation technique investigates the summation of all involved hamstring muscles, and the summation showed no decrease in central fatigue. However, normalised sEMG analyses specific muscles such as the BF_{LH} in both the current and the study of Marshall et al. (2014). Both studies indicate that BF_{LH} might fatigue to a greater degree immediately after IS compared to the other hamstring muscles, as reduced sEMG suggests either a reduction in central activation or recruitment of motor units affecting BF_{LH} muscle function, or both. For estimated peripheral fatigue, Marshall et al. (2014) used resting doublet with 100 Hz, but lower physiological frequencies were not included in the assessment. In human muscle, the torque-frequency relationship can be used to asses peripheral fatigue, and the effect of fatigue is most evident when muscles are stimulated at frequencies around 10–30 Hz (Jones, 1996, Bellemare et al., 1983). Therefore, the impaired muscle function seen in the study of Marshall et al. (2014) following the soccer match simulation might be not solely based on central fatigue, as suggested, and a major contribution may have been due to peripheral fatigue as the current study indicates.

The different results between VA and normalised sEMG of the BF_{LH} suggest that muscle impairment of BF_{LH} might not be solely caused of central fatigue as other hamstring muscles would then be affected at the same rate compared with the BF_{LH}. Both the semitendinosus and BF_{LH} share the same proximal tendon at the ischial tuberosity and there is evidence to suggest that there is a complex muscle recruitment between these both muscles

(Schuermans et al., 2016, Schuermans et al., 2014). Recent investigations revealed that the BF_{LH} has the highest EMG activity between 15 and 30° of knee flexion angles, while the other hamstring muscles showed the largest EMG activity between 90 and 105° of knee flexion, when isokinetic MVCs were performed (Onishi et al., 2002). During the deceleration of the knee extension in the swing phase of sprinting, it could be speculated that the semitendinosus and BF_{LH} have their highest EMG activity to different time points during the late swing phase. The semitendinosus might predominantly contribute to decelerate the shaft at the initial part of the late swing phase (at the time with a more flexed knee joint). During the subsequent part of the late swing phase of sprinting, the contribution to decelerate the shaft shifts from the semitendinosus to the BF_{LH} (when the knee joint extends until terminal swing). After repeated bouts of high-speed running, the semitendinosus might fatigue prematurely (Schuermans et al., 2014) and the BF_{LH} would need to substitute the impaired function of the preceding semitendinosus to decelerate the shaft. To protect the BF_{LH} against injuries, the reduced neuromuscular activation of the BF_{LH} is accompanied by changes of the running kinematic strategy as seen in the current study (see below). However, alterations in neuromuscular coordination of the biarticular BF_{LH} requires further investigation, as unpublished observations of our laboratory (Lake et al.) demonstrate an increase of the semitendinosus EMG activity (with a simultaneous decrease of the BF_{LH} EMG activity) following IS indicating a critical role of the semitendinosus for HSI.

Interestingly, peripheral fatigue (identified via the torque-frequency relationship) revealed in the current study that frequencies between 10 and 50 Hz were affected immediately after the IS-intervention, but there was a delayed recovery for higher frequencies. This suggests that IS might impair fast-twitch muscle fibres to a greater extent compared to slow-twitch fibres (Henneman et al., 1965, Gregory and Bickel, 2005), which led to an impaired force generation rather than simply fatiguing the muscle fibres with its consequential effect on the rate of relaxation at lower frequencies. Initial studies reported a left shift of the torque-frequency curve after fatiguing exercise, i.e. with the stimulation of the fatigued human adductor pollicis muscle (Bigland-Ritchie et al., 1983). However, the torque-frequency

relationship shifted to the right following IS in the current study, which is in line with other *in vivo* strenuous exercise studies (Dundon et al., 2008, Binder-Macleod et al., 1998). Presumably, investigations including protocols, which causes ultrastructural damage, might prevent muscle fibres from producing as much force at low frequency stimulation in comparison to protocols that only fatigue the muscle (and therefore having a greater influence on rate of relaxation at low frequencies than force production *per se*). Therefore, studies causing EIMD might shift the torque-frequency curve to the right rather than to the left, which might explain the discrepancy between the present study and the study by (Bigland-Ritchie et al., 1983).

3.5.2 Exercise-Induced Muscle Damage

The average strength loss was similar to other studies regarding both the hamstring (Verma et al., 2015, Chen et al., 2017) and quadriceps (Howatson and Milak, 2009) muscle groups. In the current study, both muscle groups showed similar strength loss initially POST and the hamstring muscle group showed further strength loss POST48 compared to the quadriceps muscle group. This was accompanied by supressed involuntary pRTD of the hamstrings, another indicator of peripheral fatigue. These findings suggest that the IS induced muscle damage to a different extent in each muscle group. Differences in the use of the muscles in daily activities may make the quadriceps muscles less susceptible to EIMD compared to the hamstrings (Chen et al., 2011). However, other studies did not show this additional strength loss for the hamstring muscle group POST48. Different methodological approaches might partly explain the different outcomes as sprints interspersed with longer rest intervals, which allows near complete recovery of sprint performance, might induce more muscle damage and muscle soreness compared to exercises with (submaximal but) longer high-speed running duration and shorter rest intervals (Wiewelhove et al., 2015, Balsom et al., 1992). Further, as the participants in the other studies were elite tennis and football players with training on at least three occasions per week (Verma et al., 2015, Chen et al., 2017), it could be assumed that these participants were more adapted to regular high-

speed running and that this regular sprint exercises might have protected the hamstring muscles against additional EIMD or inflammatory processes, as chronic sprint exercises have been shown to protect athletes against HSI (Malone et al., 2018). Interestingly, involuntary pRTD was slightly recovered POST48 in contrast to the additional decrease of hamstring MVC. This was similar to results of other studies, which showed a correlated but disconnected time course between MVC and voluntary pRTD following a muscle-damage intervention (Molina and Denadai, 2012). Involuntary pRTD might reflect the combined outcome of neural mechanisms underlying the early phase of MVC torque loss (Jenkins et al., 2014) as well as peripheral fatigue/damage. The early phase of MVC torque loss may be partly influenced of the inability to fully activate muscles following EIMD, which is reflected by the decrease of involuntary pRTD. However, the disconnected recovery between involuntary pRTD and MVC indicates that predominately peripheral fatigue/damage might cause the delayed MVC recovery in the later phase of recovery (i.e. 48 h post IS) and that involuntary pRTD, which reflected the early phase of MVC torque loss, is less affected by ultrastructural muscle damage (Jenkins et al., 2014).Less is known about the mechanisms of muscle strain injuries other than of the hamstring muscles. High-speed running leads to substantial activation of the upper thigh muscles, when eccentric contractions occur to decelerate the lower limb. This happens for the hamstrings during the late swing phase (= deceleration of the knee extension) (Chumanov et al., 2007) and for the quadriceps during the early/mid swing phase (= deceleration of hip extension) (Jönhagen et al., 1996, Mendiguchia et al., 2013). However, quadriceps strain injuries predominantly occur in the bi-articular rectus femoris (Cross et al., 2004), and it is thought that injuries typically occur during eccentric contractions, such as during deceleration of running and during kicking with the kicking leg (Brukner and Connell, 2015, Orchard, 2001). The likelihood to sustain a quadriceps strain injury is significantly lower compared to HSI (Ekstrand et al., 2011). If repeated eccentric contractions are one of the main causes for strain injuries, as indicated of several investigations (Malone et al., 2016, Higashihara et al., 2010), then the differences might be explained due to different amount of repetitions and of

the extent of force which the involved muscles obtain. Muscle damage in the quadriceps muscle presumably occur during the deceleration of sprinting and during the backswing phase whilst the quadriceps muscle eccentrically works to decelerate the leg with a flexed knee during the early swing phase of high-speed running, but HSI occurs with an almost extended leg during the late swing phase. This extended lever arm of force might cause higher eccentric force on the involved hamstring muscles compared to the shorter lever arm with a flexed knee on the quadriceps muscle. Continuously repeated eccentric contraction with the longer lever arm of force will potentially induce more muscle damage within the hamstrings in total during i.e. a soccer match compared to the quadriceps muscle group, which might explain the significant different strength loss POSt48 IS in the current study and potentially the different injury rate between these muscle groups.

3.5.3 Muscle Architecture

Recent investigations revealed that Nordic hamstring eccentric strength test in combination with BF_{LH} fascicle length might be predictors for HSI (Timmins et al., 2015, Timmins et al., 2016). We measured fascicle length mid-way between the ischial tuberosity and the popliteal fossa, along the line of the BF_{LH}. Interestingly, we could not find any association of the BF_{LH} fascicle length with any biomarker of fatigue. However, BF_{LH} volume and particularly PCSA were correlated with hamstring strength loss from PRE to POST. As muscle volume is the product of fascicle length (the main determinant of maximum shortening velocity) x PCSA (the main determinant of maximum force), if two muscles have the same volume but one has a larger PCSA, that muscle would be expected to have either a greater number or larger muscle fibres arranged in parallel (thus generating more force), while the other muscle would be expected to have longer muscle fascicles (thus enabling the muscle to contract faster). However, BF_{LH} PCSA did not correlate with either baseline hamstring MVC ($P > 0.05$, data not shown). Recent investigations reported an important role of the extracellular matrix regarding muscle damage (Hyldahl and Hubal, 2014, Hyldahl et al., 2015) and muscle injuries (Balius et al., 2018). The extracellular matrix consists of

different layers of connective tissue and this non-contractile structure surrounds the muscle fibres and fascicles (Kjær, 2004). We suggest that a greater BF_{LH} reflects more fibres aligned in parallel, which would be accompanied by more connective tissue (e.g. endomysium and perimysium) that transmits potentially damaging force laterally rather than longitudinally, thus protecting the muscle fibres from excessive damage during eccentric contractions. Therefore, we propose that a relatively small BF_{LH} PCSA might be a better predictor for muscle impairment following strenuous hamstring exercises compared to a single assessment of the BF_{LH} fascicle length, and that PCSA should be taken into account for further HSI investigations. Increasing BF_{LH} PCSA (rather than focusing solely on increasing fascicle length) via resistance training should therefore be a priority in sports, where HSIs are prevalent.

3.5.4 Kinematic Analyses

Neuromuscular fatigue induced by a bout of straight line sprints was shown to alter hip and knee kinematics. Concerning SLHL, the knee showed a more adducted position (less valgus angle) immediately after the IS-intervention, which is in line with another study that used an endurance protocol to cause fatigue (Benjaminse et al., 2008). This was accompanied by an increase in hip ROM in the transverse plane and a generally more externally rotated hip angle. The thigh muscles, and particularly the hamstring muscle group, are important for the control of the knee (Azmi et al., 2018), e.g. during landing. Reduction in thigh muscle strength due to neuromuscular fatigue might trigger a protective mechanism for the knee directly after IS. This fatigue control leads to a more adducted position of the knee as knee valgus moments are suggested to increase stress on the ACL (Kanamori et al., 2000, Bendjaballah et al., 1997). However, the fatigued muscles are less able to control the landing which causes greater ROM in the transverse plane. Further, as the bi-articular hamstring muscles assist with hip extension (Neumann, 2010), fatigued hamstring muscles might not be able to keep the upright posture of the upper body in position during landing, which would lead to the increased hip flexion immediately after IS.

Other studies have shown that muscle fatigue in the leg muscles permit an increased anterior tibial translation which could also lead to an increased ACL strain (Behrens et al., 2013). The protective mechanism seems predominantly to occur immediately after IS, as hip and knee angles for SLHL returned to the same values at baseline POST48 the intervention. As flexion MVC continued to deteriorate at POST48 but extension MVC started to improve, it could be that these kinematic changes are more controlled by the quadriceps rather than the hamstrings. Alternatively, as hamstring sEMG declined immediately POST IS, it could be that central fatigue rather than muscle damage *per se* might have triggered this change. However, there was a significant elevated time to decelerate the knee (time between heel contact and maximal knee flexion) during landing POST48 IS indicating that participants were still not able to fully control the landing. It is possible that accumulated fatigue over time (e.g. due to a congested match calendar) accompanied by this kinematic change could increase the risk for ACL injuries (Waldén et al., 2016). Injury prevention programmes, which included lower body strength training (i.e. Nordic hamstrings, lunges, and calf raises), were able to reduce ACL injury risk by 50 % (Webster and Hewett, 2018, Petushek et al., 2018), potentially by improving the control of the knee and by attenuating the effect of neuromuscular fatigue during match play. However, no prospective kinematic study has investigated whether EIMD is a risk factor for ACL injury (Bossuyt et al., 2016). Further investigations are needed, whether athletes with pre-damaged thigh muscles prior to the match have an increased likelihood to sustain an ACL injury.

Running kinematics were assessed at one running speed (i.e. 4.17 m s^{-1}) in the current study. Previous investigations assessed running kinematics with lower running speed between 2.5 and 3.6 m s^{-1} (Paquette et al., 2017, Tsatalas et al., 2013, Paschalis et al., 2007). However, in the current study, high-speed running was of interest and the selected speed was the fastest speed on a motorized treadmill following safety considerations. The current IS-intervention caused changes in running kinematics in the sagittal plane with reduced knee extension in the late swing phase POST. This is different to isolated quadriceps muscle damage interventions, which cause increased minimal but significant

pelvic rotation (Paschalis et al., 2007) alongside the changes on the sagittal plane (reduced knee flexion at initial contact; reduced knee ROM during stance and swing phase) (Paquette et al., 2017, Dutto and Braun, 2004, Chan-Roper et al., 2012, Tsatalas et al., 2013, Satkunskiene et al., 2015). The reduced knee extension while contracting eccentrically in the late swing phase might decrease the elongation stress on the hamstring muscles, suggesting a protective mechanism for the hamstring muscles during submaximal running speed directly after IS. Changes in running kinematics were not evident for knee flexion angle POST48 the intervention, although hamstring MVC torque showed further strength loss POST48. However, there was a non-significant tendency for prolonged stride duration POST48 compared to PRE (P = 0.08, data not shown), which is similar to the significant elevated time to decelerate the knee during SLHL POST48. Presumably, ultrastructural damage in the thigh muscles leads to decelerated movement patterns with an extended phase in both the stance and swing phase. Further investigations with additional EMG tests for the medial hamstring muscles are necessary to confirm whether intermittent sprints cause fatigue/damage only or more in specific muscles (e.g. BF_{LH}) of the hamstring muscle group and whether this has an effect on an increased HSI risk.

3.5.5 Limitations

Ecentric exercise results in a delayed peak and a slower decrease of serum IL-6 during recovery between 6 and 24 h after EIMD (Willoughby et al., 2003, Pedersen and Febbraio, 2008). Therefore, serum IL-6 expression immediately after IS indicates that intense exercise occurred but it cannot be assured that this marker indicates muscle damage in the current study. The generally low absolute value of CK activity of the current study might be explained by the fact that we have used a different method involving venous blood, which will usually be conducted in *in vitro* investigations (Owens et al., 2015). Therefore, further interventions are necessary to compare CK activity assessments *in vivo*. Recent studies suggest that fatigue alters the optimal angle during MVC in the hamstrings but not in the quadriceps (Coratella et al., 2015). This might have influenced the results of the isometric

MVC test of the hamstring muscle group. Further studies should confirm these results with isometric MVCs at different knee joint angles. Peripheral fatigue can be caused by metabolite perturbations such as the depletion of intramuscular glycogen (Bendiksen et al., 2012). However, despite the fact that participants were instructed to eat two hours before arrival, and to avoid strenuous exercise prior to the testing, the current study did not control for diet or assessed glycogen depletion during the intervention. Although the low decrement of the sprinting performance, which lasted about 25 min, indicates that glycogen depletion was potentially only a minor factor which contributed to the impaired muscle function following IS-intervention, future studies should assess and control the glycogen content of the muscles before and after IS. Further, we have only measured muscle architecture and sEMG activity of the BF_{LH}. During data collection, several published investigations (Kellis, 2015, Schuermans et al., 2016, Schuermans et al., 2014) indicated that the other hamstring muscles might have an impact on hamstring fatigue and, potentially, on HSI risk. We therefore suggest that further studies conducting IS should also take the other hamstring muscles into consideration. Other factors, such as skeletal muscle composition (Mackey et al., 2017) and genetic variation (Baumert et al., 2016a, Baumert et al., 2018) might also contribute to the prolonged recovery process following EIMD, and large-scale analyses are required to assess the individual differences between the participants which might be based on genetic variation. These studies should consider the possible influence of testing strength at different times of the day on the extent of strength loss. For instance, previous studies have shown that MVC strength is higher in the evening compared to the morning (Araujo et al., 2011). However, due to the large numbers required for genetic studies, the competition for laboratory time, and the availability of the participants, it was necessary for us to perform our measurements at different times of day (although each participant was tested at the same time of day for their respective repeated measurements). As the relative change in strength due to any interaction between the damaging exercise intervention and their genetic make-up is likely to be the same regardless of the time of day, we do not consider that this factor confounded our results.

3.6 CONCLUSION

Our data provide strong evidence that impaired muscle function following IS is caused primarily by impaired neuromuscular activation of the BF_{LH}, together with peripheral fatigue in the hamstrings immediately after the intervention, but the magnitude of fatigue and delayed recovery 48 h later is probably caused primarily by damage to the skeletal muscle tissue. Furthermore, BF_{LH} PCSA was inversely related to hamstring strength loss immediately after IS. The findings provide evidence that the structure of the muscle protects it against neuromuscular fatigue showing that non-contractile tissue within the muscle, such as the extracellular matrix, might play an important role in the complex response to muscle damaging exercises. Impaired thigh muscle function following IS alters hip and knee kinematics during running and landing immediately after the intermittent sprints, but this (likely) protective effect is less evident 48 hours after the intervention which may lead to an increased ACL or hamstring muscle injury risk. We conclude that a 48h recovery period following sports incorporating IS is insufficient, and might increase injury risk. The practical implications of this study include the assessment of BF_{LH} PCSA to estimate the neuromuscular fatigue/damage response to IS in athletes, and the inclusion of hamstring resistance training to increase hamstring muscle PCSA, thus decreasing injury risk.

.

4 Muscle Recovery Rate Following *in vitro* and *in vivo* Damage is Related to the Myoblast:non-Myoblast Ratio Derived from Human Biopsies

4.1 ABSTRACT

Introduction The aim of this study was to objectively assess whether the ratio between myoblast and fibroblasts was related to recovery rate following damage to human skeletal muscle *in vivo* (intermittent sprints) and *in vitro* (wounding assay), and whether this was sex-dependent. We hypothesised that a high myoblast:fibroblast ratio would lead to an accelerated muscle regeneration compared to a lower myoblast:fibroblast ratio, but that this would be independent of sex.

Methods Myoblast:fibroblast ratio was assessed of Isolated human skeletal muscle-derived stem cells from biopsies of six young, recreational active male Caucasians *in vitro* and the ratio was compared with the muscle damage response of the intermittent sprint intervention *in vivo* (Chapter 3). Primary muscle stem cells of an extended group of twelve young, untrained female and male Caucasian participants were subjected to mechanical wound injury to investigate the correlation of muscle regeneration regarding their individual myoblast:fibroblast ratio and sex-differences *in vitro*.

Results There was an average percentage of 46.8 % myoblasts compared to fibroblasts (range 13.3-74.5 %). There was no significant differences to any parameters regarding sex following the artificial wound healing assay (all $P>0.05$). Cells with a high myoblast:fibroblast ratio showed a delayed wound closure/ MVC torque recovery within the first 48 h both *in vitro* ($R^2=0.51$, $P=0.040$) and *in vivo* ($R^2=0.85$, $P=0.009$), but a better myotube formation seven days after the scratch assay *in vitro.*

Conclusion The current results suggest that muscle stem cell characteristics are not influenced by sex and that fibroblasts are essential for the initial days of wound closure, but a larger number of myoblasts is crucial for the latter stage of muscle regeneration, presumably when myoblasts differentiate and fuse to become myotubes indicating that non-contractile elements of the muscle play an important part in the recovery process following muscle damage.

4.2 INTRODUCTION

Unaccustomed strenuous exercise, particularly, that which involves muscle-lengthening (eccentric) contractions, can cause exercise-induced muscle damage (EIMD) within the contractile tissue (Clarkson and Hubal, 2002). Ultrastructural muscle damage is indicated with Z-line disturbance as well as disruption of the extracellular matrix (ECM), which provides structural scaffolding for muscle remodelling and has an integral role in force transmission (Tidball, 1991, Garg and Boppart, 2016). Among several other minor molecular elements, collagen type I and type III are the main components of the ECM (Davis et al., 2013), which is connected to the Z-disks of the peripheral myofibrils via costameres (Jani and Schöck, 2009). In muscle, fibrils of collagen type I are stiff structures and it is predominantly present in the perimysium (surrounding muscle fascicles), which become continuous with tendon (Passerieux et al., 2007). Type III collagen fibrils are thinner and with less stiffer properties compared to type I (Zhang et al., 2005). Both type I and III collagens are almost equal distributed in the endomysium (surrounding muscle fibres) and epimysium (surrounding the muscle) (Gillies and Lieber, 2011). It is well established, that myogenic satellite cells (skeletal muscle stem cells), play a key role in skeletal muscle regeneration and remodelling after substantial EIMD (Yin et al., 2013, Tidball, 2011). Following ultrastructural muscle damage, the nuclei in the area of the myotrauma undergo apoptosis, and activated satellite cells (myoblasts) proliferate and migrate from their niche along the basal lamina to the injury site before terminally differentiating and fusing into myotubes (Goetsch et al., 2013).

There is increasing evidence that fibroblasts, the main cell type of muscle connective tissue, have a critical role in supporting muscle regeneration (Mackey et al., 2017, Murphy et al., 2011, Joe et al., 2010). Following damage, infiltrating inflammatory cells activate muscle fibroblasts, which proliferate and migrate to the injury site to produce ECM components in the area of the myotrauma in an orchestrated and regulated fashion (Mann et al., 2011, Murphy et al., 2011). This is accompanied with increased proteolytic activity of matrix metalloproteinase (MMPs) to degrade damaged parts of the ECM and to allow cell migration

through the ECM (Mackey et al., 2004). The fine-tuned coordinated resolution and restructure of the ECM is crucial for healthy muscle remodelling (Mann et al., 2011, Kragstrup et al., 2011). Interaction of activated satellite cells with fibroblasts helps to dissolve and reorganise the ECM by supressing the master regulator of collagen biosynthesis Rrbp1 in the days and weeks after the injury to avoid long lasting unfavourable fibrosis and to support healthy muscle regeneration (Garg and Boppart, 2016, Fry et al., 2017). There is an increasing number of investigations into the effect of fibroblasts on skeletal muscle regeneration following injury, including models such as electrical stimulation or barium chloride (Mackey et al., 2017, Murphy et al., 2011). However, less is known about the effect of fibroblasts on the response to *physiological* EIMD within the first days following eccentric damage.

Further, there is a high individual variation in the response to EIMD, as some individuals reveal greater muscle strength loss and perceived muscle soreness compared to others, who have undergone the same EIMD protocol (Nosaka and Clarkson, 1996, Baumert et al., 2016b, please see Chapter III). In Chapter 3, we reported that a greater force generating capacity (larger physiological cross-sectional area) of the biceps femoris long head of the hamstring muscle group appears to protect the muscle immediately following intermittent sprints (IS). Potentially external forces can be allocated to a higher number of parallel sarcomeres in muscles with an higher physiological cross-sectional area, which might reduce the stress to each individual sarcomere leading to a lower damage response (Wisdom et al., 2015). However, this relationship was not evident two days after IS, indicating that the muscle recovery response immediately vs. days after muscle damaging exercise is differentially controlled. Some (Sewright et al., 2008), but not all human investigations (Stupka et al., 2001, Hubal and Clarkson, 2009), suggest that the different response following EIMD could be sex-dependent, potentially due to varying skeletal muscle properties such as fibre type composition or regeneration efficiency. Animal studies revealed that male serum enhanced cell proliferation and cell growth compared to female serum (Lee et al., 2011), and that primary female muscle stem cells appeared to have a

slower long-term proliferation kinetics and higher muscle regeneration efficiency compared to males (Deasy et al., 2007). However, to our knowledge, no studies have investigated sex-related differences in terms of proliferation and differentiation characteristics in human primary skeletal muscle cells *in vitro*. An understanding of the underlying mechanisms of the individual rate of skeletal muscle regeneration has implications, not only for the prevention of muscular injury (Larruskain et al., 2017) but also in the context of skeletal-muscle adaptation such as muscle hypertrophy (Cartee et al., 2016) or wasting (Volaklis et al., 2015).

Therefore, the main objective of this study was to investigate whether differences in the myoblast:fibroblast ratio was associated with the *in vivo* EIMD damage response following the IS protocol (Chapter 3) in a group of six male Caucasians, which participated in both studies. A second objective was to investigate whether inter-individual differences in the ratio between myoblast and fibroblasts affected skeletal muscle repair/recovery within the initial days after an artificial wounding (scratch) assay in an extended group of 12 young male and females. A third objective was to investigate whether this variability in the myoblast:fibroblast ratio was sex-dependent, and whether this led to sex-specific differences in the rate of muscle repair following mechanical injury. It was hypothesised that a higher myoblast:fibroblast ratio would be associated with an improved EIMD-response compared to muscle cells with a lower myoblast:fibroblast ratio following IS *in vivo*. We further hypothesised that increased percentage of fibroblasts would show an improved wound closure, and that sex would not be associated with muscle remodelling *in vitro*.

4.3 METHODS

4.3.1 Participants

Recreationally active and healthy young female (n = 4; age 25.5 ± 1.29 years; height 1.67 ± 0.08 m; body mass 61.40 ± 2.57 kg; *mean* ± *standard deviation*) and male (n = 8; age 21.25 ± 4.27 years; height 1.77 ± 0.05 m; body mass 73.78 ± 5.68 kg; *mean* ± *standard deviation*) Caucasian individals participated in this study. Six of the eight males (age 20.5 ±

4.68 years; height 1.78 ± 0.05 m; weight 73.49 ± 6.01 kg) also participated in the IS protocol (see Chapter 3) after the biopsy procedure separated by at least three weeks. Prior to starting the study, written informed consent and a pre-biopsy screening as approved by a physician was obtained from each participant, which complied with the Declaration of Helsinki and was approved by the Research Ethics Committee of Liverpool John Moores University. Participants were physically active but were excluded if they had performed strength training of the lower limbs within 6 months prior to participation in the study, as screened via interview. Further exclusion criteria were (i) any lower limb injury in the past 12 months; and (ii) age under 18 or above 35 years; as assessed via interview and health questionnaire.

4.3.2 Reagents, Chemicals, and Solvents

Growth media (GM) used for the expansion of human muscle-derived cell populations consisted of Hams F-10 nutrient mix (Lonza, Basel, Switzerland) with added L-glutamine (2.5 mM), 10% heat-inactivated fetal bovine serum (hiFBS; Gibco, Thermo Fisher Scientific, Altincham, UK), 1% penicillin-streptomycin (Life Technologies, Warrington, UK), and 1% L-Glutamine (Gibco). Differentiation media (DM) consisted of α-MEM (Lonza), 1% hiFBS, 1% penicillin-streptomycin, and 1% L-glutamine. Phosphate-buffered saline (PBS; Sigma-Aldrich) was used to wash cell monolayers. Desmin polyclonal rabbit anti-human antibody (Cat# ab15200, RRID: AB_301744) was used (1:200) from Abcam (Abcam, Cambridge, UK), and secondary antibody (TRITC polyclonal goat anti-rabbit; Cat# A16101, RRID: AB_2534775) was used (1:200) from Fisher Scientific.

4.3.3 Muscle Biopsy Procedure

Participants were advised to avoid any strenuous exercise 48 h prior to the biopsy procedure. Muscle biopsies, from the vastus lateralis muscle of the quadriceps femoris, were obtained under local anaesthesia, from each participant, using the Weil-Blakesley

conchotome technique as described previously (Baczynska et al., 2016). Briefly, participants rested in a supine position and the incision site was shaved then thoroughly cleaned with an alcohol swab and Hydrex surgical scrub (ECOLAB Leeds, UK), following which a sterile sheet was placed above the biopsy site to maintain sterility. To anesthetise the biopsy site, 1.5 ml of bupivacaine hydrochloride (AstraZeneca, Luton, UK) was administered at a concentration of 5 mg/ml. After allowing a short time for the anaesthetic to take effect, a sterile single-use scalpel was used to make a small incision through the skin and deep muscle fascia. The conchotome was inserted through the incision into the muscle belly to obtain the muscle biopsy (134 ± 82.7 mg).

4.3.4 Extraction of Human Muscle-Derived Cells

The muscle biopsies analysed in this study were isolated (Blau and Webster, 1981, Crown et al., 2000) and cultured (Owens et al., 2015) as reported previously. Briefly, biopsy samples were transferred with precooled GM from the muscle biopsy suite to the sterile tissue culture hood (Kojair Biowizard Silverline class II hood; Kojair, Vippula, Finland) within 40 min and muscle biopsy samples were washed three times with ice-cold PBS (0.01 M phosphate buffer, 0.0027 M KCl, and 0.137 M NaCl, pH 7.4, in dH2O). Visible fibrous and fat tissue were removed using sterile scissor and forceps. Samples were cut in small pieces (1 mm^3) and digested in 5 ml of trypsin-EDTA for 15 min on a magnetic stirring platform at 37°C to dissociate muscle cells. The trypsinisation process was repeated twice. Supernatant derived following each treatment was collected and pooled with hiFBS at a concentration of 10% of the total volume to inhibit further protease activity. Cell supernatant was centrifuged at 450 g for 5 min. Supernatant was discarded and the cell pellet was resuspended in GM and plated on a T25 cm^2 culture flask (Corning, Life Sciences, New York, USA) for cell population expansion. Culture flasks were previously coated with a 2 mg/l porcine gelatin solution (90–110 g, Bloom; Sigma-Aldrich, Dorset, UK) to support cell adhesion.

4.3.5 Expansion of Extracted Cells

The medium was refreshed on the fourth day after the extraction procedure and subsequently every 48 h following two brief washes with PBS. Cells were incubated in a HERAcell 150i CO_2 Incubator (Thermo Scientific, Cheshire, UK). T25 cm^2 culture flasks reached 80% confluence after aproximately 10 days and were passaged via trypsinisation. Cells were counted using Trypan Blue exclusion and re-plated on gelatinised T75 cm^2 culture flasks (Nunc, Roskilde, Denmark). The cells were expanded until passage 3 and then frozen in GM with 10% dimethyl sulfoxide (DMSO) in liquid N_2 as a cryopreservant. All experiments were performed on cells between passages 3 and 6 to avoid potential issues of senescence (Alsharidah et al., 2013, Foulstone et al., 2004).

4.3.6 Characterization of Human Muscle-Derived Cells

The mixed population of human skeletal muscle-derived myoblast and fibroblasts were characterised by immunofluorescent staining for the detection of desmin expressed by myoblasts (desmin positive) and non-myoblasts (desmin negative) to determine the percentage of myoblasts and fibroblasts (Figure 4-1). Previous investigations have determined that the non-myoblasts fraction is highly enriched in fibroblasts with up to 99 % of this fraction being fibroblasts (Agley et al., 2013, Mathew et al., 2011). Therefore, non-myoblasts (desmin negative) will hereafter be referred to as fibroblasts.

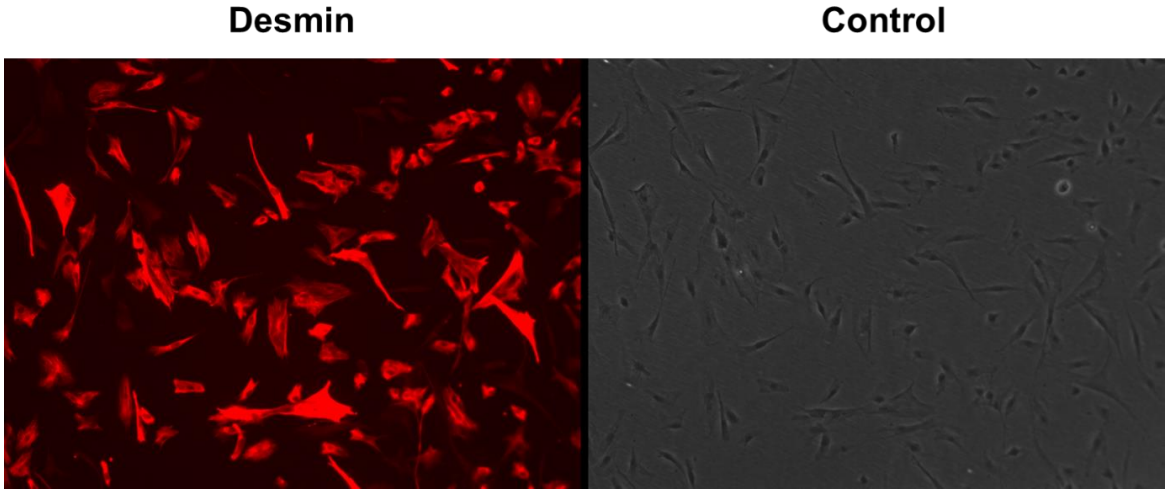

Figure 4-1 *Representative images for immunofluorescent staining for desmin of human muscle cell cultures. Myoblasts are desmin positive (red) and non-myoblasts are desmin negative. Myoblast to fibroblast ratio is 2.9. Magnification is x 10.5.*

Grohmann et al. (2005) showed that passaging does not change the percentage of myoblast and fibroblasts and all populations were included for analysis. Monolayers with a relatively low density of 30% were incubated with 25% (vol/vol methanol in Tris-buffered saline (TBS; 10 mmTris-HCl, pH 7.8, 150 mm NaCl)], 50% and 100% for 5-min to fix the cells and stored at 4°C wet in TBS until further analysis. Fixed monolayers were permeabilised and blocked for 2 h with 5% goat serum and 0.2% Triton X-100 in TBS, prior to staining. Cells were incubated overnight at 4°C with anti-Desmin antibody (1:200). After overnight incubation, the primary antibody was removed, and the cells were washed three times with TBS. Secondary TRITC polyclonal goat anti-rabbit antibody (1:200) was then applied and incubated for 2 h at 4°C. Fluorescent images were captured using live imaging microscopy (Leica DMB 6000; Magnification x 10.5) and analysed via ImageJ cell counter plug-in. A total of four randomly selected areas per well were analysed per individual. To compare the variability between the four images of the myoblast:fibroblast ratio within the same biopsy to the variability between the biopsies, we determined the intraclass correlation coefficient [ICC (3,k)] which was good with ICC=0.83 (95% CIs: 0.59-0.95).

4.3.7 Wound-Healing Assay, Migration and Differentiation Analysis

For the wound healing assay, 100,000 cells/ml were seeded in gelatinised six-well plates (Nunc, Roskilde, Denmark)). Cells were expanded as described above until cell monolayers reached a confluent state, GM was removed, monolayers were washed with PBS and cells were damaged by a vertical scrape with a 1-ml pipette tip (width of the wound area, *mean* ± *S.E.M.*: 896.4 ± 21.24 *µ*m) as our group has reported before (Brown et al., 2017, Owens et al., 2015, Dimchev et al., 2013). PBS was aspirated, damaged cell monolayers were washed twice with PBS to remove cell debris and 2 ml DM was added. Monolayers were imaged with a live imaging microscopy (Leica) for the analysis of cell migration immediately, 24h, 48h and seven days after the wound healing assay. Additional 500 µl DM was added in each well at day 4. TIF images were exported from Leica Application Suite and loaded as TIF image stacks in ImageJ with a cell counter plug-in. Cells in the outer and inner segments were counted (Figure 4-4).

Damaged monolayers were imaged at two sites per well in the wound site immediately post-damage (0 h). These image coordinates were tracked and stored to allow subsequent monitoring of the same sites on the wound to this reduce experimental bias. Myotube formation was captured on day. Captured images were exported as TIFF image files, and analysed in ImageJ. Muscle cell fusion/differentiation was assessed by myotubes per field of view and myotube hypertrophy via the assessment of myotube length, myotube diameter (the average of three diameters along the length of the myotube), myotube area (determined by manually drawing a line around the sarcolemma of each myotube) and total myotube number via ImageJ cell counter plug-in. By normalising the pixel scale to the micron scale of each image, a value expressed as μm^2 was obtained for myotube area. A total of two images per well were analysed and experiments were performed on 12 cell populations of 12 different individuals.

4.3.8 Creatine Kinase Activity

At 0 and 10 days following the mechanical scrape insult, creatine kinase (CK) activity was analysed as a marker of muscle cell differentiation/fusion into myotubes. Cell monolayers were first lysed with 300 µl/well of 50 M Tris-mes and 1% Triton-X 100, pH 7.8 (TMT). Ten microliters of TMT cell lysate was loaded in duplicate wells on a 96-well UV plate and used for quantification of CK activity. The CK reaction reagent and diluent (Catachem, Bridgeport, CT, USA) were prepared as per the manufacturer's instructions as previously described (please see subsection 3.3.13).

4.3.9 Experimental Design of the Intermittent Sprint Intervention *in vivo*

Details of the experimental design of the IS-Intervention *in vivo* are described previously (please see subsection 3.3.2.)

4.3.10 Data Analysis

Data sets were first checked for normal distribution, and where data violated the assumption of normality, an appropriate correction factor was used. We recruited young, healthy men and women to increase both the external validity and sample size of our study. However, to the best of our knowledge, there is no investigation about sex-related differences of primary skeletal muscle cells. Therefore, we initially analysed the data according to sex. All data regarding cell migration within the first 48 h and CK activity were analysed for interactions and main effects for sex (sex groups: between subjects factor) and time (within subjects factor) using two-way-mixed analysis of covariance (ANCOVA) with wound width as a cofactor. Independent T-test was used for analysing the association between sex and myotube formation (total myotube number, myotube length, average diameter, myotube area and CK activity) at day seven. As no significant differences were found between sex for any parameter (subsection 4.4.2), the data from all muscle stem cells were combined for all subsequent analyses. Linear regression analyses were used to analyse the relation

between myoblast:fibroblast ratio and migration dynamics (total cell migration, cell proportion of inner to outer segment) and myotube formation (total myotube number, myotube length, average diameter, myotube area and CK activity). Standard guidelines concerning violation of the sphericity assumption to adjust the degree of freedom of the F-test by the Huynh-Felt epsilon if epsilon is greater than 0.75 and to use the more stringent Greenhouse-Geisser adjustment if epsilon is less than 0.75 were followed (Girden, 1992). Results were expressed as mean ± SEM, with statistical significance set at $P<0.05$. SPSS 23 Software (IBM Inc., Armonk, NY: IBM Corp) was used for statistical analysis unless otherwise stated. Occasional missing data are reflected in the reported degrees of freedom.

4.4 RESULTS

4.4.1 Comparison of the Muscle Response between the Intermittent Sprint Protocol and the Muscle Stem Cell Study

Six male participants who performed the IS-intervention (see chapter 3), also volunteered a muscle biopsy for this study at least three weeks before the IS-intervention. There was an average ratio of 1.47 myoblasts to fibroblasts with a range between 0.299 and 2.93. There was a strong inverse correlation between myoblast:fibroblast ratio (assessed *in vitro*) and relative hamstring maximum voluntary contraction (MVC) torque measured between PRE and POST48 *in vivo* (R^2=-0.89, $F_{1,4}$=33.73, P=0.004; Figure 4-2 A). Participants with a high myoblast:fibroblast ratio showed a delayed MVC torque recovery 48 h after the IS-intervention compared to the participants with a low myoblast:fibroblast ratio. Further, there was an inverse correlation between myoblast:fibroblast ratio and relative hamstring MVC torque measured between POST and POST48 following the IS (R^2=-0.81, $F_{1,4}$=17.08, P=0.014; Figure 4-2 B). No relationship was found between the myoblast:fibroblast ratio and any knee extensor MVC torque or with any other EIMD biomarker following IS.

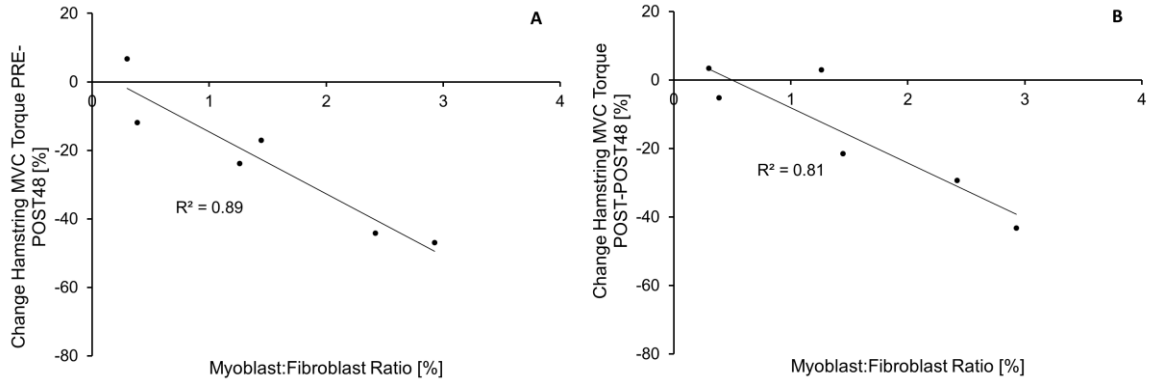

Figure 4-2 *Inverse correlation between the myoblast:fibroblast ratio, assessed in the current in vitro study and the change of hamstring MVC torque measured before and 48 h after (P=0.004) (A) and measured immediately after and 48 h after (P=0.014) (B) the intermittent sprints (see Chapter 3).*

4.4.2 Artificial Wound Healing Assay to Investigate Repair and Regeneration Regarding Sex-related Parameters

There was no main effect between myoblast:fibroblast ratio and sex (Male: 1.43 ± 1.05; Female: 1.26 ± 1.00; $F_{1,10}$=0.65, P=0.438). There were no significant differences for any parameters within the first 48 h after (P>0.05; Table 4-1).

Table 4-1 *The Effect of Sex on Artificial Wound healing for the first 48 h in vitro*.

		Time points			
	Sex	**POST24**	**POST48**	**F-Test**	**P Value**
Total number of cells migrated into the wound area [n]	Male	65.9 ± 7.44	93.7 ± 12.2	F(1.7,18) = 0.25	0.748
	Female	57.3 ± 11.27	85.1 ± 14.6		
Proportion Inner/ Outer Segment [%]	Male	15.5 ± 6.00	25.8 ± 4.23	F(1.3,20) = 0.47	0.552
	Female	17.7 ± 1.94	26.4 ± 2.39		

Further, there were no significant differences between sex for any parameters on day seven after the artificial wound healing assay (P>0.05; Table 4-2).

Table 4-2 *The Effect of Sex on Artificial Wound Healing Parameters for Day seven and CK activity at Day ten in vitro*.

	Sex			
	Male	**Female**	**F-Test**	**P Value**
Total myotubes [n]	9.74 ± 3.61	6.94 ± 1.75	F(1,11) = 2.09	0.179
Myotube length (µm)	326 ± 79.1	319 ± 30.5	F(1,11) = 0.03	0.864
Average Diameter (µm)	18.7 ± 3.53	18.4 ± 1.03	F(1,11) = 0.03	0.871
Myotube Area (µm^2)	5129 ± 2354	4677 ± 530	F(1,11) = 0.14	0.719

There was no interaction between sex and time concerning CK activity ($F_{1,9}$=2.16, P=0.176; Figure 4-3) but there was a main effect for time regarding CK activity ($F_{1,10}$=15.49, P=0.003).

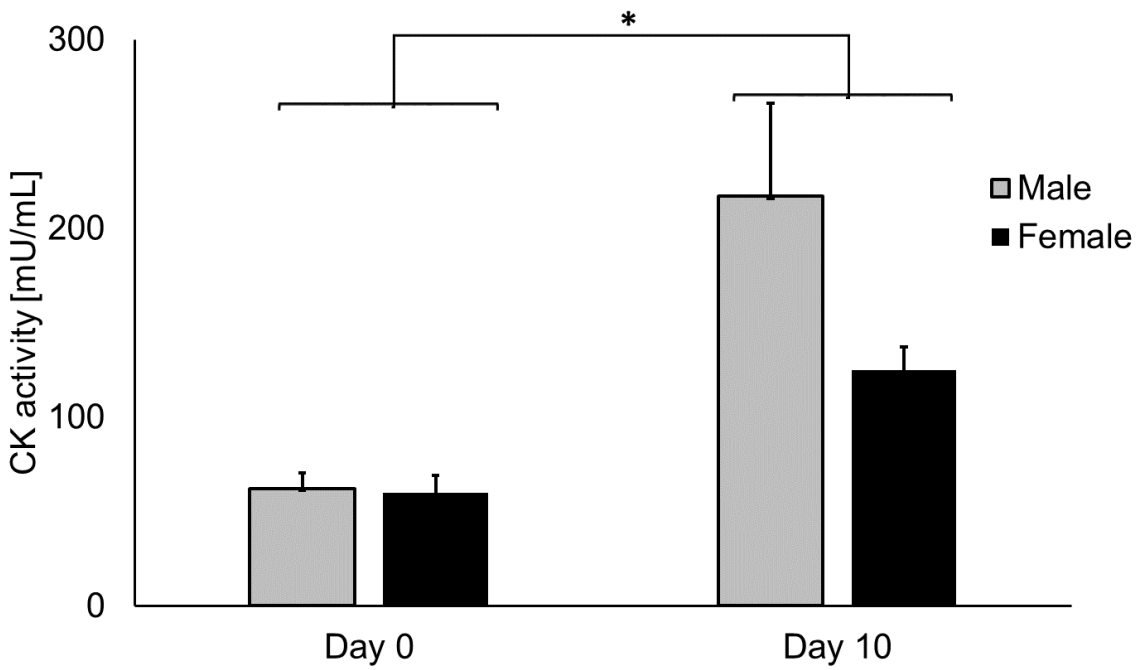

Figure 4-3 *Effect of Sex on biochemical aspects of myotube formation via Average CK activity. Results are expressed as mean ± SEM, * significant differences between Day 0 and Day 10 for both male and females (P<0.05).*

4.4.3 Artificial Wound Healing Assay to Investigate Repair and Regeneration Regarding Myoblast:Fibroblast Ratio

As no significant differences in any muscle stem cell characteristics could be detected between cells obtained from females and males, the data from all muscle cells were

combined and muscle characteristics were correlated with myoblast:fibroblast ratio. There was an average ratio of 1.26 myoblasts to fibroblasts with a range between 0.154 and 2.93 (Figure 4-4). No differences were seen regarding myoblast:fibroblast ratio and the total number of myoblast and fibroblast migration into the artificial wound after 24 h (R^2=0.20, $F_{1,10}$=2.56, P=0.141) and 48 h (R^2=0.02, $F_{1,10}$=0.19, P=0.671) after the scratch assay.

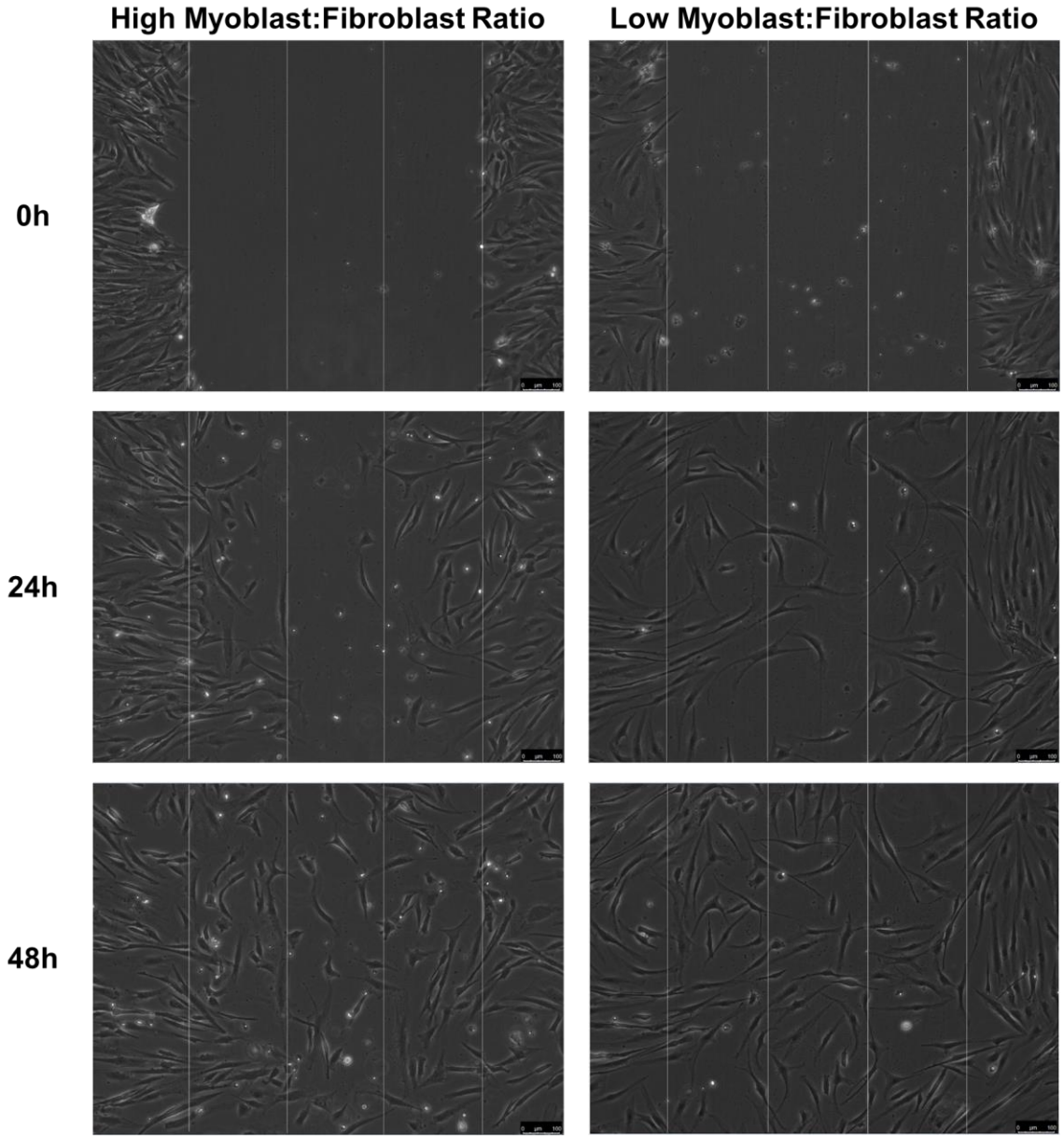

Figure 4-4 *Representative images for cell migration of muscle cells with a high myoblast:fibroblast ratio (2.4; left) and with a low percentage of myoblasts (0.3; right) into the artificial wound. The wound area is about 900 µm in width and split into 3 x 300 µm segments. Magnification is x 10.5, and scale bar is 100 µm.*

There was an inverse correlation between myoblast:fibroblast ratio and migration dynamics for the six male Caucasions, which performed the IS-intervention and also volunteered a muscle biopsy (Figure 4-5 A and B). Muscle stem cells with a low ratio of myoblasts (to fibroblasts) demonstrated more cells in the inner segment than to the outer segment compared to muscle stem cells with a medium or high myoblast:fibroblast ratio for 24 h (R^2=0.83, $F_{1,4}$=19.05, P=0.012; Figure 4-5 A) and for 48 h (R^2=0.72, $F_{1,4}$=10.22, P=0.033; Figure 4-5 B) after the artificial wound healing assay. Further, there was an inverse correlation between myoblast:fibroblast ratio and migration dynamics for the whole cohort (Figure 4-5 C and D). Muscle stem cells with a low ratio of myoblasts (to fibroblasts) demonstrated more cells in the inner segment than to the outer segment compared to muscle stem cells with a medium or high myoblast:fibroblast ratio for 24 h (R^2=0.49, $F_{1,10}$=9.53, P=0.011; Figure 4-5 C) and with a trend for 48 h (R^2=0.30, $F_{1,10}$=4.33, P=0.064; Figure 4-5 D) after the artificial wound healing assay.

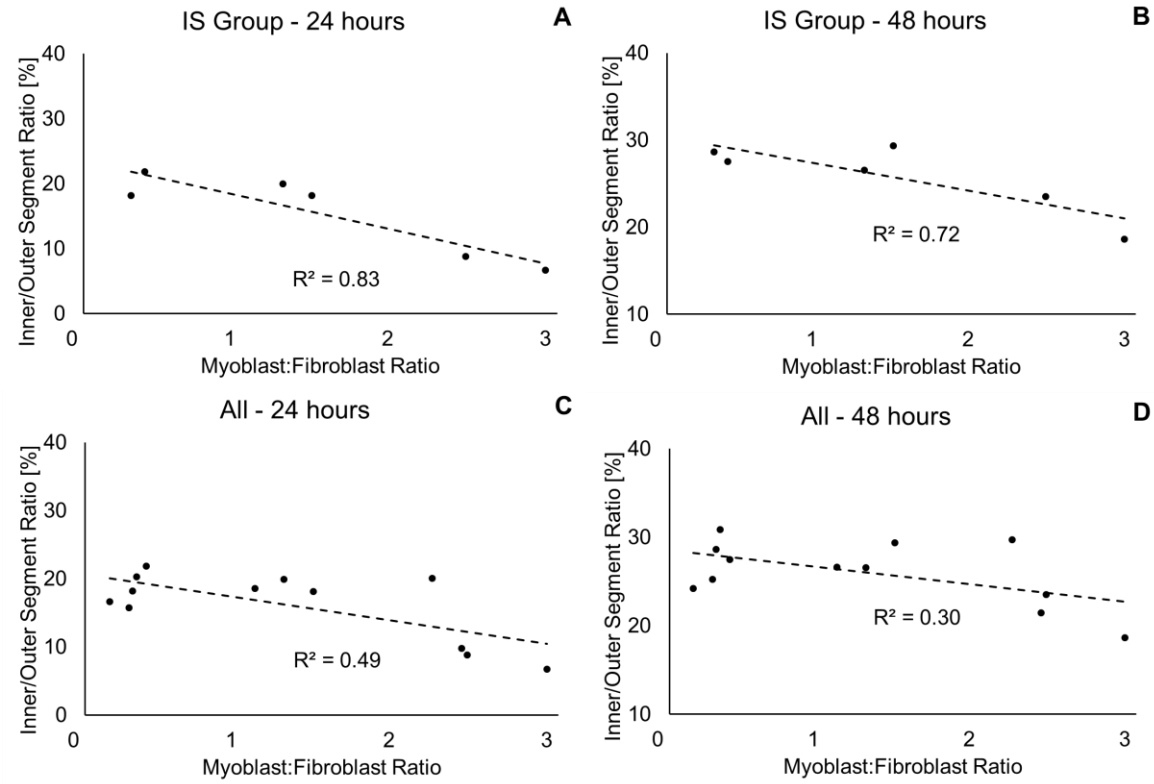

Figure 4-5 *Inverse correlation between the myoblast:fibroblast ratio and the migration dynamics of the six male participants, who performed the intermittent sprint (IS) intervention and also volunteered a muscle biopsy (A) 24 h (P=0.012), and (B) 48 h (P=0.033) after the artificial wound healing assay;*

and also for the whole cohort (C) 24 h (P=0.011) and a trend (D) 48 h (P=0.064) after the artificial wound healing assay.

There was a linear relationship between the myoblast:fibroblast ratio with all parameters at seven and ten days after the artificial wound healing assay (all P<0.05; Table 4-3).

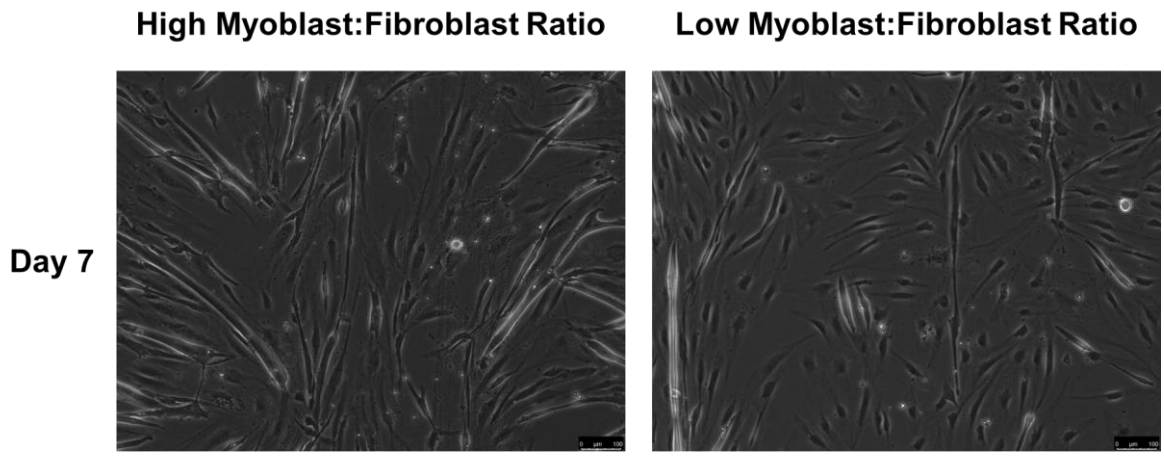

Figure 4-6 *Representative images for muscle cell differentiation with a high percentage of myoblasts (2.4; left) and with a low percentage of myoblasts (0.3; right) compared to fibroblasts at day seven. Magnification is x 10.5, and scale bar is 100 µm.*

Biopsies with a higher myoblast:fibroblast ratio showed more myotubes per field, which had a higher diameter and area compared to biopsies with a lower myoblast:fibroblast ratio (Figure 4-6).

Table 4-3 *Linear regression analysis between the myoblast:fibroblast ratio and fusion parameters at day 7 and CK activity at day 10 in vitro*.

	R^2	F-Test	P Value
Total myotubes [n]	0.345	F(1,10) = 5.27	0.045*
Myotube Length [µm]	0.339	F(1,10) = 5.13	0.047*
Myotube Diameter [µm]	0.387	F(1,10) = 5.56	0.031*
Myotube Area [μm^2]	0.404	F(1,10) = 6.79	0.026*
CK activity [mU/mL]	0.409	F(1,10) = 6.23	0.034*

* significant (P<0.05);

4.5 DISCUSSION

The purpose of this study was to investigate whether myoblast:fibroblast ratio influences functional recovery. The aim was objectively assess (i) the relationship between myoblast:fibroblast ratio and repair/recovery following *in vitro/in vivo* damage; and (ii) the association between sex with the response to *in vitro* wound healing parameters after a scratch assay *in vitro.* It was hypothesised that a high myoblast:fibroblast ratio measured *in vitro* would be related to improved recovery *in vivo* following the IS-intervention. Further, it was hypothesised that a higher myoblast:fibroblast ratio would show an improved wound closure compared to muscle cells with a low myoblast:fibroblast ratio, and that muscle remodelling would not be associated with sex differences.

In line with our hypothesis, myoblast:fibroblast ratio was related with muscle repair/recovery, both *in vivo* and *in vitro*, and that there were no significant differences in any parameters of muscle remodelling of human primary muscle stem cells, which were obtained of both males and females. However, contrary to our hypothesis, our results showed an ambiguous picture during the muscle repair process. A high myoblast:fibroblast ratio showed a delayed wound closure *in vitro* and a delayed MVC torque recovery following IS *in vivo* within the first 48 h, but a better myotube formation at seven days, and higher CK activity at ten days after the scratch assay.

4.5.1 Comparison of the Muscle Response to the Intermittent Sprint Intervention and the Artificial Wound Healing Study *in vitro*

Six of the eight male participants who volunteered a muscle biopsy for this study also performed an IS-intervention *vivo* (Chapter 3) at least three weeks after the biopsy procedure. Comparing the muscle characteristics measured in the current study *in vitro* and the response patterns of the *physiological* EIMD-intervention *in vivo* (Chapter 3), revealed a strong inverse correlation between myoblast:fibroblast ratio and the recovery of MVC torque assessed between baseline and 48 h after IS. Further, there was an inverse relationship between myoblast:fibroblast ratio and MVC torque recovery (measured

between POST and POST48), which provides further evidence that the ECM is important for recovery of maximum strength. Force produced by the sarcomeres can be transmitted both longitudinally and laterally to the overlying connective tissues and ECM, and from there to the tendon, which leads to joint movement (Kjær, 2004, Hughes et al., 2015). Potentially, ECM remodelling in the initial phase of muscle regeneration is crucial for restoring (lateral) force transmission. Skeletal muscles with an increased availability of fibroblasts around the area of myotrauma might have a better capacity to reorganise the complex ECM, which results in a faster recovery of muscle strength after EIMD. This is also in line with the time course of muscle connective tissue accumulation within the first 3-5 days (Murphy et al., 2011), potentially to restore the muscle force transmission capacity, and the recovery of MVC following EIMD-interventions (Clarkson and Hubal, 2002, Baumert et al., 2018). However, there was no significant relationship between myoblast:fibroblast ratio and any other EIMD-related biomarker, such as muscle soreness (Warren et al., 1999) or serum CK activity (Baird et al., 2012) indicating that these biomarkers might be related to other damage/repair mechanisms.

Further, there was no relationship between myoblast:fibroblast ratio and any measured parameters for the quadriceps femoris. A recent study revealed that skeletal muscles of different origin but with similar physiological functions demonstrate a high similarity of transcriptome expression patterns (Terry et al., 2018). Skeletal muscles with similar fibre type compositions were found to have a striking similar transcript expression of up to 99%. As the fibre type composition is similar between the biceps femoris long head and of the vastus lateralis (Evangelidis et al., 2016), from which the muscle biopsies were obtained in the current study, we can assume that the variation of the correlation between myoblast:fibroblast ratio and the EIMD-response of both hamstring and quadriceps skeletal muscles might be explained by the severity of ultrastructural damage caused by the IS-intervention. This is in line with the correlation between MVC torque loss of the hamstring and quadriceps muscle group (please see section 3.4.2). Increased MVC torque loss of the

hamstrings is related with an elevated loss of MVC torque of the quadriceps of the same participant.

4.5.2 Sex-related Outcomes

Animal studies have often indicated attenuated EIMD susceptibility in female animals (Amelink and Bär, 1986), and some human *in vivo* investigations have supported the observation that there are differences in the response to EIMD between male and females (Sewright et al., 2008). Further, female primary muscle stem cells of mice demonstrated a better regeneration capacity compared to male cells (Deasy et al., 2007). However, other human studies could not replicate these findings (Stupka et al., 2001), and some have indicated that any potentially sex-related differences might be explained by differences in muscle fatigability (Wüst et al., 2008) rather than differences in the susceptibility to ultrastructural muscle damage.

In the current study, human male and female muscle stem cells did not differ in any parameters regarding the early recovery rates following an artificial wound healing assay. However, the current study tested CK activity within ten days and only the muscle recovery capacity within the first seven days, which was similar compared to the results of Deasy et al. (2007), as different outcomes were mainly evident after 14 days, with female muscle stem cells exhibiting slightly slower long-term proliferation kinetics but improved regeneration capacity compared to males. Therefore, the current study suggests that human muscle stem cells characteristics are similar between male and females regarding the early recovery rates, but this needs to be confirmed for muscle regeneration capacity in the long-term, e.g. with population doubling.

4.5.3 Myoblast:Fibroblast Ratio and the Artificial Wound Healing Assay

As no significant differences were found between sex for any parameter, the data from all primary muscle cells were combined for assessing the effect of myoblast:fibroblast ratio on

cellular aspects of muscle regeneration and remodelling. The myoblast:fibroblast ratio ranged between 13 and 74%, which is in line with current literature (Schäfer et al., 2006, Owens et al., 2015).

The results of the current study indicated that muscle stem cells with a high myoblast:fibroblast ratio revealed a delay in the wound closure (less cells migrated to the inner part of the artificial injury compared to the outer part), in particular 24 h after performing the scratch assay. However, this effect was less significant 48 h after the protocol. Further, at day seven, muscle primary cells with a higher myoblast:fibroblast ratio showed an improved myotube formation. These results suggest that a larger abundance of fibroblasts has a positive effect at the beginning of muscle repair, but a larger number of myoblasts is more important for the latter stage of muscle regeneration, when myoblasts differentiate and fuse to become myotubes.

The contribution of fibroblasts to the early phase of muscle repair has previously been investigated in animal studies *in vivo* (Murphy et al., 2011). The amount of muscle ECM (measured with sirius red) increased to peak levels three days after a barium chloride-induced injury in mice. This was accompanied with rapid proliferation of muscle fibroblasts in close proximity to satellite cells and both, the amount of ECM and of muscle fibroblasts, returned to baseline levels 21 days after the injury. Transgenic mice with $Pax7^{+}$ satellite cells deficiency showed dramatically impaired muscle regeneration from day five onwards, and generated fibrosis after the chemically mediated injury. Further, genetically engineered mice with $Tcf4^{+}$ fibroblast deficiency demonstrated a premature activation and differentiation of $Pax7^{+}$ satellite cells three days after the barium chloride-induced injury, which led to a decreased number of satellite cells over the following days. These results are in line with that of the current study, suggesting the abundance and activity of fibroblasts and myoblasts play different roles, depending on the time points during muscle repair.

Recent human *in vivo* investigations confirmed the interdependence of muscle fibroblasts and satellite cells for a healthy muscle regeneration (Mackey et al., 2017). However, the time frame of muscle fibroblast accumulation around the regenerating muscle fibres

happened at a later time point (30 days) following electrical stimulations compared to Murphy et al. (2011). Further, expression of collagen I, III, and IV transcripts were unchanged in the first 48 h following eccentric lengthening contractions, but were increased 27 days later (Hyldahl et al., 2015). Presumably, the different results are based on the different organisms investigated, varying muscles studied and different injury protocols used. It can be assumed that involuntary isometric contractions induced by artificial electrical stimulation potentially damage more proteins, which anchor the actin filaments at the Z-line (Crameri et al., 2007). However, this involuntary isometric contractions might damage the muscle ECM to a lesser extent compared to physiological eccentric MVCs (Crameri et al., 2007) or barium chloride-induced injury (Hardy et al., 2016). That might explain the delayed fibroblast proliferation (Mackey et al., 2017) and production of temporary ECM components (Mackey et al., 2004) at the early stage of muscle repair compared to barium chloride-induced injury in mice (Murphy et al., 2011).

In **conclusion**, therefore, the current results suggest that fibroblasts might play an important role in supporting muscle cell migration during muscle regeneration within the first 48 h following *physiological relevant* EIMD, when a certain level of ultrastructural damage has been exceeded and the ECM has been damaged. However, following wound closure, a higher number of myoblasts will ensure improved myoblast fusion and hypertrophy *in vitro*. Future investigations with other *in vitro* models, e.g. with mechanical stretch-induced injury of the cells (Clarke and Feeback, 1996, Wozniak et al., 2005), should assess whether individuals with a high number of fibroblasts (which demonstrated advantages in muscle regeneration shortly after an acute muscle-damaging intervention) demonstrate an increased proteolytic activity after elevated fibroblast proliferation, or whether these individuals tend to develop more fibrotic scar tissue within the muscle in the long term.

4.5.4 Limitations

The current study observed significant differences between human primary muscle cell composition and skeletal muscle regeneration, but there were no significant differences

between sex regarding any parameters. Further research is necessary to confirm these results with a larger sample size. However, given the scarcity of data that have examined human muscle stem cell characteristics in association with muscle regeneration, we believe that our study represents an important advance in our understanding in this area. Further investigations are necessary to evaluate whether a generally low number of fibroblasts has a detrimental effect on MVC torque following an EIMD-intervention or whether myoblast:fibroblast ratio is essential for healthy muscle regeneration *in vivo*. We assumed that the myoblast:fibroblast ratio is similar between the vastus lateralis musle (from which we obtained the muscle biopsy) and the BF_{LH}, as Terry et al. (2018) demonstrated a high similarity of transcriptome expression patterns in skeletal muscles of rodents, when the muscles consist of similar fibre type composition and of similar physiological functions, such as the BF_{LH} (47.1% MHC-I, 35.5% MHC-IIA, and 17.4% MHC-IIX) (Evangelidis et al., 2016) and the vastus lateralis (49-59% MHC-I, 26-35% MHC-IIA, and 16-28% MHC-IIX) (Mittendorfer et al., 2005, Taylor et al., 1997). However, gene expression patterns vary between rodents and human (Su et al., 2002), and there are is a gap of research between the intra-individual variability of the myoblast:fibroblast ratio between different skeletal muscles. Therefore, further investigations needs to address this research gap to confirm our findings. Lastly, we distinguished the primary muscle cells in myoblasts (desmin-positive) and fibroblasts (desmin-negative), as previous investigations have determined that the non-myoblast fraction is highly enriched in fibroblasts, with up to 99 % of this fraction being fibroblasts (Agley et al., 2013, Mathew et al., 2011). For immnunohistochemistry analysis, we have fixed the cells with a low density of 30%, so that the cells could be easily distinguished from each other. The ICC (3, k) of 0.83 (95% CIs: 0.59-0.95) indicates a good reliability for the characterisation and the quantification of myoblasts and fibroblasts. However, analysing the entire well (instead of sub-sampling random areas of each well) and additional methodical approaches, such as cell sorting by flow cytometry, could have improved the accuracy of our myoblast:fibroblast ratio calculation. Future studies may use

additional antibodies, such as DAPI (for staining nuclei) and TE7 (for staining fibroblasts), to validate our findings.

4.6 CONCLUSION/ PERSPECTIVE

The current results suggest that individual muscle stem cell characteristics are not explicable by differences in sex and that fibroblasts are essential for optimal muscle regeneration in the initial days following injury. Skeletal muscles with an increased number of fibroblasts might have a better capacity to reorganise the complex ECM, which results in a faster MVC torque recovery after EIMD. However, a larger number of myoblasts seems to be important for the latter stage of muscle regeneration, when myoblasts differentiate and fuse to become myotubes. The results lead to further scientific questions, such as whether the individual muscle characteristics, including contraction properties (physiological cross-sectional area) (Chapter 3), and human primary muscle cell composition of the current study is associated with genetic variations. In addition, further investigations are required to demonstrate if varying regeneration capacity within the first days after EIMD underpinned by the percentage of fibroblasts is correlated with (i) the likelihood to sustain a muscle injury; and (ii) the individual differences in the response to prolonged resistance training.

5 Individual and Combined Polymorphisms are Associated with *in vivo* and *in vitro* Muscle Damage in Humans: a Genetic Approach to Elucidate the Mechanisms Underpinning the Response to Exercise-Induced Muscle Damage

*Publications resulted from this chapter**:***

1. *Baumert, P., G-REX Consortium, Lake, M. J., Drust, B., Stewart, C. E., & Erskine, R. M. (2017). TRIM63 (MuRF-1) gene polymorphism is associated with biomarkers of exercise-induced muscle damage. Physiological genomics, 50(3), 142-143.*
2. *Baumert, P., G-REX Consortium, Stewart, C. E., Lake, M. J., Drust, B., & Erskine, R. M. (2018). Variations of collagen-encoding genes are associated with exercise-induced muscle damage. Physiological genomics.*

5.1 ABSTRACT

Introduction We aimed to test the hypothesis that a combination of selected candidate SNPs forming a polygenic profile could estimate high and low responders to exercise-induced muscle damage (EIMD).

Methods Sixty-five young female and male untrained Caucasians performed 120 maximal eccentric knee-extensions to induce EIMD. Maximal quadriceps strength, range of motion (ROM), muscle soreness and serum blood biomarkers were assessed before, directly after and 48 h after the EIMD intervention and participants were genotyped for 20 candidate SNPs. Significant SNPs were further investigated regarding the muscle recovery following an artificial wound healing assay *in vitro.* SNPs that showed a gene-intervention interaction *in vivo*, were used to calculate a total genotype score in respect to the acute response following EIMD (TGS-A) and the cohort was then divided into a “preferential” (PG), “moderate” (MG), and “non-preferential” (NPG) genetic group.

Results Four SNPs, which showed an interaction/main effect with the EIMD-intervention *in vivo*, also demonstrated changes in muscle stem cell characteristics *in vitro*. Seven SNPs demonstrated significant interactions *in vivo*, and these candidate SNPs were used to compute the TGS-A. There was a main effect for isometric and isokinetic MVC torque regarding TGS-A (both $P<0.001$). Individuals of the NPG and MG group were consistently weaker compared to the PG group ($P=0.005$), and NPGs demonstrated higher muscle soreness ($P=0.003$) and decreased ROM ($P=0.006$) following the EIMD-intervention, respectively.

Conclusion Individual and combined candidate SNPs forming a polygenic profile demonstrated a genotype X intervention interaction to the response to different biomarkers following EIMD. The striking finding that NPGs regarding the EIMD-intervention also demonstrate a generally lower muscle strength indicates that a genetically determined impaired EIMD-response might result in lower muscle quality over time.

5.2 INTRODUCTION

Tender and sore muscles following unaccustomed intense exercise is a well-known phenomenon, and is referred to as delayed onset muscle soreness, or DOMS (Cheung et al., 2003). Several investigations have revealed that DOMS is mainly induced by eccentric contractions, i.e. movements that forcibly lengthen the contracting muscle (Armstrong et al., 1991). It is thought that excessive sarcomere strain due to exercise-induced muscle damage (EIMD) is the primary cause of muscle injury (Lieber and Fridén, 1999) and poorly managed training workload (e.g. identical training intensity for all team members of a football squad) with insufficient recovery between exercise sessions can lead to overuse muscle injury (Gabbett et al., 2016). In rare cases, over-exertion (e.g. marathon) can lead to exertional rhabdomyolysis with clinically relevant symptoms, such as compartment syndrome or renal failure, and can even cause death (Rawson et al., 2017).

The response to EIMD is very complex as several tissues that contribute to the transmission of force to the bone, including skeletal muscle fibres (Clarkson and Hubal, 2002), the extra-cellular matrix (ECM) (Hyldahl and Hubal, 2014), and tendon (Hicks et al., 2017), play a potential role in the damage response. Further, different muscle tissue repair mechanisms (inflammation, degradation of damaged tissues and muscle remodelling) take part in this process in a highly coordinated fashion (Tidball, 2011). Therefore, the response to EIMD can manifest in a variety of symptoms, including prolonged loss of maximal strength, decreased range of motion (ROM), ultrastructural damage, DOMS, localised inflammation and release of muscle-specific proteins into the circulatory system [e.g. creatine kinase (CK) activity, and interleukin-6 (IL-6)], which can be detected for days or even for weeks (Brown et al., 1999, Baird et al., 2012). All of these variables are considered to be biomarkers of EIMD.

Previous studies have determined a wide range of inter-individual variability in the response to EIMD, even when tested in the same human population (Nosaka and Clarkson, 1996). Investigations in the previous chapters demonstrated that the varying response to EIMD is potentially based on different muscle characteristics. Improved contractile properties (e.g.

larger muscle physiological cross-sectional area) appears to protect against immediate loss of maximal voluntary contraction (MVC) torque (see Chapter 3), and a relative high proportion of stem cells of the connective tissue/ECM (fibroblasts) to muscle stem cells (myoblasts) appear to support the recovery of MVC torque (see Chapter 4) the days after sprinting-induced muscle damage, respectively. Whilst different muscle characteristics between different human populations can be explained by factors such as age (Roth et al., 1999), ethnic origin (Sherwood et al., 1996), training status (Ploutz-Snyder et al., 2001) and sex (Sewright et al., 2008), there is evidence to suggest that some of this inter-individual variability might be explained by differences in genetic make-up (Clarkson et al., 2005b, Ahmetov et al., 2014, Baumert et al., 2016a). Indeed, several single nucleotide polymorphisms (SNPs), i.e. common variations in the sequence of nucleotides within genes that encode proteins (i) in skeletal muscle (Baumert et al., 2017, Clarkson et al., 2005b), tendon and muscle ECM (Baumert et al., 2018, Barfield et al., 2014), or (ii) are related with the inflammatory response (Funghetto et al., 2013, Ahmetov et al., 2014) have been associated with changes in various biomarkers of EIMD following eccentric exercise. Therefore, it is important to understand that any genetic association with damage to the muscle-tendon unit (MTU) is likely to be polygenic.

To our knowledge, only two studies have investigated a combination of candidate SNPs in regard to the response to muscle damaging exercises (Del Coso et al., 2017b, Del Coso et al., 2017a). Both studies investigated the association of a combination of seven candidate SNPs with plasma CK concentration in applied settings (marathon and half-ironman), i.e. the exercise is not standardised, thus making it difficult to accurately characterise the variable response to EIMD. Further, whilst a plethora of genetic association studies have been performed (Baumert et al., 2016a), only a few studies have investigated the mechanisms underpinning the effect of genetic variation in the context of exercise and EIMD (Barfield et al., 2014, Garton et al., 2013, Garton et al., 2018b). Knowledge of both genetic association with the individual response to strenuous exercise and the underlying mechanism could help develop individualised exercise training programmes that may be

used to optimise physical performance, while maximising recovery, thus reducing the risk of injury and development of MTU-related disease (van der Horst et al., 2015, Colberg et al., 2010, Erskine et al., 2010b).

Therefore, the primary aim of this study was to investigate whether a polygenic profile could distinguish between high and low responders following a controlled eccentric exercise intervention in previously untrained individuals *in vivo.* Using a two-level approach, 20 SNPs, which demonstrated a genotype X intervention interaction with the skeletal muscle (*ACTN3* C>T, rs1815739; *DES* G>C, rs12621188; *MYLK* G>A, rs2700352; *MYLK* G>T, rs28497577; *TTN-AS1* A>G, rs1001238; *TTN-AS1* G>A, rs3731749), muscle ECM/tendon (*COL1A1* G>T rs1800012; *COL1A1* T>C, rs2249492; *COL2A1* G>A, rs2070739; *COL5A1* T>C, rs12722; *MMP3* G>A, rs679620), or with the response following EIMD *(AGT* C>T, rs699; *CCL2* G>C; *IGF2-AS* A>C, rs4244808; *IL6* C>G, 1800796; *NOS3* T>C, rs2070744; *PAX7* G>A, rs485874; *TNF* T>C rs1799964; *TRIM63* A>G, rs2275950; *VDR* C>T, rs2228570) were initially investigated on an individual basis for an interaction effect with an acute bout of maximal eccentric exercise *in vivo*. SNPs that demonstrated an interaction with the response to exercise (following correction for multiple comparison) were used to calculate a “total genotype score” to compute a polygenic profile regarding the acute EIMD response (TGS-A) (Williams and Folland, 2008). The secondary aim was to further investigate candidate SNPs, which were found to be significant in the *in vivo* intervention, in an *in vitro* design. We used cultures of primary human skeletal muscle cells to assess the association of the candidate SNPs on muscle recovery following an artificial wound healing assay *in vitro*. We hypothesised that individuals with a non-preferential genetic (NPG) profile, i.e. those with a low TGS-A, would demonstrate greater muscle damage and a slower recovery rate in response to maximal eccentric exercise compared to those with a preferential genetic (PG) profile. More specifically, we hypothesised that NPGs would show a relatively greater loss of maximal muscle strength and ROM, greater perception of muscle soreness, and higher concentration/activity of circulating muscle-specific proteins,

immediately following eccentric exercise compared to PGs, and that these differences would be evident either immediately after or 48 hours after the EIMD-intervention.

5.3 METHODS

5.3.1 Cohorts

Two cohorts were included in this study and both cohorts provided written informed consent prior to participation in the study, which complied with the Declaration of Helsinki and were approved by the Research Ethics Committee of Liverpool John Moores University (Table 5-1). Participant inclusion criteria for both cohorts comprised: (i) aged 18-35 years; (ii) no history of strength training 6 months prior to the study (assessed via interview); (iii) no history of lower extremity musculoskeletal injuries 12 months prior to the study (assessed via interview and health questionnaire); (iv) medium level of habitual physical activity (Baecke et al., 1982a); and (v) use of potentially anabolic supplements. The first (Genetics of Recovery after Exercise, G-REX) cohort comprised 65 (39 females and 26 males) participants, while the second (Muscle Stem Cell) cohort comprised 12 (four females and eight males) participants (Table 5-1).

Table 5-1 *Participant Characteristics of both cohorts (mean ± SD).*

Cohort	Sex	Total (n)	Age (years)	Height (m)	Body mass kg)
G-REX	Females	39	22.3 ± 4.06	1.66 ± 0.07	65.5 ± 12.4
	Males	26	22.8 ± 4.07	1.78 ± 0.07	78.6 ± 13.6
Muscle Stem Cell	Females	4	25.5 ± 1.29	1.67 ± 0.08	61.4 ± 2.57
	Males	8	21.3 ± 4.27	1.77 ± 0.05	73.8 ± 5.68

5.3.2 Experimental Design of the G-REX Cohort (*in vivo* Study)

Participants reported to the laboratory on three separate occasions: (i) familiarisation to the isometric and isokinetic knee extension MVC assessments; (ii) maximal knee extension eccentric contractions on an isokinetic dynamometer to induce EIMD (EIMD-intervention) in the right leg plus assessments before (PRE) and directly after (POST); and (ii) 48 h after

(POST48) the EIMD-intervention. The assessments of muscle damage indices comprised isometric and isokinetic MVC torque (assessed via isokinetic dynamometry in the right leg), right leg muscle soreness [assessed via visual analogue scale (VAS)] and serum samples (for analysing IL-6 concentration and CK activity). All tests were performed at the same time point of the day for each participant. Participants were instructed to maintain their normal dietary behaviour (consumption of purported recovery supplements was not permitted), to refrain from drinking alcohol and to avoid any strenuous physical activity for at least 48 h before and throughout the study.

5.3.3 Muscle Damage Protocol

The muscle damage protocol consisted of 12 sets of 10 right leg maximal knee extension eccentric contractions on an isokinetic dynamometer [either a Humac Norm, CSMI (Massachusetts, USA) or Biodex Multi-Joint System 3 Pro (Shirley, USA)]. The two isokinetic dynamometers show similar isometric, concentric and eccentric peak torques with high to very high reproducibility (Alvares et al., 2015) and each participant performed all their strength assessments on the same dynamometer. Each set consisting of 10 maximal repetitions (30 s rest between each set). After every fourth set, the participants rested for three minutes. The angular velocity was set to 30°/s of eccentric knee contraction (from 30° to 100° knee flexion angle, with 0° as the extended position) and the participants were instructed to maximally counteract the knee flexion induced by the dynamometer.

5.3.4 Maximal Voluntary Contraction (MVC) Torque

The participant was seated on the isokinetic dynamometer in an upright position with the hips flexed to 85º (180° = supine position) and securely fastened with inextensible straps at the chest and waist whilst the arms were held crossed above the chest. The lateral tibiofemoral epicondyle was aligned with the axis of rotation of the lever arm, and the bottom of the lever arm shin pad was strapped to the leg, 2 cm above the centre of the lateral

malleolus. Prior to performing MVCs, participants underwent a standardised warm up consisting of 10 submaximal isokinetic leg extensions ($60^{o} \cdot s^{-1}$). Participants then performed three isometric MVCs (lasting 2-3 s and interspersed by 60 s of rest), with the knee joint angle set at 80º knee flexion (0° = full extension), as this has previously been shown to be the optimum joint angle for peak knee extension moment in healthy young men (Erskine et al., 2009). Three isokinetic MVCs (70° ROM, from 30° to 100° knee flexion angle; velocity $60^{o} \cdot s^{-1}$) were performed after the isometric MVCs, and the participants were instructed to perform the MVCs in a continuous fashion. The test-retest reproducibility for isometric knee extensor MVC is high, with a coefficient of variation (CV) of 3.9% (Erskine et al., 2009). Throughout the tests, participants received verbal encouragement and biofeedback (MVC outputs) were projected onto a screen in front of the participant) (Erskine et al., 2009).

5.3.5 Delayed Onset Muscle Soreness (DOMS)

Participants were asked to score their perceived muscle soreness and pain with a visual analogue scale (VAS) following a bilateral squat. Participants stood with hands on hips with feet shoulder-width apart and slowly squatted to a standardised chair height of 40 cm (~90° knee flexion) and then rose to a standing position. This was repeated twice more, after which participants were asked to rate their perceived quadriceps muscle soreness in their right leg using a VAS (scale 0-10 cm; 0 cm=no soreness; 10 cm=maximal soreness) (Sellwood et al., 2007).

5.3.6 Blood Samples

Venous blood samples were obtained from all participants (for genotyping purposes) and serum CK activity and concentration of the inflammatory cytokine interleukin-6 (IL-6) were determined from a subgroup of 38 participants of the G-REX cohort. All blood samples drawn from an antecubital vein in the forearm and collected into a 10 ml EDTA vacutainer (genotyping sample) and serum vacutainer (both BD Vacutainer systems, Plymouth, UK).

The genotyping blood sample was taken on just one occasion (familiarisation session). Serum samples were obtained at each time point and left at temperature controlled laboratory (between 22 and 24°C) for 30 min to allow clotting, and then kept on ice when necessary. Serum samples were centrifuged at 1300 g for 15 min at 4°C. All samples were then aliquoted into 1.5 mL microcentrifuge tubes [Axygen (Corning), New York, USA] and stored at -80°C until subsequent analysis (see below).

5.3.7 Serum Creatine Kinase Activity

Creatine kinase (CK) activity was assayed using a commercially available CK assay (Catachem Inc., Connecticut, NE, USA), as described in detail elsewhere (Sharples et al., 2011, Owens et al., 2014). Briefly, 10 µL blood serum of the G-REX cohort/cell lysate of the muscle cell culture study were loaded onto a 96-well UV plate. The CK reaction reagent and diluent (Catachem) were prepared as per the manufacturer's instructions and heated for 2 min at 37°C. The reconstituted reagent contained the following active ingredients: 30 mmol/l PCr, 2 mmol/l ADP, 5 mmol/l AMP, 2 mmol/l NAD, 20 mmol/l N-acetyl-L-cystine, 3,000 U/l hexokinase, 2,000 U/l G-6-PDH, 10 mmol/l 1 Mg2, 20 mmol/l D-glucose, 10 mol/l di(adenosine 5=) pentaphosphate, and 2 mmol/l EDTA, buffered to pH 6.7. The reagent mixture was then added to the samples and the change in absorbance monitored continuously over 20 min in a Thermo Multiskan Spectrum plate reader at a wavelength of 340 nm.

5.3.8 Serum Interleukin-6 (IL-6) Concentration

Serum samples were assayed for IL-6 concentration using commercially available human IL-6 enzyme linked immunosorbent assay (ELISA) kits (Quantikine®, R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Briefly, the serum samples were thawed and aliquots (200 µl) of each diluted sample, positive control or standard, with known concentrations of human IL-6 to establish standard values, were

plated on a coated (monoclonal antibody specific for human IL-6) 96-well microtitre plate for 2 h. After washing, human IL-6 conjugate (200 µL) was added to each well and incubated for 2 h at room temperature (between 22 and 24°C). After the wells were washed, substrate solution (200 µL) was added to each well at room temperature (between 22 and 24°C) and protected from light. After adding 50 µL of stop solution to each well, the intensity of the colour produced after 20 min was measured with a Thermo Multiskan Spectrum microplate reader (Thermo Fisher Scientific. Waltham, MA. USA) at 450 nm and values were calculated with Excel 365 (Microsoft, v. 365, USA) by generating a four-parameter logistic (4-PL) curve fit. The minimum detectable dose of human IL-6 was 0.70 pg/mL.

5.3.9 Genotyping

DNA extraction from whole blood was performed with a QIAamp DNA Blood Mini Kit (Qiagen, Manchester, UK), following the manufacturer's QIAamp spin column protocol for DNA purification from whole blood. Briefly, 20 *µ*L proteinase K (Qiagen) was pipetted into a 1.5 mL microcentrifuge tube. 200 *µ*L of sample and 200 *µ*L of lysis buffer (AL, Qiagen) was then added and vortexed for 15 s. this was then incubated at 56°C for 10 mins to maximise DNA yield. Following incubation, 200 *µ*L ethanol (96-100%) was added to the sample and vortexed for 15 s. This mixture was then transferred to a QIAamp Mini spin column (Qiagen) within a 2 mL collection tube. This was then centrifuged at 8000 rpm for 1 min before the mini spin column was transferred to a clean 2 mL collection tube. 500 *µ*L AW1 binding buffer (Qiagen) was then added to the spin column before being centrifuged again at 8000 rpm for 1 min. The column was again placed into a clean 2 mL collection tube and 500 *µ*L AW2 wash buffer (Qiagen) was added before centrifuging at 14 000 RPM for 3 min. The column was then placed into a clean 2 mL collection tube and centrifuged at 14 000 RPM for 1 min. Finally, the spin column was placed in a fresh 1.5 mL microcentrifuge tube, 200 *µ*L AE wash buffer (Qiagen) was added to the column and was centrifuged at 8000 RPM for 1 min following 1 min incubation at room temperature.

Real-time polymerase chain reaction (PCR) was performed using a Rotor-Gene Q PCR machine (Qiagen) to define the genotypes in each subject. Reactions were completed on a 72-well rotor-disc. Each 10 µL reaction volume contained: 5 µL Genotyping Master Mix (Applied Biosystems, Foster City, California, USA), 3.5 µL nuclease-free H_2O (Qiagen), 0.5 µL genotyping assay (Applied Biosystems), which included the SNP-specific TaqMan primers and probes, and 1 µL DNA. A list of the 20 SNPs analysed can be found in Table 5-2. For negative control wells, 1 µL nuclease-free H_2O replaced the DNA template. Positive controls were also used to provide further confidence in our results. The following PCR protocol was used: 50 cycles of incubation at 92°C for 15s (denaturation) then annealing and extension at 60°C for 1min. Lastly, genotype was determined using Rotor-Gene Q Software 2.3.1. All samples and positive controls were analysed in duplicate to ensure there was 100%.

Table 5-2 Tested Gene Polymorphisms.

Symbol	Gene	rs-number	EA> ALT	Chromosome (Position)	EAF
ACTN3	Actinin Alpha 3	rs1815739	C>T	11(66,560,624)	0.62
AGT	Angiotensinogen	rs699	C>T	1(230,710,048)	0.54
CCL2	C-C Motif Chemokine Ligand 2	rs2857656	G>C	17(34,254,988)	0.75
COL1A1	Collagen Type I Alpha 1 Chain	rs1800012	G>T	17(50,200,388)	0.83
		rs2249492	T>C	17(50,185,660)	0.54
COL2A1	Collagen Type II Alpha 1 Chain	rs2070739	G>A	4(47,974,193)	0.92
COL5A1	Collagen Type V Alpha 1 Chain	rs12722	T>C	9(134,842,570)	0.52
DES	Desmin	rs12621188	G>C	2(219,423,170)	0.60
IGF2-AS	IGF2 Antisense RNA	rs4244808	A>C	11(2,141,880)	0.51
IL6	Interleukin 6	rs1800796	C>G	7(22,727,026)	0.42
MMP3	Matrix Metallopeptidase 3	rs679620	G>A	11(102,842,889)	0.44
MYLK	Myosin Light Chain Kinase	rs2700352	G>A	3(123,831,616)	0.78
		rs28497577	G>T	3(123,793,780)	0.90
NOS3	Nitric Oxide Synthase 3	rs2070744	T>C	7(150,992,991)	0.63
PAX7	Paired Box 7	rs485874	G>A	1(18,746,432)	0.56
TNF	Tumour Necrosis Factor	rs1799964	T>C	6(31,574,531)	0.79
TRIM63	Tripartite Motif Containing 63	rs2275950	A>G	1(26,058,512)	0.77

TTN-AS1	Titin Antisense RNA 1	rs1001238	A>G	2(178,599,800)	0.72	
		rs3731749	G>A	2(178,541,464)	0.82	
VDR	Vitamin D Receptor	rs2228570	C>T	12(47,879,112)	0.63	*Healthy untrai*

EA/ALT - Effect allele and alternative allele; Trait; EAF - Effect allele frequency in this study. Chromosome positions are based on NCBI 151. The alleles all refer to the forward strand.

5.3.10 Total Genotype Score (TGS) Calculation

Using a two-level approach to compute a TGS (Williams and Folland, 2008), ten previously EIMD-associated SNPs (*ACTN3* C>T, rs1815739; *COL1A1* G>T rs1800012; *COL1A1* T>C rs2249492; *COL2A1* G>A, rs2070739; *COL5A1* T>C, rs12722; *IGF2-AS* A>C, rs4244808; *IL6* C>G, 1800796; *MYLK* G>A, rs2700352; *MYLK* G>T, rs28497577; *TRIM63* A>G, rs2275950) (Clarkson et al., 2005b, Devaney et al., 2007a, Yamin et al., 2008, Baumert et al., 2017, Baumert et al., 2018), plus ten so far EIMD-non-associated SNPs (*AGT* C>T, rs699; *CCL2* G>C; *DES* G>C, rs12621188; *MMP3* G>A, rs679620; *NOS3* T>C, rs2070744; *PAX7* G>A, rs485874; *TNF* T>C rs1799964; *TTN-AS1* A>G, rs1001238; *TTN-AS1* G>A, rs3731749; *VDR* C>T, rs2228570) were investigated for an association with the EIMD intervention on an individual basis, while controlling for multiple comparisons. Therefore, a total of 20 SNPs were analysed. Heterozygote genotypes were pooled with one of the two homozygotes which showed a similar pattern except for SNPs possessing rare homozygotes n ≤ 2 (*COL1A1*, rs1800012; *COL2A1*, rs2070739; *MYLK*, rs28497577; *MYLK*, rs2700352), for which a recessive model was used. Single nucleotide polymorphisms that showed an interaction with the response to the EIMD intervention following the application of a 20% false discovery rate (FDR) (Benjamini and Hochberg, 1995), were then used to calculate our TGS-A model (Williams and Folland, 2008). Each genotype from each SNP was, therefore, given a score between 0 and 2 based on the response to the EIMD-intervention. For each SNP, the homozygote genotype that showed a beneficial effect was given a score of 2 and the homozygote genotype associated with a detrimental effect was given a score of 0. The heterozygote genotype received a score of 1. Combining the scores of each significant SNP gave a total genotype score within a range between 0 – 100, with

the best possible polygenic genotype score defined as 100, and the worst possible as 0. Based on the TGS-A distribution, we divided the cohort into three categories, namely a "preferential genetic" (PG; high score), "moderate" (MG), and "non-preferential genetic" (NPG; low score) profile. The thresholds of these three TGS-A groups were determined according to the $\bar{x}$ of the total genotype score ± 1SD (i.e. NPG < $\bar{x}$ -1SD; MG $\bar{x}$-1SD to $\bar{x}$+1SD; PG ≥ $\bar{x}$+1SD).

5.3.11 Muscle Stem Cell Cohort (*in vitro* Study)

Twelve recreationally active and healthy young female Caucasians participated in this study. Prior to starting the study, a pre-biopsy screening (including venous blood samples were obtained from all participants for genotyping purposes) as approved by a physician was obtained from each participant. The following candidate SNPs, which demonstrated a genotype X intervention interaction with markers of EIMD in the G-REX cohort, were assessed for parameters in the muscle cell culture study: *ACTN3*, rs1815739; *COL1A1*, rs1800012; *COL1A1*, rs2249492; *COL2A1,* rs2070739; *COL5A1,* rs12722; *IGF-AS,* rs4244808; *MMP3,* rs679620; *NOS3*, rs2070744; *PAX7*, rs485874; *VDR;* rs2228570; *TRIM63,* rs2275950; and *TTN,* rs3731749.

5.3.12 Muscle Cell Culture

The methodological approach has been described in detail in section 4.3.

5.3.13 Data Analysis

Each SNP was tested for compliance with the Hardy-Weinberg equilibrium by using a χ^2 test. All parameters were normally distributed according to the Shapiro-Wilk test and by inspection of the Q-Q plots. Linkage disequilibrium (LD) was analysed via LDlink suite and data from the 1000 Genomes Project European ancestry populations (Machiela and Chanock, 2015, Consortium, 2012). Recent investigations revealed that pruning for LD

regarding polygenic approaches does not essentially change the results (Vrieze et al., 2013). Therefore, SNPs with LD were still included in the TGS-A. We recruited young, healthy men and women to increase both the external validity and sample size of the *in vivo* study, and the muscle stem cells did not show significant differences between sex for any of the investigated parameters (see Chapter 4). However, because there is limited evidence for sex-specific differences in the response eccentric exercise (Sewright et al., 2008), we initially analysed the data according to sex. All data were analysed for interactions and main effects for sex (sex groups: between subjects factor) and time (within subjects factor: PRE, POST, POST48) using two-way-mixed analysis of variance (ANOVA). As an interaction was found between sex and time regarding MVC normalised to body mass (please see below), All subsequent analyses were performed using two-way-mixed analysis of covariance (ANCOVA), with genotype (genotype groups: between subjects factor), time (within subjects factor: PRE, POST, and POST48) with sex (male/female) included as a covariate. For post hoc analyses, either, independent t-tests, or one-way ANOVAs were used where appropriate. Regarding the primary muscle stem cells study, linear regression analyses were used to analyse the relation between the following parameters: proportion of myoblast to fibroblasts, migration dynamics (total cell migration, proportion inner/ outer segment), and myotube formation (total myotubes, myotube length, average diameter, myotube area and CK activity). Standard guidelines concerning violation of the sphericity assumption to adjust the degree of freedom of the F-test by the Huynh-Felt epsilon if epsilon is greater than 0.75 and to use the more stringent Greenhouse-Geisser adjustment if epsilon is less than 0.75 were followed (Girden, 1992). All MVC torque values were normalised to body mass unless otherwise stated and analysed with AcqKnowledge software 4.4 (Biopac-Systems Inc., Goleta, USA) and SPSS 23 Software (IBM Inc., Armonk, NY: IBM Corp) was used for statistical analysis. Results were expressed as mean ± SD except otherwise stated, with statistical significance set at $p < 0.05$.

5.4 RESULTS

5.4.1 Effect of EIMD-Intervention on Biomarkers According to Sex

5.4.1.1 Isometric and Isokinetic MVC

There was an interaction between sex and time for normalised isometric ($F_{2,126}$=4.13, P=0.018) and isokinetic MVC torque ($F_{2,126}$=8.06, P=5.08x10^{-4}; Table 5-3). Therefore, subsequent genetic analyses included sex as a co-variate.

5.4.1.2 Muscle Soreness

There was no main effect of sex ($F_{1,63}$=0.15, P=0.701) and no interaction between sex and time (F value and P value) following EIMD ($F_{2,126}$=1.19, P=0.309).

5.4.1.3 Blood Biomarkers

There were no main effect of sex ($F_{1,36}$=2.77, P=0.105) and no interaction between time and sex regarding changes in either serum IL-6 concentration ($F_{2,72}$=0.66, P=0.521) regarding changes in either serum IL-6 concentration. There were also no main effect of sex ($F_{1,36}$=3.51, P=0.069) and no interaction between time and sex regarding serum CK activity ($F_{2,72}$=1.67, P=0.202).

5.4.2 Effect of EIMD-Intervention on Biomarkers

Isometric and isokinetic MVC, muscle soreness (all P<0.001) and serum CK activity (P=0.009) showed a main effect of time, indicating EIMD had occurred (Table 5-3). Specifically, both isometric and isokinetic MVC decreased pre- to post-EIMD (both P<0.05) and remained lower than baseline at 48 h post-EIMD (both P<0.05). Muscle soreness and serum CK activity increased pre- to post-EIMD (both P<0.05) and remained elevated post-EIMD compared to baseline (both P<0.05). However, serum IL-6 concentration did not show any changes at POST and POST48 compared to PRE EIMD-intervention (P>0.05).

Table 5-3 *Values for dependent variables in response to muscle damaging exercise between sex*

			Time points		
Test	Sex	Participants	PRE	POST	POST48
IMS (N·m)	Male	26	3.44 ± 1.16	2.24 ± 0.77	2.81 ± 1.08*
	Female	39	2.66 ± 0.77	1.90 ± 0.59	2.26 ± 0.75*
IKS (N·m)	Male	26	2.66 ± 0.83	1.71 ± 0.59	2.15 ± 0.74*
	Female	39	2.15 ± 0.57	1.61 ± 0.56	1.83 ± 0.56*
VAS (cm)	Male	26	0.39 ± 0.50	3.73 ± 2.49	3.17 ± 2.68
	Female	39	0.40 ± 0.65	3.45 ± 2.42	3.85 ± 2.15
ROM (°)	Male	13	121.5 ± 10.4	109.4 ± 13.8	118.3 ± 13.8
	Female	27	120.7 ± 10.2	110.8 ± 11.6	114.2 ± 9.14
CK (mU/mL)	Male	15	14.7 ± 13.4	38.9 ± 70.0	42.5 ± 58.6
	Female	23	9.04 ± 3.91	12.3 ± 8.17	22.7 ± 18.7
IL6 (pg/mL)	Male	15	1.52 ± 2.20	3.18 ± 5.35	2.83 ± 3.95
	Female	23	1.22 ± 1.49	1.75 ± 2.74	1.24 ± 1.12

IMS – normalised isometric Strength; IKS – normalised isokinetic Strength; VAS – Leg muscle soreness; ROM – Range of Motion; CK – Creatine kinase activity; IL6 – Interleukin-6 concentration. Values represent means ± SD. * Interaction effect between gender, P<0.05.

5.4.3 Hardy-Weinberg Equilibrium and Linkage Disequilibrium

The genotypes of all 20 SNPs were in Hardy-Weinberg equilibrium, except for *COL2A1* rs2070739 (X^2=6.04, P=0.014) and *PAX7* rs485874 (X^2=5.15, P=0.023). Linkage disequilibrium calculations revealed that the following SNPs were in LD: both *COL1A1* (rs1800012 and rs2249492) SNPs (D`=0.736 and $R^2$=0.077, P<0.001); *MMP3* (rs679620) and *ACTN3* (rs1815739) SNPs (D`=0.068 and R^2=0.004, P=0.046); both *MYLK* (rs2700352 and rs28497577) SNPs (D`=0.482 and $R^2$=0.08, P<0.001); *PAX7* (rs485874) and *TRIM63* (rs2275950) SNPs (D`=0.134 and R^2=0.004, P=0.004); and both *TTN-AS1* (rs1001238 and rs3731749) SNPs (D`=1.0 and R^2=0.472, P<0.001).

5.4.4 SNP Associations with Biomarkers of EIMD

From the 20 SNPs analysed, seven displayed significant interactions with time (Table 5-4) regarding muscle soreness (*COL2A1,* rs2070739; *COL5A1,* rs12722; and *TTN,* rs3731749), ROM (*COL5A1,* rs12722; *IGF2-AS,* rs4244808; *VDR;* rs2228570; and *TRIM63,* rs2275950) and isometric torque (*MMP3,* rs679620). There were no significant SNP associations with either serum CK activity or IL-6 concentration. Also, the following SNPs did not show either any interaction or main effect with any outcome variable intervention after correction for multiple testing: *AGT* rs699, *CCL2* (rs2857656), *DES* (rs12621188), *IL6* (rs1800796), both *MYLK* (rs2700352 and rs28497577), *NOS3* (rs2070744), *TNF* (rs1799964), and *TTN-AS1* (rs1001238). The responses of the G-REX cohort for both *COL1A1* (rs1800012 and rs2249492), *COL2A1* (rs2070739), *COL5A1* (rs12722) and *TRIM63* (rs2275950) SNPs to the eccentric exercise regimen have been previously reported (Baumert et al., 2017, Baumert et al., 2018). However, for each SNP in the present study, we pooled the heterozygote group with the homozygote group that showed a similar response to the heterozygote group. For this reason, the *TRIM63* rs2275950 SNP now shows an interaction between time x genotype (minor G homozygotes compared to A-allele carriers) regarding ROM, but no significant interaction in regard to soreness [as reported in Baumert et al. (2017)], where the three genotypes were analysed separately.

Table 5-4 SNP interaction with EIMD-intervention following correction for multiple comparisons.

			Group Response (n)			
Symbol	**rs-number**	**Interaction**	**Beneficial**	**Detrimental**	**F-Test**	**P Value**
COL2A1	rs2070739	Muscle Soreness	GG (56)	GA+AA (9)	$F_{2,124}$ = 6.63	0.002
COL5A1	rs12722	Muscle Soreness	CC+CT (49)	TT (16)	$F_{2,124}$ = 4.48	0.013
		Range of Motion	CC+CT (32)	TT (8)	$F_{2,74}$ = 3.42	0.038
IGF2-AS	rs4244808	Range of Motion	AA+AC (35)	CC (5)	$F_{2,74}$ = 3.99	0.023

MMP3	rs679620	Isometric Torque	AA (12)	GG+GA (53)	$F_{2,124}$ = 5.10	0.007
VDR	rs2228570	Range of Motion	CC+CT (36)	TT (4)	$F_{2,74}$ = 5.10	0.028
TRIM63	rs2275950	Range of Motion	AA+AG (38)	GG (2)	$F_{2,74}$ = 3.38	0.039
TTN-AS1	rs3731749	Muscle Soreness	GG (45)	GA+AA (20)	$F_{2,124}$ = 6.83	0.002

In addition to the interactions reported in Table 5-4, there were also main effects (Table 5-5) regarding the following SNPs for isometric torque (*COL1A1,* rs1800012; *COL1A1,* rs2249492; *TRIM63,* rs2275950), for isokinetic torque (*ACTN3,* rs1815739; *COL1A1,* rs1800012; *COL1A1,* rs2249492; *MMP3,* rs679620; *TRIM63,* rs2275950), for muscle soreness (*NOS3,* rs2070744; *TTN-AS1,* rs3731749) and for ROM (*PAX7,* rs485874).

Table 5-5 Main effect for genotype following correction for multiple comparisons.

			Group Response (n)			
Symbol	**rs-number**	**Main Effect**	**Beneficial**	**Detrimental**	**F-Test**	**P Value**
ACTN3	rs1815739	Isokinetic Torque	CT+TT (37)	CC (28)	$F_{1,62}$ = 4.65	0.035
COL1A1	rs1800012	Isometric Torque	GG (45)	GT+TT (22)	$F_{1,62}$ = 7.24	0.009
		Isokinetic Torque			$F_{1,62}$ = 5.82	0.019
COL1A1	rs2249492	Isometric Torque	CC (11)	TT+TC (54)	$F_{1,62}$ = 6.02	0.017
		Isokinetic Torque			$F_{1,62}$ = 6.41	0.014
MMP3	rs679620	Isokinetic Torque	AA (12)	GG+GA (53)	$F_{1,62}$ = 3.99	0.05
NOS3	rs2070744	Muscle Soreness	TT (40)	TC+CC (25)	$F_{1,62}$ = 5.72	0.02
PAX7	rs485874	Range of Motion	AA (10)	AG+GG (30)	$F_{1,37}$ = 7.29	0.01
TRIM63	rs2275950	Isometric Torque	AA+AG (60)	GG (5)	$F_{1,62}$ = 6.63	0.012

		Isokinetic Torque			$F_{1,62}$ = 5.60	0.021
TTN-AS1	rs3731749	Muscle Soreness	GG (45)	GA+AA (20)	$F_{1,62}$ = 6.42	0.014

5.4.5 Artificial Wound Healing Model to Assess Repair and Regeneration and Genetic Variations

The *COL2A1* G>A (rs2070739), *MMP3* G>A (rs6796200), *TRIM63* A>G (rs2275950), *TTN* G>A (rs3731749), and *VDR* C>T (rs2228570) SNPs did not show any interaction or main effect with any parameters for the *in vitro* artificial wound healing assay (all P>0.05). There were main effects (Table 5-6) regarding the following SNPs for the artificial wound healing model *in vitro: ACTN3,* rs1815739; *COL1A1,* rs1800012; *COL1A1,* rs2249492; *COL5A1,* rs12722; *IGF2-AS*, rs4244808; *PAX7,* rs485874.

Table 5-6 Main effect for genotype regarding the artificial wound healing model.

Symbol	rs-number	Main Effect	Group Response (n)	Values	F-Test	P Value
ACTN3	rs1815739	Myoblast:Fibroblast ratio (%)	CC (7)	36.1 ± 18.8	$F_{1,10}$ = 9.26	0.012
			CT+TT (5)	61.8 ± 20.9		
COL1A1	rs1800012	Total Myotubes (n)	GT+TT (2)	14.0 ± 2.47	$F_{1,10}$ = 11.4	0.007
			GG (10)	7.77 ± 2.37		
		CK activity (mU/mL)	GT+TT (2)	325.7 ± 1.36	$F_{1,9}$=6.96	0.027
			GG (9)	151.8 ± 97.4		
COL1A1	rs2249492	Myoblast:Fibroblast ratio (%)	TT+TC (10)	52.7 ± 20.3	$F_{1,10}$ = 5.50	0.041
			CC (2)	17.5 ± 5.9		
COL5A1	rs12722	Myotube length (µm)	TT (3)	257.4 ± 48.6	$F_{1,10}$ = 6.06	0.034
			CC+CT (9)	345.9 ± 55.2		
		CK activity (mU/mL)	TT (3)	76.4 ± 49.3	$F_{1,9}$ = 5.76	0.040

			CC+CT (8)	223.6 ± 102.3		
IGF2-AS	rs4244808	Total Myotubes (n)	CC (1)	15.75 ± 0.00	$F_{1,10}$ = 7.63	0.020
			AA+AC (11)	8.13 ± 2.53		
PAX7	rs485874	Total Myotubes (n	AG+GG (11)	8.18 ± 2.63	$F_{1,10}$ = 7.62	0.020
			AA (1)	15.75 ± 0.00		

There was a trend for an interaction between the *ACTN3* (rs1815739) SNP and time regarding migration rate ($F_{1.3,18}$=3.34, P=0.086). There was a tendency for more cells with the RR genotype (n=7; 24h: 18.4 ± 2.03 %; 48h: 26.9 ± 1.81 %) tended to migrate into the inner segment of the artificial injury compared to cells with the X allele (n=5; 24h: 13.1 ± 6.52 %; 48h: 24.8 ± 5.2 %). There was an interaction for *COL1A1* (rs1800012) SNP and time regarding migration rate ($F_{2,18}$=4.33, P=0.029). More cells with the G genotype (n=10; 24h: 17.6 ± 4.23 %; 48h: 26.7 ± 3.5 %) migrated into the inner segment of the artificial injury compared to cells with the T allele (n=2; 24h: 9.3 ± 0.69 %; 48h: 22.5 ± 1.46 %). There was an interaction between *TTN-AS1* (rs1001238) genotype and time regarding total cell migration into the wound zone ($F_{2,18}$=6.79, P=0.006). More cells with the AA genotype (n=8; 24h: 66.1 ± 9.56 cells; 48h: 96.9 ± 10.0 cells) migrated into the wound compared to the cells with the G allele (n=4; 24h: 57.0 ± 6.00 cells; 48h: 78.8 ± 10.1 cells). There was a trend for *NOS3* (rs2070744) SNP and time regarding CK activity measured at day 0 and day 10 ($F_{1.,9}$=5.01, P=0.052). There was a tendency that cells with the C allele showed increased CK activity (n=8; D0: 65.5 ± 21.4 mU/mL; D10: 222.5 ± 103.6 mU/mL) compared to cells with the TT genotype (n=3; D0: 53.1 ± 13.3 mU/mL; D10: 79.3 ± 53.0 mU/mL).

5.4.6 Effect of Total Genotype Score on Biomarkers of EIMD

The seven SNPs (*COL2A1,* rs2070739; *COL5A1,* rs12722; *IGF2-AS,* rs4244808; *MMP3,* rs679620; *VDR,* rs2228570; *TRIM63,* rs2275950; and *TTN-AS1,* rs3731749) that presented significant interactions with either muscle soreness, isometric torque or ROM (Table 5-4)

were used for the TGS-A analyses. Individuals were divided into three groups, depending on their TGS-A [see “Total Genotype Score calculation”, above, i.e. PG: n=10 (females=6; males=4); MG: n=37 (females=22; males=15); and NPG: n=18 (females=11; males=7)] Subsequently, there was a main effect for isometric ($F_{2,61}$=8.78, P=4.45x10^{-4}, Figure 5-1) and isokinetic ($F_{2,61}$=7.82, P=9.51x10^{-4}) MVC torque. Individuals of the NPG (1.93 ± 0.81 Nm/kg) and MG group (2.28 ± 0.69 Nm/kg) revealed weaker baseline isokinetic MVC torque values compared to PG group (2.73 ± 0.59 Nm/kg; P=0.005).

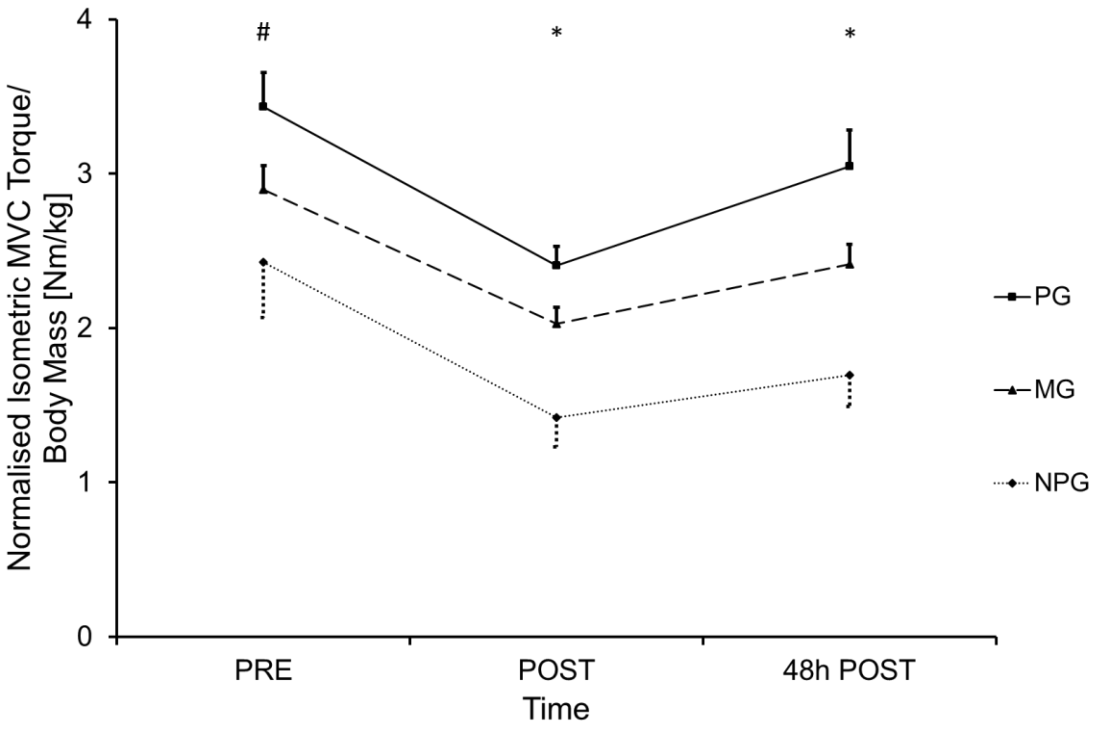

Figure 5-1 *Main effect for normalised isometric maximal voluntary contraction (MVC) torque in regard to the polygenic profile. PG – Preferential Genotype Group; MG – Moderate Genotype Group; NPG – Non-Preferential Genotype Group. # One-Way ANOVA, NPG and MG are significant different compared to PG, P<0.05; * One-Way ANOVA, significant differences between each group, P<0.05, mean ± SEM.*

There was a TGS-A group x time interaction in regard to muscle soreness ($F_{4,122}$=4.21, P=0.003, Figure 5-2), with higher muscle soreness reported for NPG compared to MG and PG at POST ($F_{2,61}$=4.87, P=0.011) and 48POST ($F_{2,61}$=6.42, P=0.003). Concerning ROM, there was a TGS-A x time interaction ($F_{4,72}$=3.40, P=0.006), whereby NPG showed greater ROM PRE-EIMD compared to MG and PG (NPG: 135.0 ± 8.66°; MG: 118.3 ± 9.69°; PG:

122.6 ± 8.70°; $F_{2,36}$=5.13, P=0.011). However, the EIMD-intervention resulted in a reduced ROM for the NPG group and, therefore, the NPG showed the same level of flexibility POST- and POST48-EIMD as the MG and PG group (P>0.05).

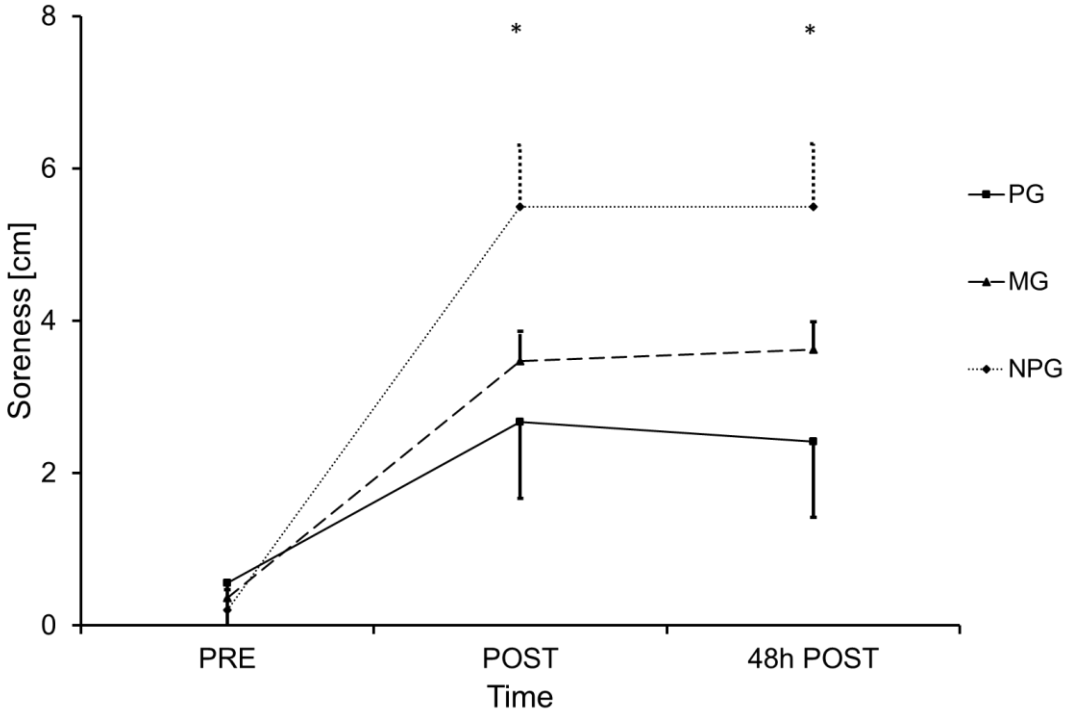

Figure 5-2 *Gene-Interaction effect (polygenic profile x time) for muscle soreness. PG – Preferential Genotype Group; MG – Moderate Genotype Group; NPG – Non-Preferential Genotype Group; * One-Way ANOVA, NPG are significant different compared to MG and PG, P<0.05, mean ± SEM.*

5.5 DISCUSSION

The aim of the current study was to investigate whether a combination of previously selected candidate SNPs could distinguish between high and low responders following a maximal eccentric exercise intervention. To achieve this aim, a total genotype score (Williams and Folland, 2008) was applied to each participant of the G-REX cohort, based on their genetic profile for the following seven candidate SNPs that demonstrated individually a genotype X intervention interaction with, at least, one EIMD-biomarker following the application of FDR. The G-REX cohort was then divided into three sub-groups, according to their TGS-A. The main finding of the current study is that the NPG group (i) demonstrated greater muscle soreness and greater loss of ROM following the EIMD-intervention, respectively; and (ii) were generally weaker compared to MG and PG groups.

Contrary to our hypothesis, we could not observe any TGS-A X intervention interaction with (i) changes in strength regarding our polygenic profile; and (ii) the concentration or activity of blood-borne biomarkers of EIMD following the intervention, neither on an individual SNP or polygenic basis. Among the individual SNPs that demonstrated an interaction with the EIMD-intervention, there were three distinct functional groups regarding the response to the EIMD-intervention. Here we discuss the potential influence of the individual SNPs that demonstrated an interaction or main effect with EIMD *in vivo* and with the wound healing assay *in vitro*, as well as the polygenic profile.

5.5.1 Functional Group of SNPs Related to Muscle Soreness

The first functional group of the protein related-SNPs comprise *COL2A1* (rs2070739), *COL5A1* (rs12722), and *TTN*-AS1 (rs3731749). The SNPs are located within genes, which contribute to the elastic properties of the MTU and may have a crucial role in storing and releasing energy during fast movements and/or stretch shortening cycles (Turrina et al., 2013). The strong type II collagen (the pro-alpha-1 chain of type II collagen is encoded by the *COL2A1* gene) fibrils are commonly expressed in cartilage (Gustafsson et al., 2003). However, chronic stress can induce an upregulation of *COL2A1* in tendon, indicating a (mal)adaptation to chronic overuse training (Archambault et al., 2007). Collagen type V (the pro-alpha-1 chain of type V collagen is encoded by *COL5A1)* is predominantly present in the endomysium (surrounding muscle fibres) and epimysium (surrounding the muscle) of the muscle ECM (Nakamura et al., 2007). The giant protein titin (encoded by the *TTN* gene) extends between the Z-line and the M-line (Bang et al., 2001) and is essential for the structure and elasticity of the sarcomere. The risk alleles of these three SNPs [A-allele of *COL2A1* (rs2070739); TT genotype of *COL5A1* (rs12722); A-allele of *TTN-AS1* (rs3731749)] demonstrated an interaction between perceived muscle soreness and time regarding the EIMD-intervention. In addition, participants of the *COL5A1* TT genotype also reported an impaired recovery of ROM *in vivo* and decreased myotube length and CK activity in the artificial wound healing model *in vitro* compared to the C-allele carriers,

presumably due to an elevated fibroblast activity. Fibroblasts are essential for efficient repair and regeneration of the ECM within muscle and tendon (Goetsch et al., 2003, Davis et al., 2013, please see Chapter IV). However, the three SNPs might affect the expression rate/stability of the corresponding gene (Lin et al., 2018, Tarpey et al., 2013, Posthumus et al., 2011). This appears to negatively influence the elastic components of the MTU with a subsequent decreased capacity to store and release energy (Stafilidis and Arampatzis, 2007, Turrina et al., 2013). An increased stiffness of the elastic components of the MTU might increase stress on the contractile components making NPGs more prone to EIMD during eccentric MVC compared to PGs. Overstretched cytoskeleton/muscle ECM may result in an increase of extracellular inflammatory mediators, which might trigger the nociceptor response within the muscle, leading to elevated muscle soreness (Hyldahl and Hubal, 2014, Hucho and Levine, 2007).

5.5.2 Functional Group of SNPs Related to Delayed Muscle Regeneration

The second functional group of SNPs, which are related to enzymes of the skeletal muscle catabolic (*MMP3*, rs679620; *TRIM63*, rs2275950) and anabolic (*VDR*, rs2228570; also referred to as *FOK1*) signaling pathways, were associated with markers of EIMD 48 h after the exercise intervention. Matrix metalloproteinases (MMPs) are enzymes which breakdown components (e.g. collagen) synthesised and secreted by fibroblasts. Fibroblasts are essential for efficient repair and regeneration of the ECM within muscle and tendon (Goetsch et al., 2003, Davis et al., 2013, please see Chapter IV). The G-allele of the missense *MMP3* (rs679620) G>A SNP is thought to decrease *MMP3* transcription (Foster et al., 2012, Ye, 2006, Taylor et al., 2008), which might supress the activity to degrade collagen III and other substrates (Sternlicht et al., 1999). The ubiquitin E3 ligase, muscle RING finger-1 (MuRF-1, expressed by the *TRIM63* gene), plays an important part in muscle atrophy via the ubiquitin–proteasome pathway (Bodine et al., 2001a). Further, recent investigations suggest that MuRF-1 interacts with one of titin's three strain-sensing locations at the M-line of the sarcomere (Centner et al., 2001, Baumert et al., 2017). Vitamin

D and the vitamin D receptor (VDR), are involved in immune function, calcium homeostasis, bone health and muscle function (Owens et al., 2018). Several polymorphisms within the *VDR* gene have been associated with changes in muscle function (Grundberg et al., 2004, Barr et al., 2010) and with the severity of musculoskeletal injuries in professional football players (Massidda et al., 2015). (Arai et al., 1997).

It is possible that the preferential alleles of three of these SNPs [A-allele of *MMP3* (rs679620); A-allele of *TRIM63* (rs2275950); C-allele of *VDR* (rs22285700)] exert their beneficial effect by increasing the expression rate of their transcript following EIMD compared to their corresponding non-preferential alleles (Foster et al., 2012, Ye, 2006, Taylor et al., 2008) (Arai et al., 1997). The increased response potentially promotes repair and supports successful skeletal muscle remodeling mirrored by a faster recovery of ROM (*TRIM63* and *VDR* SNPs) and muscle strength (*MMP3* SNP) 48 h following EIMD. Further, the preferential allele of two of these three SNPs (*MMP3* and *TRIM63 SNPs*) showed generally higher strength values. Increased gene expression of the preferential alleles and a subsequent increased degradation activity of the corresponding enzymes after intense exercises might lead to a better turnover of the target proteins and, therefore, to a higher stability of the ECM, thus potentially explaining the generally higher strength values of the G-REX participants. Regarding the *VDR* SNP, it seems that the non-preferential T-allele 'protects' non-exercising individuals against loss of muscle strength and free-fat mass (Windelinckx et al., 2007, Hopkinson et al., 2008, Roth et al., 2004). The increased VDR activity of the C-allele might lead to a higher risk of chronic low-grade inflammation resulting in accelerated muscle strength loss of sedentary elderly. However, the elevated gene activity supports muscle regeneration following EIMD, i.e. via elevated skeletal muscle myogenesis (Owens et al., 2015, Stratos et al., 2013). Therefore, in older, sedentary individuals, the C-allele is likely disadvantageous in terms of muscle function but in physically active people (young and old), this allele could be beneficial in terms of aiding the muscle recovery process following exercise.

The CC homozygotes of the insulin-like growth factor-II antisense (*IGF2-AS;* rs4244808) A>C SNP was the only signalling-related SNP that demonstrated a further reduction in ROM immediately post EIMD. Expression of *IGF2* might not change following an EIMD-intervention *per se* but IGF-II concentration might be generally higher in CC homozygotes (due to lower *IFG2-AS* gene activity) (Zhao et al., 2017). Increased *IGF2* expression might increase ECM deposition, causing a detrimental ECM structure (Keller et al., 1999, Baumert et al., 2016a), which is more prone to EIMD (Porter et al., 2002). Further, the CC genotype revealed a higher number of formed myotubes post damage *in vitro*, indicating an elevated sarcomerogenesis. This could lead to more sarcomeres in series (Wisdom et al., 2015), which potentially explains the generally greater ROM *in vivo* in CC homozygotes.

5.5.3 No Interaction of the Polygenic profile with Strength Loss following EIMD

It is thought that muscle force is transmitted predominantly laterally via the perimysium (surrounding muscle fascicles) to the tendon and ultimately to the bone (Ramaswamy et al., 2011). Collagen type I is predominantly present in the perimysial tissue, which becomes continuous with the tendon (Passerieux et al., 2007, Hughes et al., 2015, Gillies and Lieber, 2011). α-Actinin-3 (*ACTN3)* might play an exceptional role in the MTU, as α-actinin-3 anchors actin to the Z-line, which might play functionally an important role in muscle force transmission, both longitudinally and laterally (Hughes et al., 2015). The preferential alleles of the structure-related SNPs [X-allele of *ACTN3* (rs1815739); GG homozygotes *COL1A1* (rs1800012); and CC homozygotes of *COL1A* (rs2249492)] showed a main effect for a greater MVC torque generating capacity *in vivo.* CC homozygotes of *COL1A1* (rs2249492) demonstrated a higher proportion of fibroblasts to myoblasts *in vitro,* and *COL1A1* GG homozygotes (rs1800012) showed a lower number of total myotubes, which is accompanied with decreased CK activity. The beneficial alleles of both *COL1A1* SNPs might have an increased fibroblast activity, which might cause stiffer components and subsequently lead to better longitudinal and lateral force transmission. Apart from that, the

interaction of the *COL1A1* SNPs with parameters of the *in vitro* study further indicates that that the collagen SNPs do not only effect the function of the tendon, but also of the muscle ECM. However, the X-allele of the nonsense *ACTN3* R577X SNP showed a higher myoblast to fibroblast ratio *in vitro* and a non-significant tendency for a greater strength loss following the EIMD-intervention (unadjusted P = 0.022, data not shown) compared to R-allele homozygotes, supporting the literature that the X-allele is associated with an increased susceptibility to muscle damage (Seto et al., 2011, Broos et al., 2018). The absence of the stiff α-actinin-3 in fast-twitch fibres (Broos et al., 2012, North et al., 1999) might decrease the stability and rigidity of type II fibres of the X-allele carrier. Intriguingly, others found increased fibrosis and strength loss of mice with overexpression of *ACTN3* after post-natal gene editing (Garton et al., 2018a), which is in line with our results of a higher ratio of fibroblast to myoblasts in RR carriers *in vitro*. It needs further investigation whether this mirrors a lower amount of a subpopulation of muscle stem cells, which are associated with slow twitch fibres type I (Biressi and Rando, 2010). That might give a mechanistically explanation of a lower capability for an increase of muscle mass in these slow twitch fibres of R-allele carrier and, therefore, an attenuated increase of muscle strength following chronic resistance training in untrained individuals (Clarkson et al., 2005a).

Nonetheless, the polygenic profile could not detect strength loss following the EIMD-intervention indicating that the investigated SNPs might not have covered those linked genes, which encoded proteins are involved in the MVC change after the EIMD-intervention. The *MMP3* (rs679620) SNP was the only individual SNP, which demonstrated an interaction with MVC measurements. The preferential allele of *MMP3* SNP might increase the enzyme activity to degrade proteins (such as collagen type III within the perimysium) leading to a higher turnover of ECM proteins. Together with other enzyme-related SNPs such as *TRIM63* (rs2275950), potentially this mechanism results in an increased MTU integrity and a better force transmission in the long term. However, the interaction effect of the *MMP3* SNP was masked when the SNP was combined with the six other SNPs in the polygenic profile.

Recent investigations reported an important role of the ECM regarding muscle injuries and it seems that appropriate damage to the perimysium differentiates between EIMD and muscle strains (Balius et al., 2018, Mueller-Wohlfahrt et al., 2012). We therefore hypothesise that EIMD-interventions induced by an IKD with low-speed eccentric MVC are potentially not able to cause substantial damage to the stiff components of the perimysium (such as the collagen type I fibrils), and that the MVC strength loss might be more associated with damage to structural components of the myofibrillar apparatus, particularly those involved in the longitudinal force transmission (e.g. titin and nebulin) (Trappe et al., 2002). In contrast, repeated high external forces involved with high-speed contractions, such as sprinting might have a greater risk of damage to components of the perimysium (Chapter 3) compared to the current EIMD-intervention with low eccentric MVCs. Insufficient recovery of previously damaged muscle fibres might increase the risk of muscle strains (potentially caused primarily by damage to the perimysium) on a clinically relevant scale (Malone et al., 2016, Chumanov et al., 2011, Duhig et al., 2016).

The different phenotypic outcomes of the different interventions and of the SNP clusters might contribute to a deeper understanding of the mechanism of EIMD. The endo- and epimysium might have an important function to store and release elastic energy and to protect the remaining MTU from overstretching. SNPs which keep the endo- and epimysium elastic, might attenuate damage within the MTU. Other SNPs associated with the activity/function of enzymes, which are linked with the EIMD response, might support the recovery response (and potentially the maintenance of muscle quality in the long term). The stiff components of the MTU (e.g. of the perimysium) transmit force and simultaneously are the major contributor to the passive force of skeletal muscles at high strain (Gillies and Lieber, 2011). SNPs associated with the integrity of these stiff components might change the EIMD/muscle strain injury risk.

5.5.4 Limitations

We acknowledge that we have recruited both male and female participants and there is some limited evidence that men and women respond differently to eccentric exercise (Sewright et al., 2008). However, the majority of studies attempting to address this question have shown no sex differences in in the susceptibility to ultrastructural muscle damage (Sayers and Clarkson, 2001, Thompson et al., 1997, Stupka et al., 2001, Wüst et al., 2008). Furthermore, although our male participants were stronger than our female subjects in both absolute and relative (to body mass) terms, when the change in MVC at time points POST and 48POST was reported as a percentage change from baseline MVC, we observed no difference in strength loss between men and women. We, therefore, used absolute strength normalised to body mass with the covariate sex, so we were able to analyse strength differences between genotype/allele for each SNP. Moreover, each of our three polygenic groups contained a similar ratio of men to women, so it is highly unlikely that any sex differences influenced the outcome of our results. Finally, serum IL-6 levels did not increase significantly over time following the muscle damage intervention. Peak serum IL-6 values were probably not observed as the IL-6 is expected to peak between 6 and 24 hours after an eccentric-exercise intervention (Willoughby et al., 2003). Therefore, our study may have benefitted from additional time points for blood analyses to acquire a more comprehensive and accurate representation of the time course of the serum IL-6 response to our EIMD intervention.

5.6 CONCLUSION/ PERSPECTIVE

A fundamental challenge for exercise-related science is to determine the high inter-individual variability in the response to exercise for the development of inexpensive individualised health management to prevent injuries and diseases. Our study suggests that seven SNPs in seven different genes, both on an individual and combined (polygenic) basis, can anticipate the response to muscle-damaging exercises regarding different EIMD-

biomarkers (i.e. muscle soreness and ROM). The most striking outcome was that individuals possessing a non-preferential genetic profile concerning muscle changes in muscle soreness and ROM were also generally weaker than individuals with a preferential genetic profile, which is a potentially important clinical finding. Further investigations are necessary to determine whether NPGs (i) have an increased likelihood to sustain a muscle injury; (ii) also reveal an attenuated response to chronic resistance training, indicating where the anabolic signaling pathway may be impaired; (iii) might show change in the activation of the catabolic signaling pathway; and (iv) whether these differences in strength between the polygenic groups are further amplified with ageing, where size, strength and quality of the muscle and of the muscle ECM are diminished and the negative response to EIMD appears to be augmented (Jiménez-Jiménez et al., 2008, Manfredi et al., 1991). This knowledge will potentially help to develop personalised health management by providing the most suitable and effective activity plan specific to an individual as personalised exercise medicine becomes more widely accepted as an inexpensive health intervention to prevent injury and disease and maintain/improve physical performance in young and older individuals.

6 A common Polygenic Profile Linking the Acute and Chronic Responses to Resistance Exercise

It is noted that the neuromuscular physiology data (pre/post training) of the Genetics of RESIStance Training (G-ResisT) cohort have been published previously (Erskine et al., 2010a; 2010b; 2011; 2014), and constituted part of Dr Robert Erskine's PhD Thesis (2010). To investigate whether the genetic profile identified in Chapter 5 was also associated with the adaptations to chronic resistance training, the DNA samples from G-ResisT were re-analysed for the SNPs investigated in Chapter 5. .

6.1 ABSTRACT

Introduction The study aims were to investigate the hypotheses that (i) a polygenic profile (total genotype score, TGS) comprising a combination of candidate single-nucleotide polymorphisms (SNPs) previously associated with the response to *acute* resistance exercise (RE) could anticipate high and low responders to *chronic* RE; and (ii) a new TGS that demonstrate an interaction with *chronic* RE could estimate the response to *acute* RE.

Methods In cohort one (G-REX study, Chapter 5: 65 young, untrained men and women), maximal quadriceps strength, serum creatine kinase (CK) and interleukin-6 (IL-6) were measured before, after and 48 h after *acute* RE. In cohort two (51 young, untrained men), quadriceps muscle strength and size were assessed before and after nine weeks of *chronic* RE. Participants were genotyped for 20 candidate SNPs and TGSs were determined according to the response/adaptation to *acute* (TGS-A) and *chronic* (TGS-C) RE.

Results TGS-A was not associated with any adaptations to *chronic* RE. However, TGS-C was associated with changes in strength but not muscle size following *chronic* RE. TGS-C was also inversely associated with increases in serum CK and IL-6 following *acute* RE.

Conclusion A specific combination of genotypes appears to limit the damage/inflammatory response to *acute* RE, while also maximising strength gains following *chronic* RE. This offers a novel insight into the physiological mechanisms underpinning and linking the *acute* and *chronic* responses to RE. After independent replication, TGS-A and TGS-C may be used to anticipate an individual's response/adaptation to *acute/chronic* RE, thus enabling RE to be prescribed on a personalised level.

Keywords: resistance training; genetic variations; creatine kinase (CK); interleukin-6 (IL-6); exercise-induced muscle damage (EIMD); extracellular matrix (ECM)

6.2 INTRODUCTION

An acute bout of unaccustomed resistance exercise (RE) that involves muscle-lengthening (eccentric) maximal voluntary contractions (MVCs) can induce muscle damage (Clarkson and Hubal, 2002). The exercise-induced muscle damage (EIMD) is reflected by morphological changes, such as Z-line disturbance, an elevated inflammatory response including the release of muscle-specific proteins into the circulatory system [e.g. creatine kinase (CK) activity, and interleukin-6 (IL-6) concentration], and prolonged MVC loss (Tidball, 1991, Garg and Boppart, 2016). In Chapter 3, we showed that a greater muscle physiological cross-sectional area (PCSA), which is a measure of the total area of muscle fibres perpendicular to their long axes (and is therefore the main determinant of maximum force capacity), protects against immediate MVC loss following acute RE. Chronic RE, i.e. resistance training, is a potent stimulus for increasing both muscle PCSA and strength (Harries et al., 2012, Granacher et al., 2016, Erskine et al., 2010b), therefore, there may be a relationship between the change in muscle PCSA with chronic RE and the ability to resist the damaging effect of an acute bout of RE.

However, there is a large inter-individual variability in the response to acute RE (Nosaka and Clarkson, 1996, Damas et al., 2016a, Baumert et al., 2016a, Baumert et al., 2016b) as well as to chronic RE (Erskine et al., 2010a, Hubal et al., 2005), and recent investigations suggest that common genetic variations, e.g. single-nucleotide polymorphisms (SNPs), may play a role in explaining this variability (Harmon et al., 2010, Pistilli et al., 2008, Baumert et al., 2017, Baumert et al., 2018). In Chapter 5, we demonstrated that a combination of candidate SNPs (that each showed an interaction with the response to acute RE), can identify high and low responders to EIMD. Thus, by calculating a “total genotype score” (TGS) (Williams and Folland, 2008) based on the preferable/non-preferable genotype of each significantly associated SNP, we discovered that a polygenic profile (TGS-A) was associated with the variable response to acute RE. Participants with a non-preferable genetic profile (NPG), i.e. individuals with a low TGS-A, showed higher muscle soreness and decreased range of motion (ROM) following acute RE, and were generally weaker

compared to the preferable polygenic (PG) group. It has been suggested that increased muscle soreness following an acute bout of unaccustomed RE is linked to an elevated inflammatory response (Kanda et al., 2013), and that an elevated inflammatory response with chronic RE might be detrimental for muscle hypertrophy and strength gains (Mayhew et al., 2009). However, it is not known whether TGS-A NPGs with their genetic predisposition to an elevated inflammation following acute RE (Chapter 5) also demonstrate an attenuated adaptation to chronic RE in terms of gains in muscle strength and muscle size.

Muscle force is predominantly determined by its PCSA (Powell et al., 1984, Close, 1972), and it is thought that the increase in muscle PCSA following chronic RE is the result of cumulative increases in myofibrillar muscle protein synthesis in response to each successive bout of RE (Moore et al. 2009b; Brook et al. 2015). However, there is a disproportionate increase in muscle strength and size following chronic RE (Erskine et al., 2014a, Erskine et al., 2010b) and several SNPs have been linked with either chronic changes in muscle hypertrophy or muscle strength (Li et al., 2014, Harmon et al., 2010). Only a few investigations have revealed an interaction between certain SNPs and gains in both muscle mass and muscle strength, i.e. of the muscle-derived cytokine interleukin-15 (Pistilli et al., 2008) and insulin-like growth factor 1 (Kostek et al., 2010) . Further, in recent years, the extracellular matrix (ECM) within the muscle-tendon unit (MTU) gained attention for its important role in providing a structural scaffold for muscle remodelling (Mackey et al., 2017, Murphy et al., 2011) and in transmitting force laterally via the perimysium (surrounding muscle fascicles) to the tendon and bone (Ramaswamy et al., 2011). However, it is unknown whether certain SNPs might be associated with muscle ECM function and whether this might affect the response to chronic RE.

Therefore, the purpose of the current study was to test the hypotheses that (i) NPGs, i.e. those with a low TGS-A, which demonstrated greater muscle damage and a slower recovery rate with respect to the response following acute RE (Chapter 5), would also show an attenuated adaptation to chronic RE; or (ii) whether a different TGS (TGS-C) would be

associated with the neuromuscular adaptations to chronic RE; and (iii) if this TGS-C would be associated with the response to acute RE. To investigate these aims, we included the following two independent exercise cohorts. The Genetics of Recovery after EXercise (G-REX) cohort was included to compare the polygenic association with the effects of acute RE (Baumert et al., 2017, Baumert et al., 2018) with the adaptations to 9-weeks' chronic RE in a separate [Genetics of Resistance Training (G-ResisT)] cohort (Erskine et al., 2010b).

6.3 METHODS

6.3.1 General

Data from 116 young, healthy untrained Caucasians were used in the present study (Table 6-1). Men and women were recruited in the Genetics of Recovery after EXercise (G-REX) (Chapter 5), whilst only men participated in the Genetics of RESIStance Training (G-ResisT, data collection 2007 - 2009) cohort (Erskine et al., 2012).

Table 6-1 *Participant Characteristics of the two cohorts (mean ± SD).*

Cohort	Sex	Total (n)	Age (years)	Height (m)	Body mass (kg)
G-REX	Females	39	22.3 ± 4.06	1.66 ± 0.07	65.5 ± 12.4
	Males	26	22.8 ± 4.07	1.78 ± 0.07	78.6 ± 13.6
G-ResisT	Males	51	20.3 ± 3.13	1.78 ± 0.06	75.4 ± 10.6

Written informed consent was obtained from each participant prior to starting the study, which complied with the Declaration of Helsinki and was approved by the responsible local committees (the Research Ethics Committee of Liverpool John Moores University for the G-REX, and the local ethics committee of the Manchester Metropolitan University for the G-ResisT cohort).

Participants were familiarised with all test procedures and equipment before the baseline measurements and participant inclusion criteria for the three cohorts comprised: (i) age between the range of 18–35 yr (18–39 yr for G-ResisT), (ii) no history of strength training 6 months (12 months for the G-ResisT cohort) prior to the study, as determined via physical activity questionnaire and interview; (iii) no history of lower extremity musculoskeletal injuries 12 months prior to the study, as assessed via interview; (iv) no use of potentially anabolic supplements; and (v) considered not to be in ill health (determined by their responses to a health questionnaire).

6.3.2 G-REX Cohort

Please see Chapter 5 for a detailed methodology regarding these measurements in the G-REX cohort. In brief, the G-REX separate cohort of 65 untrained young men and women (G-REX cohort) performed maximum voluntary isometric and isokinetic knee extension contractions (MVCs) on an isokinetic dynamometer before (PRE), immediately after (POST) and 48 h after (48POST) performing 120 eccentric knee extension MVCs. Further, at each time point, a 10 mL venous blood sample was taken from a superficial forearm vein to measure serum interleukin-6 (IL-6) concentration, creatine kinase (CK) activity, and to determine genotype of 20 SNPs.

6.3.3 G-ResisT Cohort

6.3.3.1 Experimental Design

Maximum patellar tendon force, quadriceps femoris muscle volume, physiological cross-sectional area (PCSA), and specific force were determined in the right limb (as described in method 2 of Ref. 8) before and after 9 wk of high-intensity unilateral knee extension RE (Erskine et al., 2010b) in 51 previously untrained young healthy men (G-ResisT, a separate cohort to G-REX). In addition, all participants had blood samples isolated, which were genotyped for all 20 candidate SNPs (see below).

6.3.3.2 Progressive Resistance Exercise

The supervised chronic RE protocol has been described in detail elsewhere (Erskine et al., 2010b). In summary. The chronic RE intervention comprised a supervised unilaterally knee extension for three times per week for 9 wk. At the beginning of the training programme, the maximum training load that could be lifted once only (1-RM) throughout the full range of knee extension (110° to 20° of knee flexion; 0° full knee extension) was assessed. The 1-RM was re-evaluated at the start of each week on a standard knee extension machine (Technogym, Gambettola, Italy) and, therefore, the training intensity progressively increased throughout the 9 wk of training, as the training intensity was adjusted in relation to the 1-RM. Each training session consisted of a warm-up set of 10 knee extension repetitions (40% of 1-RM), which was followed by four sets (2 min rest between each) of 10 repetitions (80% of 1-RM). Each participant completed all 27 training sessions and, therefore, the compliance with the training protocol was 100%.

6.3.3.3 Maximum Isometric Patellar Tendon Force

The method used to assess maximum patellar tendon force has been explained in detail elsewhere (Erskine et al., 2009). In brief, participants performed isometric knee extension maximal voluntary contractions (MVCs) on an isokinetic dynamometer (Cybex Norm, Cybex International, Ronkonkoma, NY) at optimum knee strength angle (optimal force-length relationship), which ranged from 70 to 90° knee flexion (0º = full knee extension). Participants were seated in an upright position and securely fastened with inextensible straps to the strength-testing chair. The hip joint angle was set to 85° (180° = supine position). Surface electromyographic (sEMG) activity was recorded from the biceps femoris long head to determine the extent of antagonist muscle co-activation during MVCs of the respective muscle group (Reeves et al., 2004a). Two bipolar silver chloride surface electrodes (Neuroline, Medicotest, Rugmarken, Denmark) were placed 20 mm apart along the sagittal axis over the muscle belly (the location was recorded on an acetate for further

tests) and one reference electrode was positioned over the lateral tibial condyle. The root mean square of the raw EMG signal was calculated over 1 s around the peak torque during each maximum voluntary isometric knee extension and flexion at optimum joint angle and the torque produced by the hamstrings during knee extension was estimated assuming a linear relationship between torque and EMG activity (Reeves et al., 2004a). The estimated antagonist torque was obtained at the optimum knee extension joint angle and then used to calculate the maximum overall knee extension torque. To measure voluntary quadriceps femoris muscle activation capacity via the interpolated twitch technique (Rutherford et al., 1986), the participant received a supramaximal twitch (Digitimer stimulator model DS7, Welwyn Garden City, UK) via two (7.5 cm 12.5 cm) self-adhesive electrodes (Versastim, Conmed, New York, NY), which were placed distally (anode) and proximally (cathode) over the quadriceps femoris muscle, once before MVC (control twitch) and once during MVC. True maximum torque was calculated as

$$\text{True maximum torque} = \frac{\text{MVC(C)}}{1 - t/\text{T}}$$

where *t* is the amplitude of the superimposed twitch, T is the value of the twitch before the MVC, and MVC(C) is MVC corrected for antagonist muscle co-activation.

The patellar tendon moment arm (d_{PT}) was determined using a 0.2-T magnetic resonance imaging (MRI) scanner (G-Scan, Esaote Biomedica, Genoa, Italy), as previously described (Tsaopoulos et al., 2006). Sagittal and coronal-plane knee scans were acquired using a Turbo 3D T1-weighted sequence (scanning parameters: time of repetition 40 ms; time to echo 16 ms; matrix 256 x 256; field of view 180 mm x 180 mm; slice thickness 3.4 mm; interslice gap 0 mm). The participant was in the supine position and the knee fully extended, when the knee was scanned at rest. Coronal scans were used to identify the appropriate sagittal scans, which were used to locate the centre of rotation, i.e., the midpoint of the shortest distance between the two femoral condyles and the tibial plateau, and d_{PT} was defined as the perpendicular distance between the centre of rotation and the axis of the patellar tendon (Tsaopoulos et al., 2006). Previously reported ratios of d_{PT} at full extension (0°knee flexion) to d_{PT} at 70°, 80°, and 90° knee flexion (Baltzopoulos, 1995) were used to

calculate d_{PT} at optimum knee joint angle in this study. Subsequently, maximum force resolved at the patellar tendon was calculated as

$$\text{Maximum force resolved at the patellar tendon} = \frac{\text{True maximum torque}}{d\text{PT}}$$

6.3.3.4 Muscle Physiological Cross-sectional Area (PCSA)

Quadriceps femoris muscle PCSA was determined from a method that has been described in detail previously [method 2 of (Erskine et al., 2009)]. Briefly, femur length, defined as the distance from the proximal origin of the vastus lateralis muscle to the tibiofemoral contact point, was assessed by ultrasonography (MyLab25, Esaote Biomedica, Genoa, Italy). The anatomical cross-sectional area was analysed of each of the four component quadriceps femoris heads via transverse MRI scans (at 40% femur length from the distal end). Quadriceps femoris muscle volume was then calculated by adapting a previously described method (Morse et al., 2007) that incorporated femur length, the anatomical cross-sectional area of each of the four constituent quadriceps femoris heads and a series of regression equations. Vastus lateralis muscle fascicle length and pennation angle were measured during knee extension MVC at optimum knee angle using ultrasonography at 50% of the muscle length along the midsagittal plane. Dividing quadriceps femoris muscle volume by vastus lateralis muscle fascicle length provided quadriceps femoris PCSA, as vastus lateralis fascicle length has been shown to be representative of the fascicle length for the whole quadriceps femoris muscle group (Erskine et al., 2009).

6.3.3.5 In vivo Muscle Specific Force

The method used to assess *in vivo* muscle specific force has been explained in detail elsewhere (Erskine et al., 2009). Briefly, *in vivo* specific force was determined by dividing maximum force resolved at the patellar tendon by the reduced quadriceps femoris PCSA, because quadriceps femoris muscle force is reduced when resolved along the patellar tendon according to the pennation angle. Therefore, quadriceps femoris PCSA was

multiplied by the cosine of vastus lateralis pennation angle, which provided the reduced quadriceps femoris PCSA.

6.3.3.6 In vivo Peak Power Output

The method used to determine *in vivo* peak power output (W_{peak}) normalised to quadriceps femoris muscle volume (W_{peak}/muscle volume) has been described in detail elsewhere (Erskine et al., 2011). Briefly, W_{peak} of the lower right limb was assessed using a modified isokinetic cycle ergometer (Lode Standard, Groningen, The Netherlands), driven by a 2.24 kW motor through a variable-speed gearbox and with strain gauges within the pedals registering the forces at right angles to the top surface of the pedal (Sargeant et al., 1981, Beelen et al., 1994). The test session started with a 5 min warm-up at a pedal frequency of 90 r.p.m. and with the pedals connected to an external load of 60W. The external cranks were then connected to the electric motor, the external load set to 0W, and the participant performed isokinetic sprints at five predetermined, randomly assigned pedal frequencies (50, 70, 90, 110 and 130 r.p.m.), where the participant attempted (unsuccessfully) to accelerate the system by exerting maximal force during each revolution. Each sprint lasted for 6 s and was separated by a 5 min resting period. Although participants performed a two-legged sprint, W_{peak} was calculated in the right (trained) limb only. The calculation of W_{peak} was adapted by a previously described (Beelen et al., 1994) and W_{peak} was calculated according to the following equation:

$$W_{peak}\ (\text{in watts}) = \text{peak tangential force} \times \text{tangential velocity}$$

Peak tangential force and tangential velocity values were previously calculated by different equations incorporating the pennation angle of the pedal as well as both, the vertical and horizontal force and velocity, respectively.

6.3.3.7 Blood Sampling

A 10-ml blood sample was drawn into 10-ml EDTA (for genotyping) or serum (for IL-6/CK analysis in the G-REX cohort only) collection tubes (BD Vacutainer Systems, Plymouth, UK) from a superficial forearm vein. The whole blood/serum was aliquotted into 2-ml tubes (Eppendorf AG, Hamburg, Germany) and stored at -80°C until subsequent analysis.

6.3.3.8 DNA Extraction and Determination of Genotypes

DNA extraction was performed either manually (for the G-REX cohort) or with a QIAcube (Qiagen, Crawley, UK; for the G-ResisT cohort) using the QIAamp DNA Blood Kit (Qiagen, Crawley, UK), following the QIAamp spin protocol for DNA purification from whole blood. Genotyping was performed at the Liverpool John Moores University as described previously (Baumert et al., 2018, please see Chapter V). All samples, plus positive and negative controls were analysed in duplicate and there was 100% agreement between genotype for samples from the same participant.

6.3.3.9 Total Genotype Score (TGS) Calculation

Using a two-level approach to calculate a TGS (Williams and Folland, 2008), the 20 SNPs we investigated in Chapter 5 (*ACTN3* C>T, rs1815739; *AGT* C>T, rs699; *CCL2* G>C; *COL1A1* G>T rs1800012; *COL1A1* T>C, rs2249492; *COL2A1* G>A, rs2070739; *COL5A1* T>C, rs12722; *DES* G>C, rs12621188; *IGF2-AS* A>C, rs4244808; *IL6* C>G, 1800796; *MMP3* G>A, rs679620; *MYLK1* G>A, rs2700352; *MYLK1* G>T, rs28497577; *NOS3* T>C, rs2070744; *PAX7* G>A, rs485874; *TNF* T>C rs1799964; *TRIM63* A>G, rs2275950; *TTN-AS1* A>G, rs1001238; *TTN-AS1* G>A, rs3731749; and *VDR* C>T, rs2228570) were investigated for an association with the adaptations to chronic RE on an individual basis, while controlling for multiple comparisons. Heterozygote genotypes were pooled with one of the two homozygotes that showed a similar pattern except for SNPs possessing rare homozygotes n ≤ 2 (*COL1A1*, rs1800012; *COL2A1*, rs2070739; *MYLK1*, rs28497577; *MYLK1*, rs2700352), in which case a recessive model was used. Single nucleotide

polymorphisms that showed an interaction with response particular adaptation to the chronic RE intervention following the application of a 20% false discovery rate (FDR) (Benjamini and Hochberg, 1995), were then used to calculate the different TGS (TGS-A, associated with the response to acute RE and TGS-C, associated with the adaptation to chronic RE) models (Williams and Folland, 2008). Each genotype from each SNP was, therefore, given a score between 0 and 2 based on the response to the RE intervention. For each SNP, the homozygote genotype that showed a beneficial effect was given a score of 2 and the homozygote genotype associated with a detrimental effect was given a score of 0. The heterozygote genotype received a score of 1. Combining the scores of each significant SNP gave a total genotype score within a range between 0 – 100, with the best possible polygenic genotype score defined as 100, and the worst possible as 0. The contributions of the different TGSs to the inter-individual variance in the chronic RE-induced change in maximum patellar tendon force, muscle specific force and in W_{peak} were determined using linear regression analysis. As in Chapter 5, three groups were defined according to the $\bar{x}$ of the TGS ± 1SD, i.e. non-preferential genotypes (NPG) < $\bar{x}$ -1SD; moderate genotypes (MG) = $\bar{x}$-1SD to $\bar{x}$ +1SD; and preferential genotypes (PG) ≥ $\bar{x}$+1SD.

6.3.3.10 Data Analysis

Each SNP was tested for compliance with the Hardy-Weinberg equilibrium by using a χ^2 test. All parameters were normally distributed according to the Shapiro-Wilk test and by inspection of the Q-Q plots. Linkage disequilibrium (LD) was analysed via LDlink suite and data from the 1000 Genomes Project European ancestry populations (Machiela and Chanock, 2015, Consortium, 2012). Recent investigations revealed that pruning for LD regarding polygenic approaches does not essentially change the results (Vrieze et al., 2013). Therefore, SNPs with LD were still included in the TGS. Repeated-measures analysis of variance (ANOVAs) [within-subjects factor: time (pre- and post-chronic RE); between-subjects factor: genotype groups (2 group levels)] were used to detect associations between each SNP and 1-RM, isometric MVC knee joint torque, maximum

patellar tendon force, W_{peak}, quadriceps femoris muscle PCSA and muscle specific force before and after training. For post hoc analyses, either, independent t-tests, or one-way ANOVAs were used where appropriate. Standard guidelines concerning violation of the sphericity assumption to adjust the degree of freedom of the F-test by the Huynh-Felt epsilon if epsilon is greater than 0.75 and to use the more stringent Greenhouse-Geisser adjustment if epsilon is less than 0.75 were followed (Girden, 1992). 1-RM, MVC knee joint torque, and maximum patellar tendon force were normalised to body mass, unless otherwise stated, and analysed with AcqKnowledge software 4.4 (Biopac-Systems Inc., Goleta, USA). Muscle fascicle length, quadriceps femoris muscle volume and PCSA were normalised to femur length; and W_{peak} was normalised with quadriceps femoris volume.

6.4 RESULTS

6.4.1 Hardy-Weinberg Equilibrium and Linkage Disequilibrium

The genotypes of all 20 SNPs were in Hardy-Weinberg equilibrium, except for *COL2A1* rs2070739 (X^2=6.04, P=0.014) and *PAX7* rs485874 (X^2=5.15, P=0.023) in the G-REX cohort. Linkage disequilibrium calculations revealed that the following SNPs were in LD: both *COL1A1* (rs1800012 and rs2249492) SNPs (D`=0.736 and $R^2$=0.077, P<0.001); *MMP3* (rs679620) and *ACTN3* (rs1815739) SNPs (D`=0.068 and R^2=0.004, P=0.046); both *MYLK1* (rs2700352 and rs28497577) SNPs (D`=0.482 and $R^2$=0.08, P<0.001); *PAX7* (rs485874) and *TRIM63* (rs2275950) SNPs (D`=0.134 and R^2=0.004, P=0.004); and both *TTN-AS1* (rs1001238 and rs3731749) SNPs (D`=1.0 and R^2=0.472, P<0.001).

6.4.2 The Association of the Individual SNPs with the Response to Chronic Resistance Exercise

From the 20 SNPs analysed with respect to the G-ResisT cohort, eight displayed significant interactions with time (pre to post chronic RE) after correction for multiple testing (*Table*

6-2). Five of these SNPs were associated with the change in muscle specific force (*ACTN3,* rs1815739; *CCL2*, rs2857656; *COL1A1*, rs1800012; *COL1A1*, rs2249492; and *VDR,* rs2228570) and three SNPs were associated with the change in W_{peak} (*AGT*, rs699; *MYLK1*, rs2700352; and *TNF,* rs1799964).

Table 6-2 SNP interaction with chronic resistance exercise following correction for multiple comparisons.

			Group Response (n)			
Symbol	**rs-number**	**Interaction**	**Beneficial**	**Detrimental**	**F-Test**	**P Value**
ACTN3	rs1815739	Specific force	CT+TT (31)	CC (20)	$F_{1,49}$ = 5.50	0.023
AGT	rs699	W_{peak}	CC+CT (27)	TT (13)	$F_{1,38}$ = 5.07	0.030
CCL2	rs2857656	Specific force	CC (6)	GG+GC (45)	$F_{1,49}$ = 7.86	0.007
COL1A1	rs1800012	Specific force	GT+TT (20)	GG (31)	$F_{1,49}$ = 4.68	0.035
COL1A1	rs2249492	Specific force	CC (4)	TT+TC (47)	$F_{1,49}$ = 7.67	0.008
MYLK1	rs2700352	W_{peak}	AA+GA (19)	GG (21)	$F_{1,38}$ = 9.99	0.003
VDR	rs2228570	Specific force	CC+CT (43)	TT (8)	$F_{1,49}$ = 4.96	0.031
TNF	rs1799964	W_{peak}	TT (20)	CC+TC (20)	$F_{1,38}$ = 5.59	0.023

In addition to the interactions reported in *Table 6-2*, there were also main effects (*Table 6-3*) regarding the following SNPs for patellar tendon force (*TTN-AS1,* rs1001238; *TTN-AS1*, rs3731749), for fascicle length (*CCL2,* rs2857656; *COL5A1,* rs12722, and *TNF,* rs1799964), for PCSA (*CCL2,* rs2857656), and for specific force (*CCL2,* rs2857656). The following nine SNPs were not significantly associated (neither interaction nor main effect) with any outcome variable of the G-ResisT cohort after correction for multiple testing: *COL2A1* (rs2070739), *DES* (rs12621188), *IGF2-AS* (rs4244808), *IL6* (rs1800796), *MYLK1*

(rs28497577), *NOS3* (rs2070744), *MMP3* (rs679620), *PAX7* (rs485874), and *TRIM63*, rs2275950.

Table 6-3 Main effect for genotype regarding chronic resistance exercise following correction for multiple comparisons.

			Group Response (n)			
Symbol	**rs-number**	**Main Effect**	**Beneficial**	**Detrimental**	**F-Test**	**P Value**
CCL2	rs2857656	fascicle length	CC (6)	GG+GC (43)	$F_{1,47}$ = 15.97	2.25 x10^{-4}
		PCSA	GG+GC (45)	CC (6)	$F_{1,49}$ = 8.37	0.006
		specific force	CC (6)	GG+GC (45)	$F_{1,49}$ = 9.22	0.004
COL5A1	rs12722	fascicle length	TT (16)	CC+CT (33)	F_{1472} = 5.74	0.021
TNF	rs1799964	fascicle length	TT (26)	CC+CT (23)	$F_{1,47}$ = 5.47	0.024
TTN-AS1	rs1001238	patellar tendon force	GG+GA (22)	AA (29)	$F_{1,49}$ = 7.14	0.010
TTN-AS1	rs3731749	patellar tendon force	AA+AG (10)	GG (41)	$F_{1,49}$ = 10.18	0.002

6.4.3 The Correlation between TGS-A and the Response to Chronic Resistance Exercise

There was no correlation between TGS-A and changes in 1-RM, MVC knee joint torque, maximum patellar tendon force, quadriceps muscle specific force, muscle fascicle length, quadriceps femoris muscle volume, PCSA or W_{peak} following chronic RE. Two SNPs, which showed a beneficial outcome for the one allele [GG homozygotes for *TTN-AS1* (rs3731749) regarding muscle soreness; GG homozygotes for *COL1A1* (rs1800012) regarding MVC torque] following acute RE, demonstrated a detrimental association with the change in

patellar tendon force (*TTN-AS1,* rs3731749) and muscle specific force (*COL1A1,* rs3731749) following chronic RE .

6.4.4 A new Polygenic Profile (TGS-C) to Anticipate the Response to Chronic Resistance Exercise

Secondly, we calculated the TGS-C, which included all eight SNPs (*ACTN3,* rs1815739; *AGT*, rs699; *CCL2*, rs2857656; *COL1A1*, rs1800012; *COL1A1*, rs2249492; *MYLK1*, rs2700352; *TNF,* rs1799964; and *VDR,* rs2228570) that showed an individual interaction with the chronic RE intervention following correction for multiple comparisons. There was a linear correlation between TGS-C and % change in maximum patellar tendon force (Figure 6-1 A). Participants with a higher TGS-C demonstrated a greater increase in force compared to participants with a low TGS (R^2=0.08, F=4.53, P=0.038) after chronic RE. TGS-C also correlated positively with % change in muscle specific force (Figure 6-1 B), i.e. the higher the TGS, the greater the increase in muscle specific force (R^2=0.10, F=5.31, P=0.025) after chronic RE.

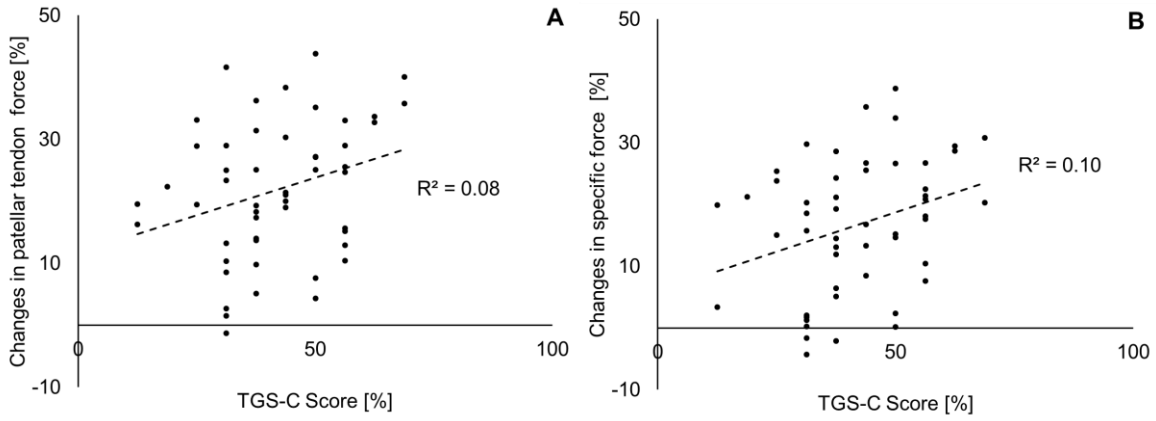

Figure 6-1 *Correlation between the polygenic profile TGS–C and maximum patellar tendon force (A) and muscle specific force (B).*

6.4.5 New Polygenic Profiles Regarding Chronic RE Estimate the Individual Response to Acute RE

Lastly, we investigated the association between the TGS-C groups and the changes in various parameters following acute RE (Chapter 5). Regarding the G-REX cohort, there was an interaction between TGS-C and time regarding serum CK activity ($F_{4,68}$=7.51, P=4.3*10^{-5}Figure 6-2 A). The serum CK activity for NPG was higher POST [for PG (n=7): 17.2 ± 13.7 mU*mL^{-1}; MG (n=29): 16.4 ± 27.1 mU*mL^{-1}; NPG (n=2): 130.3 ± 174.6 mU*mL^{-1}] as well as POST48 [for PG (n=7): 27.7 ± 26.0 mU*mL^{-1}; MG (n=29): 24.6 ± 21.0 mU*mL^{-1}; NPG (n=2): 125.8 ± 153.9 mU*mL^{-1}] compared to MG and PG. There was an interaction between TGS-C and time regarding serum IL-6 concentration ($F_{4,68}$=7.22, P=6.6*10^{-5}; Figure 6-2 B). Serum IL-6 concentration was higher for NPG [(n=2): 13.1 ± 12.9 pg*mL^{-1}] POST compared to MG [(n=29): 1.84 ± 2.46 pg*mL^{-1}] and PG [(n=7): 1.18 ± 0.63 pg*mL^{-1}].

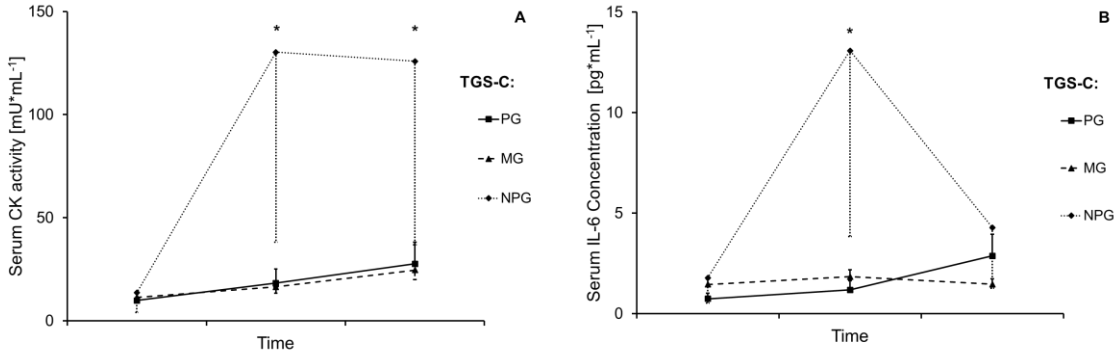

Figure 6-2 *Interaction effect for serum CK activity (of the G-REX cohort) and time regarding the polygenic profile TGS-C (A), Interaction effect for serum IL-6 concentration (of the G-REX cohort) and the polygenic profile TGS-C (B); PG – Preferential Genotype Group; MG – Moderate Genotype Group; NPG – Non-Preferential Genotype Group. * NPG is significant different compared to MG and PG, P<0.05, mean ± SEM.*

6.5 DISCUSSION

The aim of the current study was to investigate whether (i) NPGs of TGS-A, that was previously associated with biomarkers of EIMD and a slower recovery rate following acute RE (Chapter 5), would be associated with an attenuated adaptation to chronic RE; (ii) NPGs of a new TGS (TGS-C), calculated with respect to the individual associations of 20

candidate SNPs with adaptations to chronic RE, would demonstrate attenuated strength and muscle mass gains; and (iii) NPGs of the TGS-C would demonstrate a greater EIMD response to acute RE compared to their PG counterparts. Contrary to our first hypothesis, TGS-A was not related to any chronic RE adaptation. In line with our second hypothesis, however, TGS-C PGs showed greater adaptations to chronic RE compared to NPGs in terms of changes in maximum patellar tendon force and muscle specific force. In accordance with our third hypothesis, NPGs of TGS-C demonstrated a greater inflammatory response (indicated by IL-6 and CK activity) following acute RE.

6.5.1 Using TGS-A to Anticipate the Adaptation to Chronic RE

It is thought that the accumulation of repeated RE leads to an increase in muscle strength and mass. Less is known about whether muscle damage following acute RE triggers a cascade of events that can lead to muscle restructuring, greater strength gains and muscle hypertrophy (Roig et al., 2008). However, we did not find any association between TGS-A and chronic RE. The individual response following acute RE might be predominantly caused by SNPs, which are linked to the elastic components of the MTU (e.g. *COL5A1* rs12722, *TTN-AS1* rs3731749) and to inflammation and protein degradation (e.g. *TRIM63* rs2275950, *MMP3* rs67962). This is in line with recent investigations, which found that acute RE does not correlate well with the long-term effect of chronic RE regarding both RNA expression (Phillips et al., 2013, Damas et al., 2018) and myofibrillar protein synthesis (Damas et al., 2016b). However, there is no investigation that has compared collagen protein synthesis responses following acute RE and chronic RE. It is thought that the elastic capabilities of the MTU are essential to protect the muscle fibres from mechanical strain during eccentric (muscle lengthening) contractions during RE (Gillies and Lieber, 2011, Turrina et al., 2013). However, as the SNPs are not linked to the stiff perimysium, but to other parts of the ECM, they might contribute to the force transmission to a lesser extent and, therefore, might be less relevant for strength gains following chronic RE. SNPs related to protein degradation pathways might be predominantly important for the capability to

repair ultrastructural damage following acute RE (Damas et al., 2016b). Interestingly, a GWAS study regarding the individual strength in a large cohort of UK residents between 40 - 69 yrs demonstrated significant associations with *FBXO32* SNPs (e.g. rs12548263) and a non-significant trend with SNPs of the *TRIM63* gene (e.g. rs3008425 which is in strong LD with rs2275950) (Willems et al., 2017). Both genes are related to the ubiquitin-proteasome pathway, providing evidence of SNPs of these genes contributing to the individual differences in muscle strength over the life span of the general population (Willems et al., 2017). However, protein degradation pathways might be less linked with strength gains following chronic RE (Brook et al., 2016, Phillips et al., 2013, Damas et al., 2018). It needs to be further investigated if this is also true with muscle hypertrophy in humans (Zillikens et al., 2017, Verbrugge et al., 2018).

6.5.2 Using TGS-C to Estimate the Adaptation to Chronic RE

A new polygenic profile (TGS-C), which was based on the adaption to chronic RE, included eight candidate SNPs (*ACTN3,* rs1815739; *AGT*, rs699; *CCL2*, rs2857656; *COL1A1*, rs1800012; *COL1A1*, rs2249492; *MYLK1*, rs2700352; *TNF,* rs1799964; and *VDR,* rs2228570) that showed an individual interaction with the chronic RE intervention. The TGS-C was able to explain 8 % and 10% of the variability in changes of patellar tendon force and specific force following chronic RE, respectively. This is quite significant given that the heritability of maximum muscle strength is 50-70% (De Moor et al., 2007, Georgiades et al., 2017, Thomis et al., 1998). As one candidate SNP is thought to account for only a small proportion of the heritability for complex traits (McCarroll et al., 2008), the data presented including multiple SNPs might be of scientific value to identify larger proportions of the heritability for RE adaptations. Given the challenges in performing these types of comprehensive genotype-phenotype interventions, the current study sheds new light on the complex mechanism underlying the individual response following acute and chronic RE. In comparison, the most comprehensive investigated human trait is stature, which is relatively

easy and quick to assess. About 17 million genetic variations have been implicated in explaining 56% of the variability for human height, for which the heritability is 60–70% (Yang et al., 2015). Further, among the individual SNPs that were associated with changes in strength following chronic RE, there were two distinct clusters.

6.5.2.1 Cluster of SNPs Related to Wpeak

The preferential alleles of three SNPs [C-allele of *AGT (*rs699); A-allele of *MYLK1 (*rs2700352); TT genotype of *TNF* (rs1799964)] showed a beneficial interaction to W_{peak}. The preferential alleles of the myosin light chain kinase 1 (*MYLK1*), which is expressed in skeletal muscle stem cells in the early phase of differentiation (Herring et al., 2000), and of the tumour necrosis factor (*TNF) SNPs* also demonstrated a non-significant trend for generally greater quadriceps muscle volume (unadjusted P=0.031, data not shown), and a generally longer fascicle length in the vastus lateralis, respectively. However, these three SNPs did not show any interaction to acute RE, although the genes linked to the SNPs are directly and indirectly associated with muscle wasting related effects (Brink et al., 2001, Tidball, 2011, Han et al., 2012). Recent investigations demonstrated that muscle stem cell proliferation and myoblast differentiation is positively regulated by *TNF* (Zhan et al., 2007, Yang and Hu, 2018) and by components of the renin–angiotensin system, which is linked to the *AGT gene* (which encodes the precursor protein angiotensinogen) ~~(Yoshida et al., 2014, Johnston et al., 2011, Johnston et al., 2010)~~. Further, *MYLK1* contributes to cellular responses by its scaffolding activity to recruit macromolecular complexes, which might support the remodelling of the cytoskeleton (Khapchaev and Shirinsky, 2016, Wu et al., 2003, Levinson et al., 2004, Gautel, 2011a). Since maximal muscle power is a product of force and contraction velocity, the preferential alleles of these SNPs might affect the expression rate/stability of the corresponding genes resulting in an improved W_{peak} due an increased number of sarcomeres in parallel (thus increasing force) and in series (thus increasing contraction velocity).

6.5.2.2 Cluster of SNPs Related to Muscle Specific Force

In the current study, individuals carrying the preferable alleles [CC genotype of *CCL2* (rs2857656), CC genotype of *COL1A1* (rs2249492), the T-allele of *COL1A1* (rs1800012), the C-allele of *VDR* (rs2228570)] of four SNPs demonstrated an increased muscle specific force following chronic RE, potentially by an enhanced RNA expression (He et al., 2017, Mann et al., 2001, Arai et al., 1997). Collagen type I (the pro-alpha-1 chain of type I collagen is encoded by *COL1A1*) is the predominant protein in the perimysium, which is thought to be involved in lateral force transmission (Ramaswamy et al., 2011), and recent investigations suggest that the *CCL2* (Hara et al., 2013, Moore et al., 2005a) and the *VDR* (Potter et al., 2013) genes also contribute to changes of collagen type I expression. Intriguingly, the non-preferential alleles of the *COL1A1* (G-allele homozygotes, rs1800012) and of the VDR (T-allele, rs2228570) SNPs demonstrate higher baseline muscle strength in untrained cohorts, as seen in the current acute RE and in other studies (Van Pottelbergh et al., 2001, Windelinckx et al., 2007, Hopkinson et al., 2008, Roth et al., 2004). These non-preferential alleles of the *COL1A1* and the VDR SNPs seem to 'protect' against loss of muscle strength in a non-exercising population in the long term, possibly due to a generally lower gene expression and, therefore, lower unfavourable ECM deposition. However, increased gene expression of the preferable alleles of the four SNPs might support the remodelling of the muscle ECM in active individuals, which subsequently lead to a stiffer perimysium of the ECM with better force transmission capabilities. The addition of connective tissue between muscle fibres, especially of the stiff perimysium, might serve to split muscle fibres up into multiple force generating units, thus increasing the muscle specific force, i.e. the force per cross-sectional area of muscle (Jones et al., 1989). Further It is often observed that there is an additional increase of muscle strength compared to muscle size following chronic RE (Jones et al., 2008), which cannot be entirely explained by changes in PCSA, neural adaptation, tendon capability (Erskine et al., 2010b), or change in fibre type composition, or myofibrillar packing after chronic RE (Erskine et al., 2011). The association between these four SNPs and the muscle ECM indicates that the

disproportionate gains in muscle strength and mass could be explained, at least in part, by muscle ECM remodelling following RE.

Further, individuals carrying the minor X-allele of the nonsense *ACTN3* C > T (rs1815739) SNP, which results in a stop codon rather the expression of the amino acid arginine (R-allele) (Nowak et al., 1999), also demonstrated an additionally increase in muscle specific force following chronic RE, and they were generally stronger in the G-REX cohort (Chapter 5). However, the X-allele also showed a non-significant tendency for a greater strength loss following acute RE (Chapter 5) and a non-significant tendency for a generally lower quadriceps femoris muscle volume in the G-ResisT cohort (unadjusted P=0.046, data not shown). Deficiency of α-actinin-3 results in a shift in fast-twitch fibres towards oxidative metabolism due to an enhanced activation of calcineurin (Seto et al., 2013). As untrained XX homozygotes tend to have a skeletal muscle fibre type distribution of more (slow) type I fibres (Ahmetov et al., 2011, Vincent et al., 2007) compared to R-allele carriers, smaller muscles of untrained X-allele carriers might be based on the generally higher ratio of type I (smaller CSA) to type II (greater CSA) muscle fibres (Broos et al., 2016, Gilliver et al., 2009, Vincent et al., 2007). However, calcineurin activity is associated with the increase of CSA predominantly of type I skeletal muscle fibres in humans following muscle overload (Hudson and Price, 2013, Sakuma and Yamaguchi, 2010). An increased calcineurin activity of X-allele carrier, might lead to an enhanced capability to express growth factors (e.g. MyoD) for muscle remodelling (Sakuma and Yamaguchi, 2010, Vincent et al., 2010), and, subsequently, in an elevated CSA of type I fibres following chronic RE. The increased capability of muscle remodelling of X-allele carriers is mirrored by the elevated ratio of muscle stem cells to stem cells of the connective tissue *in vitro* compared to RR homozygotes (Chapter 5), which is in line with other results that found increased fibrosis and strength loss of mice with overexpression of *ACTN3* after post-natal gene editing (Garton et al., 2018a). The lower ratio of muscle stem cells of R-allele carrier might mirror a decreased amount of a subpopulation of muscle stem cells of type I fibres (Biressi and

Rando, 2010), and, therefore might give a mechanistically explanation of a lower capability for an increase of CSA in these slow twitch fibres following chronic RE

6.5.3 Using TGS-C to Anticipate the Response to Acute RE

Lastly, we investigated whether TGS-C could estimate high and low responders to acute RE. TGS-C included all 8 SNPs, which interacted with the response to chronic RE on an individual basis, and was able to anticipate high responders (NPGs) regarding a larger increase in serum IL-6 concentration and CK activity in a separate (G-REX) cohort. It was unexpected that the TGS-C would be associated with serum CK activity and IL-6 concentration following acute RE, as neither serum CK activity nor IL-6 concentration were associated with any individual SNP or with TGS-A in the G-REX cohort (Chapter 5). It seems that a high increase in serum CK activity and IL-6 concentration immediately after acute RE might be associated with attenuated adaptation regarding strength gains. Moreover, this link between an acute inflammatory/damage response and neuromuscular adaptations to chronic RE is linked to the same genetic profile. Thus, we provide evidence that genetic make-up drives the response to acute RE, which influences the longer-term adaptations to chronic RE.

A common polygenic profile that links both the acute response and chronic adaptation to RE allows us to speculate about the molecular mechanisms underpinning these responses/adaptations. The TGS-C consisted of SNPs linked to the muscle ECM and was associated with CK activity response. It has been suggested that the intramuscular enzyme, CK, might leak into the circulation particularly in high responders, when the muscle ECM is disrupted immediately after the exercise (Newham et al., 1983, Baird et al., 2012). Further, CK might also leak into the blood stream in the days after EIMD, when activated satellite cells with fibroblasts interact to dissolve and reorganise the muscle ECM with the help of the proteolytic activity of matrix metalloproteinases (MMPs) for muscle remodelling (Mackey et al., 2004). An increase in circulating levels of IL-6 during chronic RE has also been negatively associated with muscle strength (but not with muscle mass) gains in the elderly

(Hangelbroek et al., 2018). IL-6 is strongly associated with glucose transport in human skeletal muscle (Glund et al., 2007) and the delayed peak of IL-6 concentration in the circulation (Fischer, 2006, Toth et al., 2011) might be an indication of a long lasting demand for energy to remodel the damaged muscle fibres and the surrounding connective tissue following acute RE.

6.5.4 Limitations/ Perspective

We acknowledge that the G-REX cohort comprised both male and female participants, whilst the G-ResisT cohort contained only males. Therefore, we normalised strength parameters to body mass for the intervention studies and used sex as a covariate for the G-REX cohort, so we were able to analyse strength differences between the alleles of each SNP independently of sex. Another limitation was the relatively small number of subjects included in each cohort. The current investigation suggests that the muscle ECM might play an important role with respect to the response/adaption to acute/chronic RE. As recent investigations have revealed that the muscle ECM is involved in the severity of muscle injuries (Balius et al., 2018), future studies should assess, whether the loading magnitude of RE, in particular, plays a key role for muscle ECM adaptation with respect to injury prevention and rehabilitation programmes. Nordic hamstring training might be an effective injury prevention exercise, as the eccentric component of this exercise modality might lead to a stiffer muscle ECM. Further studies should investigate SNPs that are linked specifically to other perimysium-related protein-encoding genes, such as proteoglycans (e.g. decorin and biglycan) (Nakano et al., 1997, Mercado et al., 2006), different collagen types (e.g. type XII and XIV) (Listrat et al., 2000, Gillies and Lieber, 2011) and of the ECM-muscle interface (Grounds et al., 2005).

6.6 CONCLUSION

In conclusion, the current study demonstrated that a polygenic profile (TGS-A), which was based on a cohort performing an acute bout of unaccustomed eccentric RE, cannot anticipate the neuromuscular adaptations to chronic RE. TGS-A included SNPs that are linked to genes contributing to the elastic properties of the MTU and to muscle catabolic signalling pathways. These genes might have a crucial functional role regarding the response to acute RE, but are less important for long-term gains in muscle strength and hypertrophy. However, a different polygenic profile (TGS-C), based on a cohort, which performed chronic RE, was associated with changes in muscle strength (but not muscle mass), and this polygenic profile was also inversely associated with the muscle damage response in terms of blood biomarkers (CK activity and IL-6 concentration) following acute RE. Secondly, SNPs, which were associated with chronic RE, could be categorised into signalling- and muscle ECM-related (force transmission) clusters, indicating that muscle hypertrophy and muscle strength gains are, at least in part, based on independent molecular pathways. Our findings provide strong evidence for a common polygenic profile that influences both the acute and chronic responses to RE. Specifically, different combinations of particular genotypes appear to limit the damage response to acute RE, while maximising the adaptation to chronic RE. Thus, our findings further our understanding of the molecular mechanisms underpinning and ***linking*** the acute and chronic responses to RE. After independent replication, TGS-A and TGS-C may be used to estimate an individual's response/adaptation to *acute/chronic* RE, thus enabling RE to be prescribed on a personalised level to improve muscle health and function.

Our findings provide strong evidence for a common polygenic profile that influences both the acute and chronic responses to RE. Specifically, different combinations of particular genotypes appear to limit the damage response to acute RE, while maximising the adaptation to chronic RE. Thus, our findings further our understanding of the molecular mechanisms underpinning and ***linking*** the acute and chronic responses to RE. After independent replication, TGS-A and TGS-C may be used to anticipate an individual's

response/adaptation to *acute/chronic* RE, thus enabling RE to be prescribed on a personalised level to improve muscle health and function.

7 Thesis Synthesis

7.1 GENERAL DISCUSSION

The overall aim of this thesis was to investigate the physiological and genetic factors underpinning the response to exercise-induced muscle damage (EIMD). Here, we used a triangulation approach, where a combination of varying assessments from different research disciplines, including genetics, cell biology, muscle physiology and biomechanics was used to systematically investigate the mechanisms underpinning muscle damage and the variable response to EIMD. The following sections aims to discuss the key outcomes of the present thesis, the limitation of the investigations and provide practical recommendations. The outcomes of this thesis, and how they may help practitioners to prescribe "personalised exercise medicine" to their patients/athletes (thus optimising health and performance, and reducing the risk of injury), are discussed.

The objectives of this thesis were therefore:

1. To characterise the physiological mechanisms underpinning neuromuscular fatigue and the recovery pattern following intermittent maximal sprints, to acquire a better understanding of EIMD in an applied setting (Chapter 3).
2. To determine the effect that inter-individual differences in the ratio of skeletal muscle myoblast to fibroblast composition has on skeletal muscle repair/recovery after an artificial wounding (scratch) assay using primary human skeletal muscle cells *in vitro*, and the association of this ratio with recovery after intermittent maximal sprints (Chapter 4).
3. To ascertain whether a polygenic profile could distinguish between high and low responders following a controlled *in vivo* eccentric exercise (acute resistance exercise, RE) intervention in previously untrained individuals, and whether those genetic variations were also associated with the artificial wounding (scratch) assay *in vitro* (Chapter 5). This would, therefore, provide a genetic link between *in vivo* and *in vitro* muscle damage, thus potentially shedding new light on the aetiology of EIMD.

4. To determine whether the polygenic profile associated with *acute* RE from Chapter 5 could anticipate individual adaptations to *chronic* RE, in terms of gains in muscle size and strength (Chapter 6) and
5. To ascertain whether a new polygenic profile associated with the adaptations to chronic RE (gains in muscle size and strength) (Chapter 6) could estimate the individual responses to acute RE in Chapter 5 (e.g. loss of strength, increase in soreness, inflammation, etc.). Objectives 4 and 5 have implications for furthering our understanding of the genetic and physiological mechanisms underpinning and linking the *acute* and *chronic* responses to RE.

7.1.1 The contribution of the non-contractile muscle tissue to the response to EIMD

It was identified that immediate strength loss was associated with impaired hamstring muscle recruitment and markers of peripheral fatigue/damage, but the magnitude and sustained changes in maximal voluntary contraction (MVC) torque over time was predominately associated with indicators of peripheral fatigue/damage (Chapter 3; objective 1, see above). The most striking finding was that biceps femoris long head (BF_{LH}) fascicle length was not associated with the extent of hamstring fatigue between PRE and immediately POST. This finding was contrary to our hypothesis as fascicle length of the BF_{LH} (together with Nordic hamstring muscle strength) was previously inversely correlated with non-contact muscle injury risk (Timmins et al., 2016). Instead, our data showed an inverse correlation between MVC torque loss and both BF_{LH} muscle volume and (especially) BF_{LH} physiological cross-sectional area (PCSA). These results indicate that the structure of the muscle (reflected by PCSA) protects the muscle against EIMD independently of force due to the fact that baseline hamstring MVC did not correlate with PCSA, and also not with relative hamstring MVC torque loss (measured between PRE and POST). Recent investigations reported an important role of the extracellular matrix (ECM) regarding muscle damage (Hyldahl and Hubal, 2014, Hyldahl et al., 2015) and muscle injuries (Balius et al., 2018). The ECM consists of different layers of connective tissue and this non-contractive

structure surrounds the muscle fibres and fascicles (Kjær, 2004). More parallel fibres might be accompanied with more cross-sectional muscle ECM content that might have a protective effect against mechanical stress on the contractile components following eccentric lengthening contractions. Therefore, our results indicate that BF_{LH} PCSA might be a better predictor for muscle strains rather than BF_{LH} fascicle length, and we suggest that future prospective muscle strain studies should assess BF_{LH} PCSA.

As there is a lack of research of both the role of the muscle ECM and of the stem cells of this connective tissues (fibroblasts), we further analysed the components of the non-contractile muscle tissue (Chapter 4; objective 2). It was initially hypothesised that a high myoblast:fibroblast ratio would support muscle recovery *in vivo* following the IS-intervention and in the wound healing assay *in vitro* following the IS-intervention. Surprisingly, a low myoblast:fibroblast ratio was related with improved knee-flexion MVC torque muscle repair/recovery following EIMD, both *in vitro* and *in vivo*. However, contrary to our hypothesis, our results showed an ambiguous picture during the muscle repair process. A high myoblast:fibroblast ratio showed a delayed wound closure within the first 48 h, but a better myotube formation at seven days, and higher CK activity at ten days after the scratch assay, and a delayed knee-flexion MVC torque recovery following IS *in vivo*. The IS-intervention of Chapter 3 induced muscle damage with a similar underlying mechanism comparing with incidences, when hamstring muscle strain injuries occur (Ekstrand et al., 2011, Brooks et al., 2006, Woods et al., 2004). Dynamic (stretch-shortening) movements, such as IS, might have an additional damaging effect on the muscle ECM beyond traditional EIMD assessments e.g. with an isokinetic dynamometer, which potentially damage the myofibrillar content predominantly. Following IS, there was a significantly increased knee-flexion MVC torque loss POST48 compared to the knee-extension. Interestingly, there was no significant correlation between the myoblast:fibroblast ratio and *knee-extension* MVC torque recovery (between POST and POST48) indicating that a certain damage in the muscle-tendon unit (MTU) is necessary to trigger fibroblast activation for muscle regeneration. That leads to the assumption that fibroblasts play an important part during

muscle recovery, when the muscle ECM is also involved in the structural damage after strenuous exercise.

7.1.2 The association between genetic variations and the individual response/ adaptation to acute and chronic RE

The studies of chapter 3 and 4 revealed high inter-individual differences in the response to EIMD, despite the homogeneous groups *in vivo* and *in vitro.* We next aimed to understand the functional relevance in terms of whether genetic variations are associated to changes in the response to EIMD and chronic RE (Chapter 5 and 6; objectives 3, 4 and 5, as outlined above). We identified that the NPG with the non-preferential polygenic profile of TGS-A (i) demonstrated greater muscle soreness and greater loss of range of motion following the EIMD-intervention, respectively; and (ii) were generally weaker compared to moderate (MG) and PG groups (Chapter 5; objective 3). As all participants performed the same relative intensity of exercise, these findings gives further evidence that genetic variations play a role in the individual response to EIMD. However, there was no interaction between genetic variations and (i) changes in strength; and (ii) the concentration or activity of blood-borne biomarkers of EIMD following the intervention, neither on an individual SNP or polygenic basis of TGS-A.

Chronic resistance exercise (RE) is a potent stimulus for increasing strength and muscle hypertrophy and it is increasingly recognised as a cornerstone for prevention of both muscle (Petersen et al., 2011) and ACL (Webster and Hewett, 2018, Petushek et al., 2018) injury. However, as non-contact injury rate is not completely eradicated in some athletes undertaking chronic RE, it can be suggested that prevention training has a variable effect on each athlete. However, TGS-A was not related with any adaptation to chronic RE (Chapter 5 and 6; objective 5). Against that, PGs of the new computed TGS-C showed increased adaptations to chronic RE in terms of changes in normalised force and muscle specific force (Chapter 6, objective 4). When the outcome of the G-REX participants (Chapter 5) were associated with the new TGS-C, NPGs demonstrated a greater

inflammatory response following acute RE (Chapter 5 and 6, objective 5). It was unexpected that the TGS-C would be associated with serum CK activity and IL-6 concentration following acute RE, as neither serum CK activity nor IL-6 concentration were associated with any individual SNP or with TGS-A in the G-REX cohort (Chapter 5). These findings imply that a high increase in serum CK activity and IL-6 concentration immediately after unaccustomed acute RE might be associated with attenuated adaptation regarding strength gains. Moreover, this link between an acute inflammatory/damage response and neuromuscular adaptations to chronic RE is linked to the same genetic profile. Thus, we provide evidence that genetic make-up drives the response to acute RE, which influences the longer-term adaptations to chronic RE.

7.1.3 Genetic variations expand our understanding of the molecular mechanisms underpinning the individual response to acute and chronic RE

There were several distinct functional groups regarding the response to the acute and chronic RE (Chapter 5 and 6). Some of the SNPs that were associated with EIMD in Chapter 5 are located within genes, which contribute to the elastic properties of the MTU. An increased stiffness of the elastic components (e.g. endomysium) of the MTU might increase stress on the contractile components, which may result in an increase of extracellular inflammatory mediators and in an activation of the nociceptor of the overstretched cytoskeleton/muscle ECM leading to elevated muscle soreness (Chapter 5). Another functional group of SNPs associated with the acute RE intervention are related to enzymes of the skeletal muscle catabolic and anabolic signaling pathways. The beneficial alleles might increase the expression rate of their transcript following EIMD, potentially promoting MTU repair in the short term. However, as the majority of these SNPs are not associated with an increased muscle strength after chronic RE, proteins of both the elastic components of the muscle-tendon unit (MTU) and of the catabolic signaling pathway might be less important for chronic RE adaptation (Chapter 6).

There was no interaction between genetic variations and changes in strength following the acute RE (Chapter 5). It is thought that muscle force is transmitted longitudinally as a result of sarcomere shortening and laterally via the perimysium (surrounding muscle fascicles) to the tendon and ultimately to the bone (Hughes et al., 2015, Ramaswamy et al., 2011). The EIMD-intervention induced by an IKD with low-speed eccentric MVC was potentially not able to cause substantial damage to the stiff components of the perimysium (such as to the collagen type I fibrils), and the MVC strength loss might be more associated with damage to structural components of the myofibrillar apparatus, particularly those involved in the longitudinal force transmission (e.g. titin and nebulin) (Trappe et al., 2002). As no SNP, which showed an association to myoblast:fibroblast ratio in the *in vitro* scratch assay, demonstrated an interaction with strength, this gives further evidence that fibroblasts play a less important part in muscle recovery, when the muscle ECM is not substantially damaged. Repeated high external forces involved with high-speed (stretch-shortening) movements, such as with IS of Chapter 3, are potentially associated with a greater risk of damaging components of the muscle ECM/perimysium compared to EIMD-interventions with low-speed eccentric MVCs such as in Chapter 5 (objective 2; see section 7.1.1). Among the individual SNPs that were associated with improvements in muscle specific force following chronic RE (Chapter 6), were predominantly linked with proteins of the stiff part of the ECM. The association between the ECM-related SNPs and muscle specific force lead to the suggestion that the accumulation of recurring RE might lead to a better turnover of the proteins, and, therefore, subsequently to a stiffer perimysium of the muscle ECM with better force transmission capabilities.

In terms of the role of the muscle ECM, several studies investigated global mRNA expression of skeletal muscle biopsies following acute and chronic RE (Damas et al., 2018, Phillips et al., 2013) and these investigations observed strong changes in gene expression related to the cytoskeleton and muscle ECM. Studies of the adaptive responses of the rates of protein synthesis to exercise have largely so far focused on myocellular fractions, excluding collagen. However, the response of the muscle ECM to RE was confirmed in

some investigations on the protein level (Moore et al., 2005b, Miller et al., 2005), in an immunohistochemical investigation (Mackey et al., 2011), and in one metabolomics study (Fazelzadeh et al., 2016). This together with the results of this thesis indicates that strength gains might not only associated with improvements of the myofibrillar content and of the (patellar) tendon of the MTU, but that muscle ECM remodelling might play an important part in terms of strength gains following chronic RE.

To conclude, we have investigated the role of genetic variations on the response to acute and chronic strenuous exercise interventions, including assessments from the DNA to the whole muscle-tendon complex. We believe that the findings of this thesis advance the knowledge of exercise genetics. However, research in the area of exercise genetics is in its infancy. Additional large cohorts, investigating the individual response to acute and chronic RE, needs to be established so that our model can be validated in these independent cohorts. If the polygenic profiles (e.g. TGS-C) can be validated in future, practical recommendations can be deducted from these findings. Potentially, the responsiveness of strength and power athletes can be estimated with one acute RE session together with a serum sample (assessing IL-6 concentration and CK activity). This might also have valuable information for practitioners to significantly improve prevention and rehabilitation programmes and to optimise muscle strengthening programmes.

7.2 THESIS LIMITATIONS

We acknowledge that there was a relatively small number of subjects included in the *in vivo* cohorts of Chapter 5 and 6 and also from the *in vitro* cohort of Chapter 4. However, given the difficulties in performing these types of intervention studies, the data presented is of great scientific value and should be utilised as a catalyst to reliably validate and replicate these findings.

Further, we have recruited both male and female participants in Chapter 4 and 5, and there is some limited evidence that men and women respond differently to eccentric exercise (Sewright et al., 2008). However, the *in vitro* study of Chapter 4 and the majority of previous

investigations attempting to address this question have shown no sex differences in in the susceptibility to ultrastructural muscle damage (Sayers and Clarkson, 2001, Thompson et al., 1997, Stupka et al., 2001, Wüst et al., 2008). Furthermore, although our male participants were stronger than our female subjects in both absolute and relative (to body mass) terms, when the change in MVC at time points POST and 48POST was reported as a percentage change from baseline MVC, we observed no difference in strength loss between men and women. We, therefore, used absolute strength normalised to body mass with the covariate sex, so we were able to analyse strength differences between genotype/allele for each SNP. Moreover, each of our three polygenic groups contained a similar ratio of men to women, so it is highly unlikely that any sex differences influenced the outcome of our results.

Eccentric exercise results in a delayed peak and a slower decrease of serum IL-6 during recovery between 6 and 24 h after EIMD (Willoughby et al., 2003, Pedersen and Febbraio, 2008). Therefore, serum IL-6 expression immediately after the investigations indicates that intense exercise occurred but it cannot be assured that this marker indicates muscle damage in the current study. The generally low absolute value of CK activity of the *in vivo* studies (Chapter 3 and 5) of this thesis might be explained by the fact that we have used a different method involving venous blood, which will usually be conducted in *in vitro* investigations (Owens et al., 2015). Therefore, further interventions are necessary to compare CK activity assessments *in vivo*, and the investigations may have benefitted from additional time points for blood analyses to acquire a more comprehensive and accurate representation of the time course of e.g. serum IL-6 response and of additional muscle damage biomarkers to our EIMD intervention.

Peripheral fatigue can be caused by metabolite perturbations such as the depletion of intramuscular glycogen (Bendiksen et al., 2012). However, despite the fact that participants were instructed to eat two hours before arrival, and to avoid strenuous exercise prior to the testing, the two *in vivo* EIMD-studies (Chapter 3 and 5) did not control for diet or assessed glycogen depletion during the intervention. Although the EIMD interventions lasted less than

25 min, indicating that glycogen depletion might be only a minor factor which contributed to the impaired muscle function following the EIMD-interventions, future studies should assess and control the glycogen content of the muscles before and after EIMD-interventions.

Recent studies suggest that fatigue alters the optimal angle during MVC in the hamstrings but not in the quadriceps (Coratella et al., 2015). This might have influenced the results of the isometric MVC test of the hamstring muscle group in Chapter 3. Further studies should confirm these results with isometric MVCs at different knee joint angles. Further, we have only measured muscle architecture and sEMG activity of the BF_{LH}. During data collection, several published investigations (Kellis, 2015, Schuermans et al., 2016, Schuermans et al., 2014) indicated that the other hamstring muscles might have an impact on hamstring fatigue and, potentially, on hamstring strain injury risk. We therefore suggest that further studies conducting IS should also take the other hamstring muscles into consideration. Other factors, such as skeletal muscle fibre type composition might also contribute to the prolonged recovery process following EIMD.

We assumed that the myoblast:fibroblast ratio is similar between the vastus lateralis musle (from which we obtained the muscle biopsy) and the BF_{LH}, as Terry et al. (2018) demonstrated a high similarity of transcriptome expression patterns in skeletal muscles of rodents, when the muscles consist of similar fibre type composition and of similar physiological functions, such as the BF_{LH} (47.1% MHC-I, 35.5% MHC-IIA, and 17.4% MHC-IIX) (Evangelidis et al., 2016) and the vastus lateralis (49-59% MHC-I, 26-35% MHC-IIA, and 16-28% MHC-IIX) (Mittendorfer et al., 2005, Taylor et al., 1997). However, gene expression patterns vary between rodents and human (Su et al., 2002), and there are is a gap of research between the intra-individual variability of the myoblast:fibroblast ratio between skeletal muscles. Therefore, further investigations needs to address this research gap to confirm our findings.

Lastly, we distinguished the primary muscle cells in myoblasts (desmin-positive) and fibroblasts (desmin negative) with a desmin antibody in the in *vitro study* of Chapter 4 and 5, as previous investigations have determined that the non-myoblasts fraction is highly

enriched in fibroblasts with up to 99 % of this fraction being fibroblasts (Agley et al., 2013, Mathew et al., 2011). For immnunohistochemistry analysis, we have fixed the cells with a low density of 30%, so that the cells could be easily distinguished from each other. The ICC (3, k) of 0.83 indicates a good reliability for the characterisation and the quantification of myoblasts and fibroblasts. However, analysing the entire well (instead of sub-sampling random areas of each well) and additional methodical approaches, such as cell sorting by flow cytometry, could have improved the accuracy of our myoblast:fibroblast ratio calculation. Future studies may use additional antibodies, such as DAPI (for staining nuclei) and TE7 (for staining fibroblasts), to validate our findings.

7.3 FUTURE DIRECTIONS

Previous investigations have identified over 200 genes, in which common genetic variants are located, involved in the genetic predisposition to physical performance (Moran and Pitsiladis, 2017). However, the small overlap of SNPs related to athletes/ performance and of GWAS studies of the general population including a wide range of age and lifestyle and behavioural exposures (Willems et al., 2017, Zillikens et al., 2017) indicating the importance of homogenous cohorts. Presumably, SNPs, which are associated with changes in muscle mass and strength in the normal population over different age ranges might be more influenced by SNPs regarding muscle atrophy and motor neuron genes. In future, large-scale studies with homogeneous (young) cohorts should be conducted as an interdisciplinary and multi-centre project including several “omics” measurements such as transcriptome sequencing and metabolomics together with muscle biopsies to identify the cause and consequences of different recovery patterns of participants following acute and chronic RE. Here, large-scale investigations including primary human muscle stem cells derived from the same participants who performed the *in vivo* intervention, could shed light on the association between the myoblast:fibroblast ratio and the genetic variations on the response/adaptation following acute and chronic RE.

The results of this thesis lead to the assumption that strength might be linked with muscle ECM remodelling following chronic RE. Further, the stiffness of the ECM can be seen as a

key sensor of external forces to appropriate respond and adapt to external stress, a process known as mechanotransduction (Gattazzo et al., 2014). Molecular skeletal mechanotransducers of the ECM (e.g. integrins and costameres) might be associated with muscle ECM remodelling and, subsequently, with the increased ECM stiffness following chronic RE. However, the investigation of the mechanoreceptors of the ECM was beyond the scope of the current thesis. As the mechanosensory pathway seems to be independent of the Akt/mammalian target of rapamycin/ S6K1 pathway (Eftestøl et al., 2016), and recent investigations revealed that increased ECM stiffness is accompanied with the activity of components (YAP/TAZ) of the Hippo Pathway (Engler et al., 2006, Piccolo et al., 2014), further investigations should assess the association between the mechanotransduction and muscle ECM remodelling following RE. Further, muscle strength declines during disuse at approximately three times the rate that muscle tissue is lost (Farthing et al., 2009). Future studies should investigate whether the loss of muscle ECM integrity plays a role of the disproportional loss of strength compared to muscle mass during disuse and they should replicate the SNPs of the GWAS studies regarding muscle strength (Willems et al., 2017) and muscle mass (Zillikens et al., 2017) in hypertrophy and atrophy interventions.

There is evidence to suggest that there is a dose-response relationship for training volume of RE and gain of muscle mass, but increases in muscle strength remain similar and seems not to be affected by the total number of repetitions (Schoenfeld et al., 2018). Muscle strength is further augmented by higher intensities, whereas exceeding a threshold of about 40% of 1RM is a sufficient stimulus for increasing muscle mass (Lasevicius et al., 2018). Power athletes, usually exercising with high intensity and lower volume, demonstrate a smaller muscle fibre CSA but a higher fibre specific tension compared to body builders training with high volume but low/moderate intensity (Meijer et al., 2015). The investigations of this thesis suggest that the muscle ECM might play an important role in respect to changes in strength following acute and chronic RE. However, no studies exist about muscle ECM training so far. Further investigations need to assess, whether the current knowledge of tendon training (exercise regime with at least of 70% of 1-RM) (Bohm et al.,

2015) can be extrapolated to muscle ECM training, and whether chronic RE potentially improve the material (remodelling) within the muscle ECM rather than a solely increase of CSA.

Further, it is well known that a history of previous injuries is evidenced as a risk factor for future sport-related injuries (Freckleton and Pizzari, 2013). As recent investigations have revealed that muscle ECM is involved in the severity of muscle injuries (Balius et al., 2018), high recurrence rate of muscle strains might be partly caused of exercising only with low or moderate intensities during the rehabilitation exercise programmes, and, therefore, missing the intensity threshold of at least 70 % (1-RM) to exercise the muscle ECM. RE with high intensities might lead to a stiffer muscle ECM and to a higher 'protection' against structural ECM damage. We therefore suggest that future investigations in respect to rehabilitation exercise programmes should assess whether progressive RE, reaching high training intensities towards the end of the programme, decrease muscle re-injuries.

7.4 FINAL CONCLUSION

This thesis has generated novel findings as to the role genetic variation plays in the response to acute and chronic strenuous exercise. Further, by using a triangulation approach of combining different assessments from varying research disciplines, including genetic, cellular, physiological, and biomechanical analyses, this thesis sheds light onto the role of the muscle ECM regarding the response/adaptation following acute and chronic RE. There is evidence to suggest that fibroblasts have an important role in muscle remodelling, especially within the first days following eccentric exercises induced by fast and intense dynamic movements. The results of this thesis will help to identify individuals who are unlikely to respond to resistance exercise, or who require longer to recover from a bout of strenuous exercise. This may help practitioners to prescribe "personalised exercise medicine" to their patients/athletes, thus optimising health and performance, and reducing the risk of injury. The results will pave the way for public health services and sporting

organisations to effectively manage their patients/athletes personalised programmes of prevention and rehabilitation in the future.

8 References

AGLEY, C. C., ROWLERSON, A. M., VELLOSO, C. P., LAZARUS, N. R. & HARRIDGE, S. D. 2013. Human skeletal muscle fibroblasts, but not myogenic cells, readily undergo adipogenic differentiation. *J Cell Sci***,** jcs. 132563.

AHMETOV, I. I., DRUZHEVSKAYA, A. M., LYUBAEVA, E. V., POPOV, D. V., VINOGRADOVA, O. L. & WILLIAMS, A. G. 2011. The dependence of preferred competitive racing distance on muscle fibre type composition and ACTN3 genotype in speed skaters. *Experimental physiology,* 96**,** 1302-1310.

AHMETOV, I. I., NAUMOV, V. A., DONNIKOV, A. E., MACIEJEWSKA-KARŁOWSKA, A., KOSTRYUKOVA, E. S., LARIN, A. K., MAYKOVA, E. V., ALEXEEV, D. G., FEDOTOVSKAYA, O. N. & GENEROZOV, E. V. 2014. SOD2 gene polymorphism and muscle damage markers in elite athletes. *Free Radical Res,* 48**,** 948-955.

AKIMOTO, A. K., MIRANDA-VILELA, A. L., ALVES, P. C., PEREIRA, L. C., LORDELO, G. S., HIRAGI CDE, O., DA SILVA, I. C., GRISOLIA, C. K. & KLAUTAU-GUIMARAES, M. D. N. 2010. Evaluation of gene polymorphisms in exercise-induced oxidative stress and damage. *Free Radical Res,* 44**,** 322-31.

AL-SHANTI, N. & STEWART, C. E. 2009. Ca2+/calmodulin-dependent transcriptional pathways: potential mediators of skeletal muscle growth and development. *Biol Rev,* 84**,** 637-52.

ALEXANDER, R. M. 1975. The dimensions of knee and ankle muscles and the forces they exert. *J. Hum. Move. Stud.,* 1**,** 115-123.

ALLEN, D. G., WHITEHEAD, N. P. & YEUNG, E. W. 2005. Mechanisms of stretch-induced muscle damage in normal and dystrophic muscle: role of ionic changes. *J Physiol,* 567**,** 723-35.

ALLEN, N. E., DAVEY, G. K., KEY, T. J., ZHANG, S. & NAROD, S. A. 2002. Serum insulin-like growth factor I (IGF-I) concentration in men is not associated with the cytosine-adenosine repeat polymorphism of the IGF-I gene. *Cancer Epidemiology Biomarkers & Prevention,* 11**,** 319-320.

ALSHARIDAH, M., LAZARUS, N. R., GEORGE, T. E., AGLEY, C. C., VELLOSO, C. P. & HARRIDGE, S. D. 2013. Primary human muscle precursor cells obtained from young and old donors produce similar proliferative, differentiation and senescent profiles in culture. *Aging cell,* 12**,** 333-344.

ALVARES, J. B. D. A. R., RODRIGUES, R., DE AZEVEDO FRANKE, R., DA SILVA, B. G. C., PINTO, R. S., VAZ, M. A. & BARONI, B. M. 2015. Inter-machine reliability of the Biodex and Cybex isokinetic dynamometers for knee flexor/extensor isometric, concentric and eccentric tests. *Physical Therapy in Sport,* 16**,** 59-65.

AMELINK, G. & BÄR, P. 1986. Exercise-induced muscle protein leakage in the rat: effects of hormonal manipulation. *Journal of the neurological sciences,* 76**,** 61-68.

ANDERSEN, H., NIELSEN, S., MOGENSEN, C. E. & JAKOBSEN, J. 2004. Muscle strength in type 2 diabetes. *Diabetes,* 53**,** 1543-1548.

ANDERSEN, M. B., PINGEL, J., KJAER, M. & LANGBERG, H. 2011. Interleukin-6: a growth factor stimulating collagen synthesis in human tendon. *J Appl Physiol,* 110**,** 1549-54.

ANDREASSEN, C. S., JAKOBSEN, J., RINGGAARD, S., EJSKJAER, N. & ANDERSEN, H. 2009. Accelerated atrophy of lower leg and foot muscles—a follow-up study of long-term diabetic polyneuropathy using magnetic resonance imaging (MRI). *Diabetologia,* 52**,** 1182-91.

APPELL, H.-J., SOARES, J. M. C. & DUARTE, J. A. R. 1992. Exercise, muscle damage and fatigue. *Sports Med,* 13**,** 108-115.

APPS, C., STERZING, T., O'BRIEN, T., DING, R. & LAKE, M. 2017. Biomechanical locomotion adaptations on uneven surfaces can be simulated with a randomly deforming shoe midsole. *Footwear Science,* 9**,** 65-77.

ARAI, H., MIYAMOTO, K. I., TAKETANI, Y., YAMAMOTO, H., IEMORI, Y., MORITA, K., TONAI, T., NISHISHO, T., MORI, S. & TAKEDA, E. 1997. A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *Journal of Bone and Mineral Research,* 12**,** 915-921.

ARAUJO, L. G., WATERHOUSE, J., EDWARDS, B., SANTOS, E. H. R., TUFIK, S. & TÚLIO DE MELLO, M. 2011. Twenty-four-hour rhythms of muscle strength with a consideration of some methodological problems. *Biological rhythm research,* 42**,** 473-490.

ARCHAMBAULT, J. M., JELINSKY, S. A., LAKE, S. P., HILL, A. A., GLASER, D. L. & SOSLOWSKY, L. J. 2007. Rat supraspinatus tendon expresses cartilage markers with overuse. *J Orthop Res,* 25**,** 617-24.

ARMSTRONG, R. B. 1984. Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Medicine and science in sports and exercise,* 16**,** 529-538.

ARMSTRONG, R. B. 1990. Initial events in exercise-induced muscular injury. *Medicine and science in sports and exercise,* 22**,** 429-435.

ARMSTRONG, R. B., WARREN, G. L. & WARREN, J. A. 1991. Mechanisms of exercise-induced muscle fibre injury. *Sports Medicine,* 12**,** 184-207.

ARNOLD, L., HENRY, A., PORON, F., BABA-AMER, Y., VAN ROOIJEN, N., PLONQUET, A., GHERARDI, R. K. & CHAZAUD, B. 2007. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med,* 204**,** 1057-69.

ASP, S., DAUGAARD, J. R., KRISTIANSEN, S., KIENS, B. & RICHTER, E. A. 1996. Eccentric exercise decreases maximal insulin action in humans: muscle and systemic effects. *The Journal of physiology,* 494**,** 891-898.

ASP, S., DAUGAARD, J. R. & RICHTER, E. A. 1995. Eccentric exercise decreases glucose transporter GLUT4 protein in human skeletal muscle. *The Journal of physiology,* 482**,** 705-712.

AZMI, N. L., DING, Z., XU, R. & BULL, A. M. J. 2018. Activation of biceps femoris long head reduces tibiofemoral anterior shear force and tibial internal rotation torque in healthy subjects. *PLoS One,* 13**,** e0190672.

BABENKO, V., GRAVEN-NIELSEN, T., SVENSSON, P., DREWES, A. M., JENSEN, T. S. & ARENDT-NIELSEN, L. 1999. Experimental human muscle pain and muscular hyperalgesia induced by combinations of serotonin and bradykinin. *Pain,* 82**,** 1-8.

BACZYNSKA, A. M., SHAW, S., ROBERTS, H. C., COOPER, C., SAYER, A. A. & PATEL, H. P. 2016. Human Vastus Lateralis Skeletal Muscle Biopsy Using the Weil-Blakesley Conchotome. *Journal of Visualized Experiments***,** e53075-e53075.

BAECKE, J., BUREMA, J. & FRIJTERS, J. 1982a. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *The American journal of clinical nutrition,* 36**,** 936-942.

BAECKE, J. A., BUREMA, J. & FRIJTERS, J. 1982b. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *The American journal of clinical nutrition,* 36**,** 936-942.

BAIRD, M. F., GRAHAM, S. M., BAKER, J. S. & BICKERSTAFF, G. F. 2012. Creatine-kinase- and exercise-related muscle damage implications for muscle performance and recovery. *Journal of nutrition and metabolism,* 2012**,** 1-13.

BALIUS, R., ALOMAR, X., PEDRET, C., BLASI, M., RODAS, G., PRUNA, R., PEÑA-AMARO, J. & FERNÁNDEZ-JAÉN, T. 2018. Role of the Extracellular Matrix in Muscle Injuries: Histoarchitectural Considerations for Muscle Injuries. *Orthopaedic Journal of Sports Medicine,* 6**,** 2325967118795863.

BALNAVE, C. & THOMPSON, M. 1993. Effect of training on eccentric exercise-induced muscle damage. *Journal of Applied Physiology,* 75**,** 1545-1551.

BALNAVE, C. D. & ALLEN, D. G. 1995. Intracellular calcium and force in single mouse muscle fibres following repeated contractions with stretch. *The Journal of Physiology,* 488**,** 25-36.

BALSOM, P., SEGER, J., SJÖDIN, B. & EKBLOM, B. 1992. Maximal-intensity intermittent exercise: effect of recovery duration. *International journal of sports medicine,* 13**,** 528-528.

BALTZOPOULOS, V. 1995. A videofluoroscopy method for optical distortion correction and measurement of knee-joint kinematics. *Clinical Biomechanics,* 10**,** 85-92.

BANG, M.-L., CENTNER, T., FORNOFF, F., GEACH, A. J., GOTTHARDT, M., MCNABB, M., WITT, C. C., LABEIT, D., GREGORIO, C. C. & GRANZIER, H. 2001. The complete gene sequence of titin, expression of an unusual≈ 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. *Circulation research,* 89**,** 1065-1072.

BANSAL, D., MIYAKE, K., VOGEL, S. S., GROH, S., CHEN, C.-C., WILLIAMSON, R., MCNEIL, P. L. & CAMPBELL, K. P. 2003. Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature,* 423**,** 168-172.

BARFIELD, W. L., UAESOONTRACHOON, K., WU, C.-S., LIN, S., CHEN, Y., WANG, P. C., KANAAN, Y., BOND, V. & HOFFMAN, E. P. 2014. Eccentric muscle challenge shows osteopontin polymorphism modulation of muscle damage. *Human molecular genetics,* 23**,** 4043-4050.

BARKER, T., HENRIKSEN, V. T., MARTINS, T. B., HILL, H. R., KJELDSBERG, C. R., SCHNEIDER, E. D., DIXON, B. M. & WEAVER, L. K. 2013. Higher serum 25-hydroxyvitamin D concentrations associate with a faster recovery of skeletal muscle strength after muscular injury. *Nutrients,* 5**,** 1253-1275.

BARR, R., MACDONALD, H., STEWART, A., MCGUIGAN, F., ROGERS, A., EASTELL, R., FELSENBERG, D., GLÜER, C., ROUX, C. & REID, D. 2010. Association between vitamin D receptor gene polymorphisms, falls, balance and muscle power: results from two independent studies (APOSS and OPUS). *Osteoporosis international,* 21**,** 457-466.

BARTON-DAVIS, E. R., SHOTURMA, D. I., MUSARO, A., ROSENTHAL, N. & SWEENEY, H. L. 1998. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proceedings of the National Academy of Sciences of the United States of America,* 95**,** 15603-15607.

BAUMERT, P., LAKE, M. J., DRUST, B., STEWART, C. & ERSKINE, R. M. 2017. TRIM63 (MuRF-1) Gene Polymorphism is Associated with Biomarkers of Exercise-Induced Muscle Damage. *Physiological genomics***,** physiolgenomics. 00103.2017-physiolgenomics. 00103.2017.

BAUMERT, P., LAKE, M. J., STEWART, C. E., DRUST, B. & ERSKINE, R. M. 2016a. Genetic variation and exercise-induced muscle damage: implications for athletic

performance, injury and ageing. *European journal of applied physiology,* 116**,** 1595-1625.

BAUMERT, P., LAKE, M. J., STEWART, C. E., DRUST, B. & ERSKINE, R. M. 2016b. Inter-individual variability in the response to maximal eccentric exercise. *European journal of applied physiology,* 116**,** 2055-2056.

BAUMERT, P., LAKE, M. J., STEWART, C. E., DRUST, B. & ERSKINE, R. M. 2018. Variations of Collagen-Encoding Genes are Associated with Exercise-Induced Muscle Damage. *Physiological genomics*.

BEDAIR, H. S., KARTHIKEYAN, T., QUINTERO, A., LI, Y. & HUARD, J. 2008. Angiotensin II receptor blockade administered after injury improves muscle regeneration and decreases fibrosis in normal skeletal muscle. *American journal of sports medicine,* 36**,** 1548-1554.

BEELEN, A., SARGEANT, A. & WIJKHUIZEN, F. 1994. Measurement of directional force and power during human submaximal and maximal isokinetic exercise. *European journal of applied physiology and occupational physiology,* 68**,** 177-181.

BEHRENS, M., MAU-MOELLER, A., WASSERMANN, F. & BRUHN, S. 2013. Effect of fatigue on hamstring reflex responses and posterior-anterior tibial translation in men and women. *PloS one,* 8**,** e56988.

BELCASTRO, A. N., SHEWCHUK, L. D. & RAJ, D. A. 1998. Exercise-induced muscle injury: a calpain hypothesis. *Mol Cell Biochem,* 179**,** 135-45.

BELL, R. D., SHULTZ, S. J., WIDEMAN, L. & HENRICH, V. C. 2012. Collagen gene variants previously associated with anterior cruciate ligament injury risk are also associated with joint laxity. *Sports Health: A Multidisciplinary Approach,* 4**,** 312-318.

BELLEMARE, F., WOODS, J., JOHANSSON, R. & BIGLAND-RITCHIE, B. 1983. Motor-unit discharge rates in maximal voluntary contractions of three human muscles. *Journal of neurophysiology,* 50**,** 1380-1392.

BEN-ZAKEN, S., ELIAKIM, A., NEMET, D., KASSEM, E. & MECKEL, Y. 2013. Increased prevalence of MnSOD genetic polymorphism in endurance and power athletes. *Free radical research,* 47**,** 1002-1008.

BEN-ZAKEN, S., ELIAKIM, A., NEMET, D., RABINOVICH, M., KASSEM, E. & MECKEL, Y. 2015. ACTN3 Polymorphism: Comparison Between Elite Swimmers and Runners. *Sports medicine-open,* 1**,** 13.

BENDIKSEN, M., BISCHOFF, R., RANDERS, M. B., MOHR, M., ROLLO, I., SUETTA, C., BANGSBO, J. & KRUSTRUP, P. 2012. The Copenhagen Soccer Test: physiological response and fatigue development. *Med Sci Sports Exerc,* 44**,** 1595-603.

BENDJABALLAH, M., SHIRAZI-ADL, A. & ZUKOR, D. 1997. Finite element analysis of human knee joint in varus-valgus. *Clinical biomechanics,* 12**,** 139-148.

BENJAMINI, Y. & HOCHBERG, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the royal statistical society. Series B (Methodological)***,** 289-300.

BENJAMINSE, A., HABU, A., SELL, T. C., ABT, J. P., FU, F. H., MYERS, J. B. & LEPHART, S. M. 2008. Fatigue alters lower extremity kinematics during a single-leg stop-jump task. *Knee Surg Sports Traumatol Arthrosc,* 16**,** 400-7.

BENNERMO, M., HELD, C., STEMME, S., ERICSSON, C. G., SILVEIRA, A., GREEN, F. & TORNVALL, P. 2004. Genetic predisposition of the interleukin-6 response to inflammation: implications for a variety of major diseases? *Clin Chem,* 50**,** 2136-40.

BENOIT, D. L. & DOWLING, J. J. 2006. In vivo assessment of elbow flexor work and activation during stretch-shortening cycle tasks. *J Electromyogr Kinesiol,* 16**,** 352-64.

BHAT, M. & ISMAIL, A. 2015. Vitamin D treatment protects against and reverses oxidative stress induced muscle proteolysis. *J Steroid Biochem Mol Biol,* 152**,** 171-9.

BIGLAND-RITCHIE, B., JOHANSSON, R., LIPPOLD, O. & WOODS, J. 1983. Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *Journal of neurophysiology,* 50**,** 313-324.

BIJUR, P. E., SILVER, W. & GALLAGHER, E. J. 2001. Reliability of the visual analog scale for measurement of acute pain. *Academic emergency medicine,* 8**,** 1153-1157.

BINDER-MACLEOD, S. A., LEE, S. C., FRITZ, A. D. & KUCHARSKI, L. J. 1998. New look at force-frequency relationship of human skeletal muscle: effects of fatigue. *Journal of neurophysiology,* 79**,** 1858-1868.

BIOLO, G., WILLIAMS, B. D., FLEMING, R. Y. & WOLFE, R. R. 1999. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes,* 48**,** 949-957.

BIOQUE, G., CRUSIUS, J. B. A., KOUTROUBAKIS, I., BOUMA, G., KOSTENSE, P. J., MEUWISSEN, S. G. M. & PENA, A. S. 1995. Allelic polymorphism in IL-1 beta and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. *Clin Exp Immunol,* 102**,** 379-83.

BIRESSI, S. & RANDO, T. A. Heterogeneity in the muscle satellite cell population. Seminars in cell & developmental biology, 2010. Elsevier, 845-854.

BLANCHARD, A., OHANIAN, V. & CRITCHLEY, D. 1989. The structure and function of α-actinin. *Journal of muscle research and cell motility,* 10**,** 280-289.

BLAU, H. M. & WEBSTER, C. 1981. Isolation and characterization of human muscle cells. *Proceedings of the National Academy of Sciences,* 78**,** 5623-5627.

BODINE, S. C., LATRES, E., BAUMHUETER, S., LAI, V. K.-M., NUNEZ, L., CLARKE, B. A., POUEYMIROU, W. T., PANARO, F. J., NA, E. & DHARMARAJAN, K. 2001a. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science,* 294**,** 1704-1708.

BODINE, S. C., LATRES, E., BAUMHUETER, S., LAI, V. K.-M., NUNEZ, L., CLARKE, B. A., POUEYMIROU, W. T., PANARO, F. J., NA, E., DHARMARAJAN, K., PAN, Z. Q., VALENZUELA, D. M., DECHIARA, T. M., STITT, T. N., YANCOPOULOS, G. D. & GLASS, D. J. 2001b. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science,* 294**,** 1704-8.

BOESEN, A. P., DIDERIKSEN, K., COUPPÉ, C., MAGNUSSON, S. P., SCHJERLING, P., BOESEN, M., KJÆR, M. & LANGBERG, H. 2013. Tendon and skeletal muscle matrix gene expression and functional responses to immobilisation and rehabilitation in young males: effect of growth hormone administration. *The Journal of physiology,* 591**,** 6039-6052.

BOHM, S., MERSMANN, F. & ARAMPATZIS, A. 2015. Human tendon adaptation in response to mechanical loading: a systematic review and meta-analysis of exercise intervention studies on healthy adults. *Sports medicine-open,* 1**,** 7.

BOPPART, M. D., VOLKER, S. E., ALEXANDER, N., BURKIN, D. J. & KAUFMAN, S. J. 2008. Exercise promotes alpha7 integrin gene transcription and protection of skeletal muscle. *Am J Physiol Regul Integr Comp Physiol,* 295**,** R1623-30.

BORG, G. A. 1982. Psychophysical bases of perceived exertion. *Med sci sports exerc,* 14**,** 377-381.

BORINSKAYA, S. A., GUREEV, A. S., ORLOVA, A. A., SANINA, E. D., KIM, A. A., GASEMIANRODSARI, F., SHIRMANOV, V. I., BALANOVSKY, O. P., REBRIKOV, D. V., KOSHECHKIN, A. V. & YANKOVSKY, N. K. 2013. Allele frequency distributions of -174G/C polymorphism in regulatory region of interleukin 6 gene (IL6) in Russian and worldwide populations. *Genetika,* 49**,** 113-24.

BORST, S. E. 2004. The role of TNF-α in insulin resistance. *Endocrine,* 23**,** 177-182.

BOSSUYT, F. M., GARCIA-PINILLOS, F., RAJA AZIDIN, R. M., VANRENTERGHEM, J. & ROBINSON, M. A. 2016. The Utility of a High-intensity Exercise Protocol to Prospectively Assess ACL Injury Risk. *Int J Sports Med,* 37**,** 125-33.

BRADLEY, P. S., DI MASCIO, M., PEART, D., OLSEN, P. & SHELDON, B. 2010. High-intensity activity profiles of elite soccer players at different performance levels. *The Journal of Strength & Conditioning Research,* 24**,** 2343-2351.

BRADLEY, P. S., SHELDON, W., WOOSTER, B., OLSEN, P., BOANAS, P. & KRUSTRUP, P. 2009. High-intensity running in English FA Premier League soccer matches. *Journal of sports sciences,* 27**,** 159-168.

BRANCACCIO, P., MAFFULLI, N. & LIMONGELLI, F. M. 2007. Creatine kinase monitoring in sport medicine. *British medical bulletin,* 81**,** 209-230.

BRING, D. K. I., PAULSON, K., RENSTROM, P., SALO, P., HART, D. A. & ACKERMANN, P. W. 2012. Residual substance P levels after capsaicin treatment correlate with tendon repair. *Wound Repair and Regeneration,* 20**,** 50-60.

BRINK, M., PRICE, S. R., CHRAST, J., BAILEY, J. L., ANWAR, A., MITCH, W. E. & DELAFONTAINE, P. 2001. Angiotensin II induces skeletal muscle wasting through enhanced protein degradation and down-regulates autocrine insulin-like growth factor I. *Endocrinology,* 142**,** 1489-1496.

BROCKETT, C. L., MORGAN, D. L. & PROSKE, U. 2004. Predicting hamstring strain injury in elite athletes. *Medicine & Science in Sports & Exercise,* 36**,** 379-387.

BROOK, M. S., WILKINSON, D. J., SMITH, K. & ATHERTON, P. J. 2016. The metabolic and temporal basis of muscle hypertrophy in response to resistance exercise. *Eur J Sport Sci,* 16**,** 633-44.

BROOKS, J. H., FULLER, C. W., KEMP, S. P. & REDDIN, D. B. 2006. Incidence, risk, and prevention of hamstring muscle injuries in professional rugby union. *The American journal of sports medicine,* 34**,** 1297-1306.

BROOS, S., MALISOUX, L., THEISEN, D., FRANCAUX, M., DELDICQUE, L. & THOMIS, M. A. 2012. Role of alpha-actinin-3 in contractile properties of human single muscle fibers: a case series study in paraplegics. *Plos One,* 7**,** 1-6.

BROOS, S., MALISOUX, L., THEISEN, D., VAN THIENEN, R., FRANCAUX, M., THOMIS, M. A. & DELDICQUE, L. 2018. The stiffness response of type IIa fibres after eccentric exercise-induced muscle damage is dependent on ACTN3 r577X polymorphism. *Eur J Sport Sci***,** 1-10.

BROOS, S., MALISOUX, L., THEISEN, D., VAN THIENEN, R., RAMAEKERS, M., JAMART, C., DELDICQUE, L., THOMIS, M. A. & FRANCAUX, M. 2016. Evidence for ACTN3 as a speed gene in isolated human muscle fibers. *PloS one,* 11**,** e0150594.

BROWN, A. D., CLOSE, G. L., SHARPLES, A. P. & STEWART, C. E. 2017. Murine myoblast migration: influence of replicative ageing and nutrition. *Biogerontology,* 18**,** 947-964.

BROWN, S., DAY, S. & DONNELLY, A. 1999. Indirect evidence of human skeletal muscle damage and collagen breakdown after eccentric muscle actions. *Journal of sports sciences,* 17**,** 397-402.

BROWN, S. J., CHILD, R. B., DAY, S. H. & DONNELLY, A. E. 1997a. Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. *Journal of sports sciences,* 15**,** 215-222.

BROWN, S. J., CHILD, R. B., DAY, S. H. & DONNELLY, A. E. 1997b. Indices of skeletal muscle damage and connective tissue breakdown following eccentric muscle contractions. *Eur J Appl Physiol Occup Physiol,* 75**,** 369-74.

BRUKNER, P. & CONNELL, D. 2015. 'Serious thigh muscle strains': beware the intramuscular tendon which plays an important role in difficult hamstring and quadriceps muscle strains. *British journal of sports medicine***,** bjsports-2015-095136.

BRUNNER, H. G., KORNELUK, R. G., COERWINKEL-DRIESSEN, M., MACKENZIE, A., SMEETS, H., LAMBERMON, H. M. M., VAN OOST, B. A., WIERINGA, B. & ROPERS, H.-H. 1989. Myotonic dystrophy is closely linked to the gene for muscle-type creatine kinase (CKMM). *Human genetics,* 81**,** 308-310.

BUCHHEIT, M. & MENDEZ-VILLANUEVA, A. 2013. Reliability and stability of anthropometric and performance measures in highly-trained young soccer players: effect of age and maturation. *Journal of Sports Sciences,* 31**,** 1332-1343.

BURDEN, A. 2010. How should we normalize electromyograms obtained from healthy participants? What we have learned from over 25 years of research. *Journal of electromyography and kinesiology,* 20**,** 1023-1035.

BUSH, M., BARNES, C., ARCHER, D. T., HOGG, B. & BRADLEY, P. S. 2015. Evolution of match performance parameters for various playing positions in the English Premier League. *Human movement science,* 39**,** 1-11.

BUTTERFIELD, T. A. 2010. Eccentric exercise in vivo: strain-induced muscle damage and adaptation in a stable system. *Exercise and sport sciences reviews,* 38**,** 51-60.

BYRNE, C., TWIST, C. & ESTON, R. 2004. Neuromuscular function after exercise-induced muscle damage. *Sports medicine,* 34**,** 49-69.

CANTINI, M., GIURISATO, E., RADU, C., TIOZZO, S., PAMPINELLA, F., SENIGAGLIA, D., ZANIOLO, G., MAZZOLENI, F. & VITIELLO, L. 2002. Macrophage-secreted myogenic factors: a promising tool for greatly enhancing the proliferative capacity of myoblasts in vitro and in vivo. *Neurol Sci,* 23**,** 189-94.

CARTEE, G. D., HEPPLE, R. T., BAMMAN, M. M. & ZIERATH, J. R. 2016. Exercise Promotes Healthy Aging of Skeletal Muscle. *Cell Metab,* 23**,** 1034-1047.

CATOIRE, M. & KERSTEN, S. 2015. The search for exercise factors in humans. *The FASEB Journal,* 29**,** 1615-1628.

CAUCI, S., DI SANTOLO, M., RYCKMAN, K. K., WILLIAMS, S. M. & BANFI, G. 2010. Variable number of tandem repeat polymorphisms of the interleukin-1 receptor antagonist gene IL-1RN: a novel association with the athlete status. *BMC medical genetics,* 11**,** 29.

CENTNER, T., YANO, J., KIMURA, E., MCELHINNY, A. S., PELIN, K., WITT, C. C., BANG, M.-L., TROMBITAS, K., GRANZIER, H. & GREGORIO, C. C. 2001. Identification of muscle specific ring finger proteins as potential regulators of the titin kinase domain1. *Journal of molecular biology,* 306**,** 717-726.

CHAMBERS, R. L. & MCDERMOTT, J. C. 1996. Molecular basis of skeletal muscle regeneration. *Can J Appl Physiol,* 21**,** 155-84.

CHAN-ROPER, M., HUNTER, I., MYRER, J. W., EGGETT, D. L. & SEELEY, M. K. 2012. Kinematic changes during a marathon for fast and slow runners. *Journal of sports science & medicine,* 11**,** 77.

CHAN, S., SETO, J. T., HOUWELING, P. J., YANG, N., NORTH, K. N. & HEAD, S. I. 2011. Properties of extensor digitorum longus muscle and skinned fibers from adult and aged male and female Actn3 knockout mice. *Muscle Nerve,* 43**,** 37-48.

CHEN, C.-H., YE, X., WANG, Y.-T., CHEN, Y.-S. & TSENG, W.-C. 2017. Differential Effects of Different Warm-up Protocols on Repeated Sprints-Induced Muscle Damage. *The Journal of Strength & Conditioning Research*.

CHEN, T. C., LIN, K.-Y., CHEN, H.-L., LIN, M.-J. & NOSAKA, K. 2011. Comparison in eccentric exercise-induced muscle damage among four limb muscles. *European journal of applied physiology,* 111**,** 211-223.

CHENG, L., ZHANG, D., ZHOU, L., ZHAO, J. & CHEN, B. 2015. Association between SLC30A8 rs13266634 Polymorphism and Type 2 Diabetes Risk: A Meta-Analysis. *Medical Science Monitor,* 21**,** 2178-2189.

CHEUNG, K., HUME, P. A. & MAXWELL, L. 2003. Delayed onset muscle soreness. *Sports Medicine,* 33**,** 145-164.

CHILDERS, M. K. & MCDONALD, K. S. 2004. Regulatory light chain phosphorylation increases eccentric contraction-induced injury in skinned fast-twitch fibers. *Muscle & nerve,* 29**,** 313-317.

CHIMIENTI, F., DEVERGNAS, S., PATTOU, F., SCHUIT, F., GARCIA-CUENCA, R., VANDEWALLE, B., KERR-CONTE, J., VAN LOMMEL, L., GRUNWALD, D., FAVIER, A. & SEVE, M. 2006. In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. *J Cell Sci,* 119**,** 4199-206.

CHUMANOV, E. S., HEIDERSCHEIT, B. C. & THELEN, D. G. 2007. The effect of speed and influence of individual muscles on hamstring mechanics during the swing phase of sprinting. *Journal of biomechanics,* 40**,** 3555-3562.

CHUMANOV, E. S., SCHACHE, A. G., HEIDERSCHEIT, B. C. & THELEN, D. G. 2011. Hamstrings are most susceptible to injury during the late swing phase of sprinting. *British journal of sports medicine***,** bjsports90176.

CLANSEY, A. C., HANLON, M., WALLACE, E. S. & LAKE, M. J. 2012. Effects of fatigue on running mechanics associated with tibial stress fracture risk. *Medicine and science in sports and exercise,* 44**,** 1917-1923.

CLARKE, M. & FEEBACK, D. L. 1996. Mechanical load induces sarcoplasmic wounding and FGF release in differentiated human skeletal muscle cultures. *The FASEB journal,* 10**,** 502-509.

CLARKSON, P. M., DEVANEY, J. M., GORDISH-DRESSMAN, H., THOMPSON, P. D., HUBAL, M. J., URSO, M., PRICE, T. B., ANGELOPOULOS, T. J., GORDON, P. M. & MOYNA, N. M. 2005a. ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women. *Journal of Applied Physiology,* 99**,** 154-163.

CLARKSON, P. M., HOFFMAN, E. P., ZAMBRASKI, E., GORDISH-DRESSMAN, H., KEARNS, A., HUBAL, M., HARMON, B. & DEVANEY, J. M. 2005b. ACTN3 and MLCK genotype associations with exertional muscle damage. *J Appl Physiol (1985),* 99**,** 564-9.

CLARKSON, P. M. & HUBAL, M. J. 2002. Exercise-induced muscle damage in humans. *American journal of physical medicine & rehabilitation,* 81**,** S52-S69.

CLARKSON, P. M., NOSAKA, K. & BRAUN, B. 1992. Muscle function after exercise-induced muscle damage and rapid adaptation. *Medicine and science in sports and exercise,* 24**,** 512-520.

CLOSE, R. 1972. Dynamic properties of mammalian skeletal muscles. *Physiological reviews,* 52**,** 129-197.

COLBERG, S. R., SIGAL, R. J., FERNHALL, B., REGENSTEINER, J. G., BLISSMER, B. J., RUBIN, R. R., CHASAN-TABER, L., ALBRIGHT, A. L. & BRAUN, B. 2010. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. *Diabetes care,* 33**,** e147-e167.

COLLINS, M. & RALEIGH, S. M. 2009. Genetic risk factors for musculoskeletal soft tissue injuries. *Medicine and Sport Science,* 54**,** 136-149.

CONSORTIUM, G. P. 2012. An integrated map of genetic variation from 1,092 human genomes. *Nature,* 491**,** 56-65.

CORATELLA, G., BELLIN, G., BEATO, M. & SCHENA, F. 2015. Fatigue affects peak joint torque angle in hamstrings but not in quadriceps. *Journal of sports sciences,* 33**,** 1276-1282.

COSTA, A. M., SILVA, A. J., GARRIDO, N. D., LOURO, H., DE OLIVEIRA, R. J. & BREITENFELD, L. 2009. Association between ACE D allele and elite short distance swimming. *European journal of applied physiology,* 106**,** 785-790.

COSTILL, D. L., PASCOE, D. D., FINK, W. J., ROBERGS, R. A., BARR, S. I. & PEARSON, D. 1990. Impaired muscle glycogen resynthesis after eccentric exercise. *Journal of Applied Physiology,* 69**,** 46-50.

CRAMERI, R., AAGAARD, P., QVORTRUP, K., LANGBERG, H., OLESEN, J. & KJÆR, M. 2007. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *The Journal of physiology,* 583**,** 365-380.

CRANE, J. D., ABADI, A., HETTINGA, B. P., OGBORN, D. I., MACNEIL, L. G., STEINBERG, G. R. & TARNOPOLSKY, M. A. 2013. Elevated mitochondrial oxidative stress impairs metabolic adaptations to exercise in skeletal muscle. *PloS One* 8**,** e81879.

CREMA, M. D., JARRAYA, M., ENGEBRETSEN, L., ROEMER, F. W., HAYASHI, D., DOMINGUES, R., SKAF, A. Y. & GUERMAZI, A. 2017. Imaging-detected acute muscle injuries in athletes participating in the Rio de Janeiro 2016 Summer Olympic Games. *Br J Sports Med***,** bjsports-2017-098247.

CROSS, T. M., GIBBS, N., HOUANG, M. T. & CAMERON, M. 2004. Acute quadriceps muscle strains: magnetic resonance imaging features and prognosis. *The American journal of sports medicine,* 32**,** 710-719.

CROWN, A., HE, X., HOLLY, J., LIGHTMAN, S. & STEWART, C. 2000. Characterisation of the IGF system in a primary adult human skeletal muscle cell model, and comparison of the effects of insulin and IGF-I on protein metabolism. *Journal of endocrinology,* 167**,** 403-415.

DA CUNHA NASCIMENTO, D., DE SOUSA, N. M. F., DE SOUSA NETO, I. V., TIBANA, R. A., DE SOUZA, V. C., VIEIRA, D. C. L., CAMARÇO, N. F., DE OLIVEIRA, S., DE ALMEIDA, J. A. & NAVALTA, J. 2015. Classification of pro-inflammatory status for interleukin-6 affects relative muscle strength in obese elderly women. *Aging clinical and experimental research,* 27**,** 791-797.

DAMAS, F., NOSAKA, K., LIBARDI, C. A., CHEN, T. C. & UGRINOWITSCH, C. 2016a. Susceptibility to Exercise-Induced Muscle Damage: a Cluster Analysis with a Large Sample. *Int J Sports Med,* 37**,** 633-40.

DAMAS, F., PHILLIPS, S. M., LIBARDI, C. A., VECHIN, F. C., LIXANDRÃO, M. E., JANNIG, P. R., COSTA, L. A., BACURAU, A. V., SNIJDERS, T. & PARISE, G. 2016b. Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage. *The Journal of physiology,* 594**,** 5209-5222.

DAMAS, F., UGRINOWITSCH, C., LIBARDI, C. A., JANNIG, P. R., HECTOR, A. J., MCGLORY, C., LIXANDRÃO, M. E., VECHIN, F. C., MONTENEGRO, H. & TRICOLI, V. 2018. Resistance training in young men induces muscle transcriptome-wide changes associated with muscle structure and metabolism refining the response to exercise-induced stress. *European Journal of Applied Physiology***,** 1-10.

DANSER, A. J., SCHALEKAMP, M. A., BAX, W. A., VAN DEN BRINK, A. M., SAXENA, P. R., RIEGGER, G. A. & SCHUNKERT, H. 1995. Angiotensin-converting enzyme in the human heart effect of the deletion/insertion polymorphism. *Circulation,* 92**,** 1387-1388.

DAVIS, M. E., GUMUCIO, J. P., SUGG, K. B., BEDI, A. & MENDIAS, C. L. 2013. MMP inhibition as a potential method to augment the healing of skeletal muscle and tendon extracellular matrix. *Journal of Applied Physiology,* 115**,** 884-891.

DE MOOR, M. H., SPECTOR, T. D., CHERKAS, L. F., FALCHI, M., HOTTENGA, J. J., BOOMSMA, D. I. & DE GEUS, E. J. 2007. Genome-wide linkage scan for athlete status in 700 British female DZ twin pairs. *Twin Research and Human Genetics,* 10**,** 812-820.

DEASY, B. M., LU, A., TEBBETS, J. C., FEDUSKA, J. M., SCHUGAR, R. C., POLLETT, J. B., SUN, B., URISH, K. L., GHARAIBEH, B. M. & CAO, B. 2007. A role for cell sex in stem cell–mediated skeletal muscle regeneration: female cells have higher muscle regeneration efficiency. *The Journal of cell biology,* 177**,** 73-86.

DEL COSO, J., SALINERO, J. J., LARA, B., GALLO-SALAZAR, C., ARECES, F., HERRERO, D. & PUENTE, C. 2017a. Polygenic Profile and Exercise-Induced Muscle Damage by a Competitive Half-Ironman. *Journal of strength and conditioning research*.

DEL COSO, J., VALERO, M., SALINERO, J. J., LARA, B., GALLO-SALAZAR, C. & ARECES, F. 2017b. Optimum polygenic profile to resist exertional rhabdomyolysis during a marathon. *PloS one,* 12**,** e0172965.

DELELLIS, K., INGLES, S., KOLONEL, L., MCKEAN-COWDIN, R., HENDERSON, B., STANCZYK, F. & PROBST-HENSCH, N. M. 2003. IGF1 genotype, mean plasma level and breast cancer risk in the Hawaii/Los Angeles multiethnic cohort. *British journal of cancer,* 88**,** 277-282.

DENDORFER, A., WOLFRUM, S., WAGEMANN, M., QADRI, F. & DOMINIAK, P. 2001. Pathways of bradykinin degradation in blood and plasma of normotensive and hypertensive rats. *Am J Physiol Heart Circ Physiol,* 280**,** H2182-8.

DENNIS, R. A., TRAPPE, T. A., SIMPSON, P., CARROLL, C., HUANG, B. E., NAGARAJAN, R., BEARDEN, E., GURLEY, C., DUFF, G. W. & EVANS, W. J. 2004. Interleukin-1 polymorphisms are associated with the inflammatory response in human muscle to acute resistance exercise. *The Journal of physiology,* 560**,** 617-626.

DESCHEPPER, C. F. 1994. Angiotensinogen: hormonal regulation and relative importance in the generation of angiotensin II. *Kidney Int,* 46**,** 1561-3.

DEUSTER, P. A., CONTRERAS-SESVOLD, C. L., O'CONNOR, F. G., CAMPBELL, W. W., KENNEY, K., CAPACCHIONE, J. F., LANDAU, M. E., MULDOON, S. M., RUSHING, E. J. & HELED, Y. 2013. Genetic polymorphisms associated with exertional rhabdomyolysis. *Eur J Appl Physiol,* 113**,** 1997-2004.

DEVANEY, J. M., HOFFMAN, E. P., GORDISH-DRESSMAN, H., KEARNS, A., ZAMBRASKI, E. & CLARKSON, P. M. 2007a. IGF-II gene region polymorphisms related to exertional muscle damage. *J Appl Physiol (1985),* 102**,** 1815-23.

DEVANEY, J. M., HOFFMAN, E. P., GORDISH-DRESSMAN, H., KEARNS, A., ZAMBRASKI, E. & CLARKSON, P. M. 2007b. IGF-II gene region polymorphisms related to exertional muscle damage. *Journal of applied physiology,* 102**,** 1815-1823.

DEWBERRY, R., HOLDEN, H., CROSSMAN, D. & FRANCIS, S. 2000. Interleukin-1 receptor antagonist expression in human endothelial cells and atherosclerosis. *Arterioscler Thromb Vasc Biol,* 20**,** 2394-400.

DI GIOVINE, F. S., TAKHSH, E., BLAKEMORE, A. I. F. & DUFF, G. W. 1992. Single base polymorphism at -511 in the human interleukin-1 beta gene (IL1 beta). *Hum Mol Genet,* 1**,** 450-450.

DIMCHEV, G. A., AL-SHANTI, N. & STEWART, C. E. 2013. Phospho-tyrosine phosphatase inhibitor Bpv (Hopic) enhances C2C12 myoblast migration in vitro. Requirement of PI3K/AKT and MAPK/ERK pathways. *Journal of muscle research and cell motility,* 34**,** 125-136.

DINARELLO, C. A. 2009. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol,* 27**,** 519-50.

DOBROWOLNY, G., GIACINTI, C., PELOSI, L., NICOLETTI, C., WINN, N., BARBERI, L., MOLINARO, M., ROSENTHAL, N. & MUSARÒ, A. 2005. Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. *The Journal of cell biology,* 168**,** 193-199.

DOESSING, S., HOLM, L., HEINEMEIER, K. M., FELDT-RASMUSSEN, U., SCHJERLING, P., QVORTRUP, K., LARSEN, J. O., NIELSEN, R. H., FLYVBJERG, A. & KJAER, M. 2010. GH and IGF1 levels are positively associated with musculotendinous collagen expression: experiments in acromegalic and GH deficiency patients. *European Journal of Endocrinology,* 163**,** 853-862.

DOUSSET, E., AVELA, J., ISHIKAWA, M., KALLIO, J., KUITUNEN, S., KYROLAINEN, H., LINNAMO, V. & KOMI, P. V. 2007. Bimodal recovery pattern in human skeletal muscle induced by exhaustive stretch-shortening cycle exercise. *Medicine and science in sports and exercise,* 39**,** 453-460.

DUAN, C., REN, H. & GAO, S. 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. *Gen Comp Endocrinol,* 167**,** 344-51.

DUANCE, V. C., RESTALL, D. J., BEARD, H., BOURNE, F. J. & BAILEY, A. J. 1977. The location of three collagen types in skeletal muscle. *FEBS letters,* 79**,** 248-252.

DUHIG, S., SHIELD, A. J., OPAR, D., GABBETT, T. J., FERGUSON, C. & WILLIAMS, M. 2016. Effect of high-speed running on hamstring strain injury risk. *Br J Sports Med,* 50**,** 1536-1540.

DUNDON, J. M., CIRILLO, J. & SEMMLER, J. G. 2008. Low-frequency fatigue and neuromuscular performance after exercise-induced damage to elbow flexor muscles. *Journal of Applied Physiology,* 105**,** 1146-1155.

DURAND-BUSH, N. & SALMELA, J. H. 2002. The development and maintenance of expert athletic performance: Perceptions of world and Olympic champions. *Journal of applied sport psychology,* 14**,** 154-171.

DUTTO, D. J. & BRAUN, W. A. 2004. DOMS-associated changes in ankle and knee joint dynamics during running. *Medicine and science in sports and exercise,* 36**,** 560-566.

EFTESTØL, E., EGNER, I. M., LUNDE, I. G., ELLEFSEN, S., ANDERSEN, T., SJÅLAND, C., GUNDERSEN, K. & BRUUSGAARD, J. C. 2016. Increased hypertrophic response with

increased mechanical load in skeletal muscles receiving identical activity patterns. *American Journal of Physiology-Cell Physiology,* 311**,** C616-C629.
EIRALE, C., TOL, J., FAROOQ, A., SMILEY, F. & CHALABI, H. 2013. Low injury rate strongly correlates with team success in Qatari professional football. *Br J Sports Med,* 47**,** 807-808.
EKSTRAND, J. 2013. Keeping your top players on the pitch: the key to football medicine at a professional level. BMJ Publishing Group Ltd and British Association of Sport and Exercise Medicine.
EKSTRAND, J., HÄGGLUND, M. & WALDÉN, M. 2011. Epidemiology of muscle injuries in professional football (soccer). *The American journal of sports medicine,* 39**,** 1226-1232.
EKSTRAND, J., WALDÉN, M. & HÄGGLUND, M. 2016. Hamstring injuries have increased by 4% annually in men's professional football, since 2001: a 13-year longitudinal analysis of the UEFA Elite Club injury study. *British journal of sports medicine,* 50**,** 731-737.
ENGLER, A. J., SEN, S., SWEENEY, H. L. & DISCHER, D. E. 2006. Matrix elasticity directs stem cell lineage specification. *Cell,* 126**,** 677-689.
ERSHLER, W. B. & KELLER, E. T. 2000. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annual review of medicine,* 51**,** 245-270.
ERSKINE, R. M., FLETCHER, G. & FOLLAND, J. P. 2014a. The contribution of muscle hypertrophy to strength changes following resistance training. *European journal of applied physiology,* 114**,** 1239-1249.
ERSKINE, R. M., JONES, D. A., MAFFULLI, N., WILLIAMS, A. G., STEWART, C. E. & DEGENS, H. 2011. What causes in vivo muscle specific tension to increase following resistance training? *Experimental physiology,* 96**,** 145-155.
ERSKINE, R. M., JONES, D. A., MAGANARIS, C. N. & DEGENS, H. 2009. In vivo specific tension of the human quadriceps femoris muscle. *European journal of applied physiology,* 106**,** 827-838.
ERSKINE, R. M., JONES, D. A., WILLIAMS, A. G., STEWART, C. E. & DEGENS, H. 2010a. Inter-individual variability in the adaptation of human muscle specific tension to progressive resistance training. *European journal of applied physiology,* 110**,** 1117-1125.
ERSKINE, R. M., JONES, D. A., WILLIAMS, A. G., STEWART, C. E. & DEGENS, H. 2010b. Resistance training increases in vivo quadriceps femoris muscle specific tension in young men. *Acta physiologica,* 199**,** 83-89.
ERSKINE, R. M., WILLIAMS, A. G., JONES, D. A., STEWART, C. E. & DEGENS, H. 2012. Do PTK2 gene polymorphisms contribute to the interindividual variability in muscle strength and the response to resistance training? A preliminary report. *Journal of applied physiology,* 112**,** 1329-1334.
ERSKINE, R. M., WILLIAMS, A. G., JONES, D. A., STEWART, C. E. & DEGENS, H. 2014b. The individual and combined influence of ACE and ACTN3 genotypes on muscle phenotypes before and after strength training. *Scandinavian journal of medicine & science in sports,* 24**,** 642-648.
ESFORMES, J. I., NARICI, M. V. & MAGANARIS, C. N. 2002. Measurement of human muscle volume using ultrasonography. *European journal of applied physiology,* 87**,** 90-92.
EVANGELIDIS, P., MASSEY, G. J., FERGUSON, R. A., WHEELER, P. C., PAIN, M. T. & FOLLAND, J. P. 2016. The functional significance of hamstrings composition: is it

really a ‘fast’muscle group? *Scandinavian journal of medicine & science in sports,* 27**,** 1181-1189.
EVANGELIDIS, P. E., MASSEY, G. J., PAIN, M. & FOLLAND, J. P. 2014. Biceps Femoris Aponeurosis Size: A Potential Risk Factor for Strain Injury? *Medicine and science in sports and exercise*.
EYNON, N., HANSON, E. D., LUCIA, A., HOUWELING, P. J., GARTON, F., NORTH, K. N. & BISHOP, D. J. 2013. Genes for elite power and sprint performance: ACTN3 leads the way. *Sports Medicine,* 43**,** 803-817.
FARTHING, J. P., KRENTZ, J. R. & MAGNUS, C. R. 2009. Strength training the free limb attenuates strength loss during unilateral immobilization. *J Appl Physiol (1985),* 106**,** 830-6.
FAZELZADEH, P., HANGELBROEK, R. W., TIELAND, M., DE GROOT, L. C., VERDIJK, L. B., VAN LOON, L. J., SMILDE, A. K., ALVES, R. D., VERVOORT, J. & MÜLLER, M. J. J. O. P. R. 2016. The muscle metabolome differs between healthy and frail older adults. *Journal of Proteome Research,* 15**,** 499-509.
FERNÁNDEZ, A. M., DUPONT, J., FARRAR, R. P., LEE, S., STANNARD, B. & LE ROITH, D. 2002. Muscle-specific inactivation of the IGF-I receptor induces compensatory hyperplasia in skeletal muscle. *Journal of Clinical Investigation,* 109**,** 347-355.
FIELDING, R. A., MANFREDI, T. J., DING, W. E. N. J. I. N. G., FIATARONE, M. A., EVANS, W. J. & CANNON, J. G. 1993. Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 265**,** R166-R172.
FIELDING, R. A., MEREDITH, C. N., O'REILLY, K. P., FRONTERA, W. R., CANNON, J. G. & EVANS, W. J. 1991. Enhanced protein breakdown after eccentric exercise in young and older men. *Journal of Applied Physiology,* 71**,** 674-679.
FISCHER, C. P. 2006. Interleukin-6 in acute exercise and training: what is the biological relevance? *Exerc Immunol Rev,* 12**,** 6-33.
FISCHER, C. P., HISCOCK, N. J., PENKOWA, M., BASU, S., VESSBY, B., KALLNER, A., SJÖBERG, L. B. & PEDERSEN, B. K. 2004. Supplementation with vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. *The journal of physiology,* 558**,** 633-645.
FISHMAN, D., FAULDS, G., JEFFERY, R., MOHAMED-ALI, V., YUDKIN, J. S., HUMPHRIES, S. & WOO, P. 1998. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest,* 102**,** 1369-76.
FITZSIMONS, M., DAWSON, B., WARD, D. & WILKINSON, A. 1993. Cycling and running tests of repeated sprint ability. *Australian Journal of Science and Medicine in Sport,* 25**,** 82-82.
FLANN, K. L., LASTAYO, P. C., MCCLAIN, D. A., HAZEL, M. & LINDSTEDT, S. L. 2011. Muscle damage and muscle remodeling: no pain, no gain? *The Journal of experimental biology,* 214**,** 674-679.
FLORINI, J. R., EWTON, D. Z. & COOLICAN, S. A. 1996. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev,* 17**,** 481-517.
FLÜCK, M., CHIQUET, M., SCHMUTZ, S., MAYET-SORNAY, M.-H. & DESPLANCHES, D. 2003. Reloading of atrophied rat soleus muscle induces tenascin-C expression around damaged muscle fibers. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 284**,** R792-R801.

FOSTER, B., MORSE, C. I., ONAMBELE-PEARSON, G., AHMETOV, I. I. & WILLIAMS, A. G. 2012. Genetic variation, protein composition and potential influences on tendon properties in humans. *The open sports medicine journal,* 6**,** 8-21.

FOULSTONE, E. J., HUSER, C., CROWN, A. L., HOLLY, J. M. & STEWART, C. E. 2004. Differential signalling mechanisms predisposing primary human skeletal muscle cells to altered proliferation and differentiation: roles of IGF-I and TNFα. *Experimental cell research,* 294**,** 223-235.

FRECKLETON, G. & PIZZARI, T. 2013. Risk factors for hamstring muscle strain injury in sport: a systematic review and meta-analysis. *British journal of sports medicine,* 47**,** 351-358.

FREITAS, S. R., MARMELEIRA, J., VALAMATOS, M. J., BLAZEVICH, A. & MIL-HOMENS, P. 2018. Ultrasonographic Measurement of the Biceps Femoris Long-Head Muscle Architecture. *Journal of Ultrasound in Medicine,* 37**,** 977-986.

FRIDEN, J., KJÖRELL, U. & THORNELL, L.-E. 1984. Delayed muscle soreness and cytoskeletal alterations: an immunocytological study in man. *International journal of sports medicine,* 5**,** 15-18.

FRIDEN, J. & LIEBER, R. L. 1992. Structural and mechanical basis of exercise-induced muscle injury. *Med Sci Sports Exerc,* 24**,** 521-30.

FRIDEN, J. & LIEBER, R. L. 2001. Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. *Acta Physiologica Scandinavica,* 171**,** 321-326.

FRIDEN, J., SJÖSTRÖM, M. & EKBLOM, B. 1981. A morphological study of delayed muscle soreness. *Experientia,* 37**,** 506-507.

FRY, C. S., KIRBY, T. J., KOSMAC, K., MCCARTHY, J. J. & PETERSON, C. A. 2017. Myogenic progenitor cells control extracellular matrix production by fibroblasts during skeletal muscle hypertrophy. *Cell stem cell,* 20**,** 56-69.

FUJITA, S., RASMUSSEN, B. B., CADENAS, J. G., GRADY, J. J. & VOLPI, E. 2006. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. *American Journal of Physiology-Endocrinology and Metabolism,* 291**,** E745-E754.

FUNGHETTO, S. S., PRESTES, J., SILVA ADE, O., FARIAS, D. L., TEIXEIRA, T. G., VIEIRA, D. C., SOUZA, V. C., SOUSA, N. M., NAVALTA, J. W., MELO, G. F. & KARNIKOWSKI, M. G. 2013. Interleukin-6 -174G/C gene polymorphism affects muscle damage response to acute eccentric resistance exercise in elderly obese women. *Exp Gerontol,* 48**,** 1255-9.

GABBETT, T. J., HULIN, B. T., BLANCH, P. & WHITELEY, R. 2016. High training workloads alone do not cause sports injuries: how you get there is the real issue. BMJ Publishing Group Ltd and British Association of Sport and Exercise Medicine.

GANDEVIA, S. C. 2001. Spinal and supraspinal factors in human muscle fatigue. *Physiological reviews,* 81**,** 1725-1789.

GARG, K. & BOPPART, M. D. 2016. Influence of exercise and aging on extracellular matrix composition in the skeletal muscle stem cell niche. *Journal of Applied Physiology,* 121**,** 1053-1058.

GARTON, F., SETO, J., QUINLAN, K., YANG, N., HOUWELING, P. & NORTH, K. 2013. α-Actinin-3 deficiency alters muscle adaptation in response to denervation and immobilization. *Human molecular genetics***,** ddt580.

GARTON, F. C., HOUWELING, P. J., VUKCEVIC, D., MEEHAN, L. R., LEE, F. X., LEK, M., ROESZLER, K. N., HOGARTH, M. W., TIONG, C. F. & ZANNINO, D. 2018a. The Effect

of ACTN3 Gene Doping on Skeletal Muscle Performance. *The American Journal of Human Genetics,* 102**,** 845-857.
GARTON, F. C., HOUWELING, P. J., VUKCEVIC, D., MEEHAN, L. R., LEE, F. X. Z., LEK, M., ROESZLER, K. N., HOGARTH, M. W., TIONG, C. F., ZANNINO, D., YANG, N., LESLIE, S., GREGOREVIC, P., HEAD, S. I., SETO, J. T. & NORTH, K. N. 2018b. The Effect of ACTN3 Gene Doping on Skeletal Muscle Performance. *Am J Hum Genet,* 102**,** 845-857.
GATTAZZO, F., URCIUOLO, A. & BONALDO, P. 2014. Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochimica et Biophysica Acta (BBA)-General Subjects,* 1840**,** 2506-2519.
GAUTEL, M. 2011a. Cytoskeletal protein kinases: titin and its relations in mechanosensing. *Pflügers Archiv-European Journal of Physiology,* 462**,** 119-134.
GAUTEL, M. 2011b. The sarcomeric cytoskeleton: who picks up the strain? *Current opinion in cell biology,* 23**,** 39-46.
GAYAGAY, G., YU, B., HAMBLY, B., BOSTON, T., HAHN, A., CELERMAJER, D. S. & TRENT, R. J. 1998. Elite endurance athletes and the ACE I allele--the role of genes in athletic performance. *Hum Genet,* 103**,** 48-50.
GEORGIADES, E., KLISSOURAS, V., BAULCH, J., WANG, G. & PITSILADIS, Y. 2017. Why nature prevails over nurture in the making of the elite athlete. *BMC genomics,* 18**,** 835.
GIACHELLI, C. M., LOMBARDI, D., JOHNSON, R. J., MURRY, C. E. & ALMEIDA, M. 1998. Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli in vivo. *Am J Pathol,* 152**,** 353-8.
GIACOPELLI, F., MARCIANO, R., PISTORIO, A., CATARSI, P., CANINI, S., KARSENTY, G. & RAVAZZOLO, R. 2004. Polymorphisms in the osteopontin promoter affect its transcriptional activity. *Physiol Genomics,* 20**,** 87-96.
GILLIES, A. R. & LIEBER, R. L. 2011. Structure and function of the skeletal muscle extracellular matrix. *Muscle & nerve,* 44**,** 318-331.
GILLIVER, S., DEGENS, H., RITTWEGER, J., SARGEANT, A. & JONES, D. 2009. Variation in the determinants of power of chemically skinned human muscle fibres. *Experimental physiology,* 94**,** 1070-1078.
GIRARD, O., MENDEZ-VILLANUEVA, A. & BISHOP, D. 2011. Repeated-Sprint Ability—Part I. *Sports medicine,* 41**,** 673-694.
GIRDEN, E. R. 1992. *ANOVA: Repeated measures*, Sage.
GLAISTER, M., HOWATSON, G., PATTISON, J. R. & MCINNES, G. 2008. The reliability and validity of fatigue measures during multiple-sprint work: an issue revisited. *The Journal of Strength & Conditioning Research,* 22**,** 1597-1601.
GLUND, S., DESHMUKH, A., LONG, Y. C., MOLLER, T., KOISTINEN, H. A., CAIDAHL, K., ZIERATH, J. R. & KROOK, A. 2007. Interleukin-6 directly increases glucose metabolism in resting human skeletal muscle. *Diabetes,* 56**,** 1630-1637.
GOETSCH, K. P., MYBURGH, K. H. & NIESLER, C. U. 2013. In vitro myoblast motility models: investigating migration dynamics for the study of skeletal muscle repair. *Journal of muscle research and cell motility,* 34**,** 333-347.
GOETSCH, K. P. & NIESLER, C. U. 2011. Optimization of the scratch assay for in vitro skeletal muscle wound healing analysis. *Analytical biochemistry,* 411**,** 158-160.
GOETSCH, S. C., HAWKE, T. J., GALLARDO, T. D., RICHARDSON, J. A. & GARRY, D. J. 2003. Transcriptional profiling and regulation of the extracellular matrix during muscle regeneration. *Physiological genomics,* 14**,** 261-271.

GOMEZ-CABRERA, M.-C., DOMENECH, E. & VIÑA, J. 2008. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radical Biology and Medicine,* 44**,** 126-131.

GORDON, P. M., LIU, D., SARTOR, M. A., IGLAYREGER, H. B., PISTILLI, E. E., GUTMANN, L., NADER, G. A. & HOFFMAN, E. P. 2012. Resistance exercise training influences skeletal muscle immune activation: a microarray analysis. *Journal of applied physiology,* 112**,** 443-453.

GORDON, S. E., DAVIS, B. S., CARLSON, C. J. & BOOTH, F. W. 2001. ANG II is required for optimal overload-induced skeletal muscle hypertrophy. *American Journal of Physiology-Endocrinology And Metabolism,* 280**,** E150-E159.

GRANACHER, U., LESINSKI, M., BÜSCH, D., MUEHLBAUER, T., PRIESKE, O., PUTA, C., GOLLHOFER, A. & BEHM, D. G. 2016. Effects of resistance training in youth athletes on muscular fitness and athletic performance: a conceptual model for long-term athlete development. *Frontiers in physiology,* 7**,** 164.

GREALY, R., HERRUER, J., SMITH, C. L., HILLER, D., HASELER, L. J. & GRIFFITHS, L. R. 2015. Evaluation of a 7-Gene Genetic Profile for Athletic Endurance Phenotype in Ironman Championship Triathletes. *PloS one,* 10**,** e0145171.

GREGORY, C. M. & BICKEL, C. S. 2005. Recruitment patterns in human skeletal muscle during electrical stimulation. *Physical therapy,* 85**,** 358-364.

GRIFFIN, C. A., APPONI, L. H., LONG, K. K. & PAVLATH, G. K. 2010. Chemokine expression and control of muscle cell migration during myogenesis. *Journal of cell science,* 123**,** 3052-3060.

GROBLER, L., COLLINS, M. & LAMBERT, M. I. 2004. Remodelling of skeletal muscle following exercise-induced muscle damage: review article. *International SportMed Journal,* 5**,** 67-83.

GROHMANN, M., FOULSTONE, E., WELSH, G., HOLLY, J., SHIELD, J., CROWNE, E. & STEWART, C. 2005. Isolation and validation of human prepubertal skeletal muscle cells: maturation and metabolic effects of IGF-I, IGFBP-3 and TNFα. *The Journal of physiology,* 568**,** 229-242.

GROUNDS, M., SOROKIN, L. & WHITE, J. 2005. Strength at the extracellular matrix–muscle interface. *Scandinavian journal of medicine & science in sports,* 15**,** 381-391.

GRUNDBERG, E., BRANDSTROM, H., RIBOM, E. L., LJUNGGREN, O., MALLMIN, H. & KINDMARK, A. 2004. Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. *European Journal of Endocrinology,* 150**,** 323-328.

GUEX, K., GOJANOVIC, B. & MILLET, G. P. 2012. Influence of hip-flexion angle on hamstrings isokinetic activity in sprinters. *J Athl Train,* 47**,** 390-5.

GUSTAFSSON, E., ASZÓDI, A., ORTEGA, N., HUNZIKER, E. B., DENKER, H. W., WERB, Z. & FÄSSLER, R. 2003. Role of collagen type II and perlecan in skeletal development. *Annals of the New York Academy of Sciences,* 995**,** 140-150.

GYSEL, T., CALDERS, P., CAMBIER, D., ROMAN DE METTELINGE, T., KAUFMAN, J., TAES, Y., ZMIERCZAK, H.-G. & GOEMAERE, S. 2014. Association between insulin resistance, lean mass and muscle torque/force in proximal versus distal body parts in healthy young men. *Journal of musculoskeletal & neuronal interactions,* 14**,** 41-49.

HÄGGLUND, M., WALDÉN, M., MAGNUSSON, H., KRISTENSON, K., BENGTSSON, H. & EKSTRAND, J. 2013. Injuries affect team performance negatively in professional football: an 11-year follow-up of the UEFA Champions League injury study. *Br J Sports Med***,** bjsports-2013-092215.

HAN, Y. J., MA, S.-F., WADE, M. S., FLORES, C. & GARCIA, J. G. 2012. An intronic MYLK variant associated with inflammatory lung disease regulates promoter activity of the smooth muscle myosin light chain kinase isoform. *Journal of molecular medicine,* 90**,** 299-308.

HANGELBROEK, R. W., KNUIMAN, P., TIELAND, M. & DE GROOT, L. C. J. E. G. 2018. Attenuated strength gains during prolonged resistance exercise training in older adults with high inflammatory status. 106**,** 154-158.

HANSEN, M., BOESEN, A., HOLM, L., FLYVBJERG, A., LANGBERG, H. & KJÆR, M. 2013. Local administration of insulin-like growth factor-I (IGF-I) stimulates tendon collagen synthesis in humans. *Scandinavian journal of medicine & science in sports,* 23**,** 614-619.

HARA, A., SAKAI, N., FURUICHI, K., SAKAI, Y., TAKEYA, M., BUCALA, R., MUKAIDA, N., TAKUWA, Y., MATSUSHIMA, K. & KANEKO, S. 2013. CCL2/CCR2 augments the production of transforming growth factor-beta1, type 1 collagen and CCL2 by human CD45-/collagen 1-positive cells under high glucose concentrations. *Clinical and experimental nephrology,* 17**,** 793-804.

HARDY, D., BESNARD, A., LATIL, M., JOUVION, G., BRIAND, D., THÉPENIER, C., PASCAL, Q., GUGUIN, A., GAYRAUD-MOREL, B. & CAVAILLON, J.-M. 2016. Comparative study of injury models for studying muscle regeneration in mice. *PloS one,* 11**,** e0147198.

HARMON, B. T., ORKUNOGLU-SUER, E. F., ADHAM, K., LARKIN, J. S., GORDISH-DRESSMAN, H., CLARKSON, P. M., THOMPSON, P. D., ANGELOPOULOS, T. J., GORDON, P. M. & MOYNA, N. M. 2010. CCL2 and CCR2 variants are associated with skeletal muscle strength and change in strength with resistance training. *Journal of applied physiology,* 109**,** 1779-1785.

HARRIES, S. K., LUBANS, D. R. & CALLISTER, R. 2012. Resistance training to improve power and sports performance in adolescent athletes: A systematic review and meta-analysis. *Journal of Science and Medicine in Sport,* 15**,** 532-540.

HARRISON, S. & GEPPETTI, P. 2001. Substance p. *The international journal of biochemistry & cell biology,* 33**,** 555-576.

HAWKE, T. J. & GARRY, D. J. 2001. Myogenic satellite cells: physiology to molecular biology. *J Appl Physiol (1985),* 91**,** 534-51.

HE, J., CHEN, Y., LIN, Y., ZHANG, W., CAI, Y., CHEN, F., LIAO, Q., YIN, Z., WANG, Y., TAO, S., LIN, X., HUANG, P., CUI, L. & SHAO, Y. 2017. Association study of MCP-1 promoter polymorphisms with the susceptibility and progression of sepsis. *PLoS One,* 12**,** e0176781.

HEAD, S. I., CHAN, S., HOUWELING, P. J., QUINLAN, K. G. R., MURPHY, R., WAGNER, S., FRIEDRICH, O. & NORTH, K. N. 2015. Altered Ca2+ kinetics associated with α-actinin-3 deficiency may explain positive selection for ACTN3 null allele in human evolution. *PLoS genetics,* 11.

HELED, Y., BLOOM, M. S., WU, T. J., STEPHENS, Q. & DEUSTER, P. A. 2007. CM-MM and ACE genotypes and physiological prediction of the creatine kinase response to exercise. *Journal of Applied Physiology,* 103**,** 504-510.

HENNEMAN, E., SOMJEN, G. & CARPENTER, D. O. 1965. Functional significance of cell size in spinal motoneurons. *Journal of neurophysiology,* 28**,** 560-580.

HERMENS, H. J., FRERIKS, B., DISSELHORST-KLUG, C. & RAU, G. 2000. Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of electromyography and Kinesiology,* 10**,** 361-374.

HERRING, B. P., DIXON, S. & GALLAGHER, P. J. 2000. Smooth muscle myosin light chain kinase expression in cardiac and skeletal muscle. *American Journal of Physiology-Cell Physiology,* 279**,** C1656-C1664.

HICKS, K. M., ONAMBELE-PEARSON, G. L., WINWOOD, K. & MORSE, C. I. 2017. Muscle-Tendon Unit Properties during Eccentric Exercise Correlate with the Creatine Kinase Response. *Front Physiol,* 8**,** 657.

HIGASHIHARA, A., ONO, T., KUBOTA, J., OKUWAKI, T. & FUKUBAYASHI, T. 2010. Functional differences in the activity of the hamstring muscles with increasing running speed. *Journal of sports sciences,* 28**,** 1085-1092.

HILL, M. & GOLDSPINK, G. 2003. Expression and Splicing of the Insulin-Like Growth Factor Gene in Rodent Muscle is Associated with Muscle Satellite (stem) Cell Activation following Local Tissue Damage. *The Journal of physiology,* 549**,** 409-418.

HIRATA, A., MASUDA, S., TAMURA, T., KAI, K., OJIMA, K., FUKASE, A., MOTOYOSHI, K., KAMAKURA, K., MIYAGOE-SUZUKI, Y. & TAKEDA, S. 2003. Expression profiling of cytokines and related genes in regenerating skeletal muscle after cardiotoxin injection: a role for osteopontin. *Am J Pathol,* 163**,** 203-15.

HOFFMAN, E. P., GORDISH-DRESSMAN, H., MCLANE, V. D., DEVANEY, J. M., THOMPSON, P. D., VISICH, P., GORDON, P. M., PESCATELLO, L. S., ZOELLER, R. F. & MOYNA, N. M. 2013. Alterations in osteopontin modify muscle size in females in both humans and mice. *Medicine and science in sports and exercise,* 45**,** 1060.

HOLMES, A. G., WATT, M. J. & FEBBRAIO, M. A. 2004. Suppressing lipolysis increases interleukin-6 at rest and during prolonged moderate-intensity exercise in humans. *Journal of Applied Physiology,* 97**,** 689-696.

HOPKINSON, N. S., LI, K. W., KEHOE, A., HUMPHRIES, S. E., ROUGHTON, M., MOXHAM, J., MONTGOMERY, H. & POLKEY, M. I. 2008. Vitamin D receptor genotypes influence quadriceps strength in chronic obstructive pulmonary disease–. *The American journal of clinical nutrition,* 87**,** 385-390.

HORNBERGER, T. A., MCLOUGHLIN, T. J., LESZCZYNSKI, J. K., ARMSTRONG, D. D., JAMESON, R. R., BOWEN, P. E., HWANG, E. S., HOU, H., MOUSTAFA, M. E., CARLSON, B. A., HATFIELD, D. L., DIAMOND, A. M. & ESSER, K. A. 2003. Selenoprotein-deficient transgenic mice exhibit enhanced exercise-induced muscle growth. *J Nutr,* 133**,** 3091-7.

HOWATSON, G. & MILAK, A. 2009. Exercise-induced muscle damage following a bout of sport specific repeated sprints. *The Journal of Strength & Conditioning Research,* 23**,** 2419-2424.

HOWATSON, G. & VAN SOMEREN, K. A. 2008. The prevention and treatment of exercise-induced muscle damage. *Sports Medicine,* 38**,** 483-503.

HUANG, P., FENG, L., OLDHAM, E. A., KEATING, M. J. & PLUNKETT, W. 2000. Superoxide dismutase as a target for the selective killing of cancer cells. *Nature,* 407**,** 390-5.

HUBAL, M. J., CHEN, T. C., THOMPSON, P. D. & CLARKSON, P. M. 2008. Inflammatory gene changes associated with the repeated-bout effect. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 294**,** R1628-R1637.

HUBAL, M. J. & CLARKSON, P. M. 2009. Counterpoint: Estrogen and sex do not significantly influence post-exercise indexes of muscle damage, inflammation, and repair. *Journal of applied physiology,* 106**,** 1012-1014.

HUBAL, M. J., DEVANEY, J. M., HOFFMAN, E. P., ZAMBRASKI, E. J., GORDISH-DRESSMAN, H., KEARNS, A. K., LARKIN, J. S., ADHAM, K., PATEL, R. R. & CLARKSON, P. M. 2010.

CCL2 and CCR2 polymorphisms are associated with markers of exercise-induced skeletal muscle damage. *Journal of Applied Physiology,* 108**,** 1651-1658.
HUBAL, M. J., GORDISH-DRESSMAN, H., THOMPSON, P. D., PRICE, T. B., HOFFMAN, E. P., ANGELOPOULOS, T. J., GORDON, P. M., MOYNA, N. M., PESCATELLO, L. S. & VISICH, P. S. 2005. Variability in muscle size and strength gain after unilateral resistance training. *Med Sci Sports Exerc,* 37**,** 964-72.
HUCHO, T. & LEVINE, J. D. 2007. Signaling pathways in sensitization: toward a nociceptor cell biology. *Neuron,* 55**,** 365-376.
HUDSON, M. B. & PRICE, S. R. 2013. Calcineurin: a poorly understood regulator of muscle mass. *The international journal of biochemistry & cell biology,* 45**,** 2173-2178.
HUERTA-ALARDÍN, A. L., VARON, J. & MARIK, P. E. 2005. Bench-to-bedside review: rhabdomyolysis—an overview for clinicians. *Crit care,* 9**,** 158-169.
HUGHES, D. C., DAY, S. H., AHMETOV, I. I. & WILLIAMS, A. G. 2011. Genetics of muscle strength and power: polygenic profile similarity limits skeletal muscle performance. *Journal of sports sciences,* 29**,** 1425-1434.
HUGHES, D. C., WALLACE, M. A. & BAAR, K. 2015. Effects of aging, exercise and disease on force transfer in skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism,* 309**,** E1-E10.
HUGHES, S. M. & BLAU, H. M. 1990. Migration of myoblasts across basal lamina during skeletal muscle development. *Nature,* 345**,** 350-353.
HYLDAHL, R. D. & HUBAL, M. J. 2014. Lengthening our Perspective: Morphological, Cellular and Molecular Respones to Eccentric Exercise *Muscle & Nerve,* 49**,** 155-170.
HYLDAHL, R. D., NELSON, B., XIN, L., WELLING, T., GROSCOST, L., HUBAL, M. J., CHIPKIN, S., CLARKSON, P. M. & PARCELL, A. C. 2015. Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. *The FASEB Journal,* 29**,** 2894-2904.
INOUE, M., TOKUYAMA, S., NAKAYAMADA, H. & UEDA, H. 1998. In vivo signal transduction of tetrodotoxin-sensitive nociceptive responses by substance P given into the planta of the mouse hind limb. *Cellular and molecular neurobiology,* 18**,** 555-561.
IWAO-KOIZUMI, K., OTA, T., HAYASHIDA, M., YONETANI, Y. & NAKATA, K. 2014. The ACTN3 Gene is a Potential Biomarker for the Risk of Non-Contact Sports Injury in Female Athletes. *J Mol Biomark Diagn S,* 6**,** 2.
JAMKRAJANG, P., ROBINSON, M. A., LIMROONGREUNGRAT, W. & VANRENTERGHEM, J. 2017. Can segmental model reductions quantify whole-body balance accurately during dynamic activities? *Gait Posture,* 56**,** 37-41.
JANI, K. & SCHÖCK, F. 2009. Molecular mechanisms of mechanosensing in muscle development. *Developmental Dynamics,* 238**,** 1526-1534.
JENKINS, N. D., HOUSH, T. J., TRAYLOR, D. A., COCHRANE, K. C., BERGSTROM, H. C., LEWIS, R. W., SCHMIDT, R. J., JOHNSON, G. O. & CRAMER, J. T. 2014. The rate of torque development: a unique, non-invasive indicator of eccentric-induced muscle damage? *Int J Sports Med,* 35**,** 1190-5.
JIAO, S., REN, H., LI, Y., ZHOU, J., DUAN, C. & LU, L. 2013. Differential regulation of IGF-I and IGF-II gene expression in skeletal muscle cells. *Molecular and cellular biochemistry,* 373**,** 107-113.
JIMÉNEZ-JIMÉNEZ, R., CUEVAS, M. J., ALMAR, M., LIMA, E., GARCÍA-LÓPEZ, D., DE PAZ, J. A. & GONZÁLEZ-GALLEGO, J. 2008. Eccentric training impairs NF-κB activation and

over-expression of inflammation-related genes induced by acute eccentric exercise in the elderly. *Mechanisms of ageing and development,* 129**,** 313-321.
JOE, A. W., YI, L., NATARAJAN, A., LE GRAND, F., SO, L., WANG, J., RUDNICKI, M. A. & ROSSI, F. M. 2010. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nature cell biology,* 12**,** 153.
JOFFE, Y. T., COLLINS, M. & GOEDECKE, J. H. 2013. The relationship between dietary fatty acids and inflammatory genes on the obese phenotype and serum lipids. *Nutrients,* 5**,** 1672-1705.
JOHNSTON, A. P., BAKER, J., BELLAMY, L. M., MCKAY, B. R., DE LISIO, M. & PARISE, G. 2010. Regulation of muscle satellite cell activation and chemotaxis by angiotensin II. *PloS one,* 5**,** e15212.
JOHNSTON, A. P., BAKER, J., DE LISIO, M. & PARISE, G. 2011. Skeletal muscle myoblasts possess a stretch-responsive local angiotensin signalling system. *Journal of the Renin-Angiotensin-Aldosterone System,* 12**,** 75-84.
JONES, D. 1996. High- and low-frequency fatigue revisited. *Acta Physiologica,* 156**,** 265-270.
JONES, D., RUTHERFORD, O. & PARKER, D. J. Q. J. O. E. P. 1989. Physiological changes in skeletal muscle as a result of strength training. 74**,** 233-256.
JONES, E. J., BISHOP, P. A., WOODS, A. K. & GREEN, J. M. 2008. Cross-sectional area and muscular strength. *Sports Medicine,* 38**,** 987-994.
JÖNHAGEN, S., ERICSON, M. O., NEMETH, G. & ERIKSSON, E. 1996. Amplitude and timing of electromyographic activity during sprinting. *Scandinavian journal of medicine & science in sports,* 6**,** 15-21.
KAHLES, F., FINDEISEN, H. M. & BRUEMMER, D. 2014. Osteopontin: A novel regulator at the cross roads of inflammation, obesity and diabetes. *Molecular Metabolism,* 3**,** 384-393.
KANAMORI, A., WOO, S. L., MA, C. B., ZEMINSKI, J., RUDY, T. W., LI, G. & LIVESAY, G. A. 2000. The forces in the anterior cruciate ligament and knee kinematics during a simulated pivot shift test: a human cadaveric study using robotic technology. *Arthroscopy,* 16**,** 633-639.
KANDA, K., SUGAMA, K., HAYASHIDA, H., SAKUMA, J., KAWAKAMI, Y., MIURA, S., YOSHIOKA, H., MORI, Y. & SUZUKI, K. 2013. Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exerc. Immunol. Rev,* 19**,** 72-85.
KARIMI, M., GOLDIE, L. C., CRUICKSHANK, M. N., MOSES, E. K. & ABRAHAM, L. J. 2009. A critical assessment of the factors affecting reporter gene assays for promoter SNP function: a reassessment of −308 TNF polymorphism function using a novel integrated reporter system. *European journal of human genetics,* 17**,** 1454-1462.
KELLER, H. L., ST PIERRE SCHNEIDER, B., EPPIHIMER, L. A. & CANNON, J. G. 1999. Association of IGF-I and IGF-II with myofiber regeneration in vivo. *Muscle & nerve,* 22**,** 347-354.
KELLIS, E. 2015. Biceps femoris and semitendinosus tendon/aponeurosis strain during passive and active (isometric) conditions. *Journal of Electromyography and Kinesiology*.
KELLIS, E. & BALTZOPOULOS, V. 1999. In vivo determination of the patella tendon and hamstrings moment arms in adult males using videofluoroscopy during submaximal knee extension and flexion. *Clinical Biomechanics,* 14**,** 118-124.

KHAPCHAEV, A. Y. & SHIRINSKY, V. 2016. Myosin light chain kinase MYLK1: Anatomy, interactions, functions, and regulation. *Biochemistry (Moscow),* 81**,** 1676-1697.
KIBLER, W. B., CHANDLER, T. J. & STRACENER, E. S. 1992. 4: Musculoskeletal Adaptations and Injuries Due to Overtraining. *Exercise and sport sciences reviews,* 20**,** 99-126.
KIRCHHOFF, K., MACHICAO, F., HAUPT, A., SCHAFER, S. A., TSCHRITTER, O., STAIGER, H., STEFAN, N., HARING, H. U. & FRITSCHE, A. 2008. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia,* 51**,** 597-601.
KIRK, E. A. & RICE, C. L. 2016. Contractile function and motor unit firing rates of the human hamstrings. *Journal of Neurophysiology***,** jn. 00620.2016.
KIRWAN, J. P., BOUREY, R. E., KOHRT, W. M., STATEN, M. A. & HOLLOSZY, J. O. 1991. Effects of treadmill exercise to exhaustion on the insulin response to hyperglycemia in untrained men. *Journal of applied physiology,* 70**,** 246-250.
KJÆR, M. 2004. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiological reviews,* 84**,** 649-698.
KNOCHEL, J. P. 1990. Catastrophic medical events with exhaustive exercise:"white collar rhabdomyolysis.". *Kidney Int,* 38**,** 709-19.
KNOLL, R., BUYANDELGER, B. & LAB, M. 2011. The sarcomeric Z-disc and Z-discopathies. *J Biomed Biotechnol,* 2011**,** 1-12.
KOSKINEN, S. O. A., WANG, W., AHTIKOSKI, A. M., KJAER, M., HAN, X. Y., KOMULAINEN, J., KOVANEN, V. & TAKALA, T. E. S. 2001. Acute exercise induced changes in rat skeletal muscle mRNAs and proteins regulating type IV collagen content. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 280**,** R1292-R1300.
KOSTEK, M. C., DEVANEY, J. M., GORDISH-DRESSMAN, H., HARRIS, T. B., THOMPSON, P. D., CLARKSON, P. M., ANGELOPOULOS, T. J., GORDON, P. M., MOYNA, N. M. & PESCATELLO, L. S. 2010. A polymorphism near IGF1 is associated with body composition and muscle function in women from the Health, Aging, and Body Composition Study. *European journal of applied physiology,* 110**,** 315-324.
KRAGSTRUP, T. W., KJAER, M. & MACKEY, A. 2011. Structural, biochemical, cellular, and functional changes in skeletal muscle extracellular matrix with aging. *Scandinavian journal of medicine & science in sports,* 21**,** 749-757.
KUIPERS, H. 1994. Exercise-induced muscle damage. *International journal of sports medicine,* 15**,** 132-135.
KUJALA, U. M., SARNA, S., KAPRIO, J., TIKKANEN, H. O. & KOSKENVUO, M. 2000. Natural selection to sports, later physical activity habits, and coronary heart disease. *British journal of sports medicine,* 34**,** 445-449.
KUROSAKA, M. & MACHIDA, S. 2012. Exercise and skeletal muscle regeneration. *The Journal of Physical Fitness and Sports Medicine,* 1**,** 537-540.
LAGUETTE, M.-J., ABRAHAMS, Y., PRINCE, S. & COLLINS, M. 2011. Sequence variants within the 3'-UTR of the COL5A1 gene alters mRNA stability: Implications for musculoskeletal soft tissue injuries. *Matrix Biology,* 30**,** 338-345.
LANGBERG, H., OLESEN, J. L., GEMMER, C. & KJÆR, M. 2002. Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. *The Journal of physiology,* 542**,** 985-990.
LANZA, M. B., BALSHAW, T. G., MASSEY, G. J. & FOLLAND, J. P. 2018. Does normalization of voluntary EMG amplitude to MMAX account for the influence of electrode location and adiposity? *Scand J Med Sci Sports*.

LAPPALAINEN, J. 2009. IL6 genotype and creatine kinase response to exercise. *European journal of applied physiology,* 106**,** 315-315.

LARRUSKAIN, J., LEKUE, J. A., DIAZ, N., ODRIOZOLA, A. & GIL, S. M. 2017. A comparison of injuries in elite male and female football players: A five-season prospective study. *Scand J Med Sci Sports*.

LASEVICIUS, T., UGRINOWITSCH, C., SCHOENFELD, B. J., ROSCHEL, H., TAVARES, L. D., DE SOUZA, E. O., LAURENTINO, G. & TRICOLI, V. 2018. Effects of different intensities of resistance training with equated volume load on muscle strength and hypertrophy. *Eur J Sport Sci,* 18**,** 772-780.

LE GRAND, F. & RUDNICKI, M. A. 2007. Skeletal muscle satellite cells and adult myogenesis. *Current opinion in cell biology,* 19**,** 628-633.

LEE, D.-M., BAJRACHARYA, P., LEE, E. J., KIM, J.-E., LEE, H.-J., CHUN, T., KIM, J., CHO, K. H., CHANG, J. & HONG, S. 2011. Effects of gender-specific adult bovine serum on myogenic satellite cell proliferation, differentiation and lipid accumulation. *In Vitro Cellular & Developmental Biology-Animal,* 47**,** 438-444.

LEE, H. J., KIM, K. J., PARK, M. H., KIMM, K., PARK, C., OH, B. & LEE, J. Y. 2005. Single-nucleotide polymorphisms and haplotype LD analysis of the 29-kb IGF2 region on chromosome 11p15.5 in the Korean population. *Hum Hered,* 60**,** 73-80.

LEMAIRE, K., RAVIER, M. A., SCHRAENEN, A., CREEMERS, J. W., VAN DE PLAS, R., GRANVIK, M., VAN LOMMEL, L., WAELKENS, E., CHIMIENTI, F., RUTTER, G. A., GILON, P., IN'T VELD, P. A. & SCHUIT, F. C. 2009. Insulin crystallization depends on zinc transporter ZnT8 expression, but is not required for normal glucose homeostasis in mice. *Proc Natl Acad Sci U S A,* 106**,** 14872-7.

LEVINSON, H., MOYER, K. E., SAGGERS, G. C. & PAUL EHRLICH, H. 2004. Calmodulin-myosin light chain kinase inhibition changes fibroblast-populated collagen lattice contraction, cell migration, focal adhesion formation, and wound contraction. *Wound repair and regeneration,* 12**,** 505-511.

LI, X., WANG, S.-J., TAN, S. C., CHEW, P. L., LIU, L., WANG, L., WEN, L. & MA, L. 2014. The A55T and K153R polymorphisms of MSTN gene are associated with the strength training-induced muscle hypertrophy among Han Chinese men. *Journal of sports sciences,* 32**,** 883-891.

LI, Y. P., CHEN, Y., JOHN, J., MOYLAN, J., JIN, B., MANN, D. L. & REID, M. B. 2005. TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogin1/MAFbx in skeletal muscle. *FASEB J,* 19**,** 362-70.

LI, Y. P., LECKER, S. H., CHEN, Y., WADDELL, I. D., GOLDBERG, A. L. & REID, M. B. 2003. TNF-alpha increases ubiquitin-conjugating activity in skeletal muscle by up-regulating UbcH2/E220k. *FASEB J,* 17**,** 1048-57.

LIEBER, R. L. & FRIDÉN, J. 1999. Mechanisms of muscle injury after eccentric contraction. *Journal of Science and Medicine in Sport,* 2**,** 253-265.

LIN, C., ZHANG, S., WANG, Y., WANG, Y., NICE, E., GUO, C., ZHANG, E., YU, L., LI, M., LIU, C., HU, L., HAO, J., QI, W. & XU, H. 2018. Functional Role of a Novel Long Noncoding RNA TTN-AS1 in Esophageal Squamous Cell Carcinoma Progression and Metastasis. *Clin Cancer Res,* 24**,** 486-498.

LING, P. R., SCHWARTZ, J. H. & BISTRIAN, B. R. 1997. Mechanisms of host wasting induced by administration of cytokines in rats. *American Journal of Physiology-Endocrinology And Metabolism,* 272**,** E333-E339.

LISTRAT, A., LETHIAS, C., HOCQUETTE, J., RENAND, G., MENISSIER, F., GEAY, Y. & PICARD, B. 2000. Age-related changes and location of types I, III, XII and XIV collagen during

development of skeletal muscles from genetically different animals. *The Histochemical Journal,* 32**,** 349-356.

LLOVERA, M., GARCIA-MARTINEZ, C., LOPEZ-SORIANO, J., CARBO, N., AGELL, N., LOPEZ-SORIANO, F. J. & ARGILES, J. M. 1998. Role of TNF receptor 1 in protein turnover during cancer cachexia using gene knockout mice. *Mol Cell Endocrinol,* 142**,** 183-9.

LOSSIE, J., KÖHNCKE, C., MAHMOODZADEH, S., STEFFEN, W., CANEPARI, M., MAFFEI, M., TAUBE, M., LARCHEVÊQUE, O., BAUMERT, P., HAASE, H., BOTTINELLI, R., REGITZ-ZAGROSEK, V. & MORANO, I. 2014. Molecular mechanism regulating myosin and cardiac functions by ELC. *Biochemical and Biophysical Research Communications,* 450**,** 464-469.

LOVERING, R. M. & DE DEYNE, P. G. 2004. Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *American Journal of Physiology-Cell Physiology,* 286**,** C230-C238.

LUCIA, A., GOMEZ-GALLEGO, F., CHICHARRO, J. L., HOYOS, J., CELAYA, K., CORDOVA, A., VILLA, G., ALONSO, J. M., BARRIOPEDRO, M., PEREZ, M. & EARNEST, C. P. 2005. Is there an association between ACE and CKMM polymorphisms and cycling performance status during 3-week races? *Int J Sports Med,* 26**,** 442-7.

LUGER, T. A. 2002. Neuromediators—a crucial component of the skin immune system. *Journal of dermatological science,* 30**,** 87-93.

MA, F., YANG, Y., LI, X., ZHOU, F., GAO, C., LI, M. & GAO, L. 2013. The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis. *PloS one,* 8**,** e54685.

MACARTHUR, D. G. & NORTH, K. N. 2004. A gene for speed? The evolution and function of α-actinin-3. *Bioessays,* 26**,** 786-795.

MACARTHUR, D. G., SETO, J. T., CHAN, S., QUINLAN, K. G., RAFTERY, J. M., TURNER, N., NICHOLSON, M. D., KEE, A. J., HARDEMAN, E. C., GUNNING, P. W., COONEY, G. J., HEAD, S. I., YANG, N. & NORTH, K. N. 2008. An Actn3 knockout mouse provides mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. *Hum Mol Genet,* 17**,** 1076-86.

MACARTHUR, D. G., SETO, J. T., RAFTERY, J. M., QUINLAN, K. G., HUTTLEY, G. A., HOOK, J. W., LEMCKERT, F. A., KEE, A. J., EDWARDS, M. R. & BERMAN, Y. 2007. Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans. *Nature genetics,* 39**,** 1261-1265.

MACHIELA, M. J. & CHANOCK, S. J. 2015. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics,* 31**,** 3555-3557.

MACKEY, A. L., BRANDSTETTER, S., SCHJERLING, P., BOJSEN-MOLLER, J., QVORTRUP, K., PEDERSEN, M. M., DOESSING, S., KJAER, M., MAGNUSSON, S. P. & LANGBERG, H. 2011. Sequenced response of extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. *The FASEB Journal,* 25**,** 1943-1959.

MACKEY, A. L., DONNELLY, A. E., TURPEENNIEMI-HUJANEN, T. & ROPER, H. P. 2004. Skeletal muscle collagen content in humans after high-force eccentric contractions. *Journal of applied physiology,* 97**,** 197-203.

MACKEY, A. L., MAGNAN, M., CHAZAUD, B. & KJAER, M. 2017. Human skeletal muscle fibroblasts stimulate in vitro myogenesis and in vivo muscle regeneration. *J Physiol,* 595**,** 5115-5127.

MAHONEY, D. J., SAFDAR, A., PARISE, G., MELOV, S., FU, M., MACNEIL, L., KACZOR, J., PAYNE, E. T. & TARNOPOLSKY, M. A. 2008. Gene expression profiling in human skeletal muscle during recovery from eccentric exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 294**,** R1901-R1910.

MAIWALD, C., STERZING, T., MAYER, T. & MILANI, T. 2009. Detecting foot-to-ground contact from kinematic data in running. *Footwear Science,* 1**,** 111-118.

MALM, C., NYBERG, P., ENGSTRÖM, M., SJÖDIN, B., LENKEI, R., EKBLOM, B. & LUNDBERG, I. 2000. Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *The Journal of physiology,* 529**,** 243-262.

MALONE, S., HUGHES, B., DORAN, D. A., COLLINS, K. & GABBETT, T. J. 2018. Can the workload–injury relationship be moderated by improved strength, speed and repeated-sprint qualities? *Journal of Science and Medicine in Sport*.

MALONE, S., ROE, M., DORAN, D. A., GABBETT, T. J. & COLLINS, K. 2016. High chronic training loads and exposure to bouts of maximal velocity running reduce injury risk in elite Gaelic football. *Journal of Science and Medicine in Sport*.

MANFREDI, T. G., FIELDING, R. A., O'REILLY, K. P., MEREDITH, C. N., LEE, H. Y. & EVANS, W. J. 1991. Plasma creatine kinase activity and exercise-induced muscle damage in older men. *Medicine and science in sports and exercise,* 23**,** 1028-1034.

MANN, C. J., PERDIGUERO, E., KHARRAZ, Y., AGUILAR, S., PESSINA, P., SERRANO, A. L. & MUÑOZ-CÁNOVES, P. 2011. Aberrant repair and fibrosis development in skeletal muscle. *Skeletal muscle,* 1**,** 21.

MANN, V., HOBSON, E. E., LI, B., STEWART, T. L., GRANT, S. F., ROBINS, S. P., ASPDEN, R. M. & RALSTON, S. H. 2001. A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *The Journal of clinical investigation,* 107**,** 899-907.

MANSFIELD, J. C., HOLDEN, H., TARLOW, J. K., DI GIOVINE, F. S., MCDOWELL, T. L., WILSON, A. G., HOLDSWORTH, C. D. & DUFF, G. W. 1994. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology,* 106**,** 637-42.

MARSH, D. R., CRISWELL, D. S., HAMILTON, M. T. & BOOTH, F. W. 1997. Association of insulin-like growth factor mRNA expressions with muscle regeneration in young, adult, and old rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 273**,** R353-R358.

MARSHALL, P. W., LOVELL, R., JEPPESEN, G. K., ANDERSEN, K. & SIEGLER, J. C. 2014. Hamstring muscle fatigue and central motor output during a simulated soccer match. *PLoS One* 10**,** e102753.

MARTIN, J. A., BRANDON, S. C., KEULER, E. M., HERMUS, J. R., EHLERS, A. C., SEGALMAN, D. J., ALLEN, M. S. & THELEN, D. G. 2018. Gauging force by tapping tendons. *Nature communications,* 9**,** 1592.

MARTINEZ AMAT, A., MARCHAL CORRALES, J. A., RODRIGUEZ SERRANO, F., BOULAIZ, H., PRADOS SALAZAR, J. C., HITA CONTRERAS, F., CABA PEREZ, O., CARRILLO DELGADO, E., MARTIN, I. & ARANEGA JIMENEZ, A. 2007. Role of alpha-actin in muscle damage of injured athletes in comparison with traditional markers. *Br J Sports Med,* 41**,** 442-6.

MASSIDDA, M., CORRIAS, L., BACHIS, V., CUGIA, P., PIRAS, F., SCORCU, M. & CALÒ, C. M. 2015. Vitamin D receptor gene polymorphisms and musculoskeletal injuries in

professional football players. *Experimental and Therapeutic Medicine,* 9**,** 1974-1978.
MATHENY, W., MERRITT, E., ZANNIKOS, S. V., FARRAR, R. P. & ADAMO, M. L. 2009. Serum IGF-I-deficiency does not prevent compensatory skeletal muscle hypertrophy in resistance exercise. *Experimental Biology and Medicine,* 234**,** 164-170.
MATHEW, S. J., HANSEN, J. M., MERRELL, A. J., MURPHY, M. M., LAWSON, J. A., HUTCHESON, D. A., HANSEN, M. S., ANGUS-HILL, M. & KARDON, G. 2011. Connective tissue fibroblasts and Tcf4 regulate myogenesis. *Development,* 138**,** 371-384.
MAYHEW, D. L., KIM, J.-S., CROSS, J. M., FERRANDO, A. A. & BAMMAN, M. M. J. J. O. A. P. 2009. Translational signaling responses preceding resistance training-mediated myofiber hypertrophy in young and old humans. 107**,** 1655-1662.
MCCARROLL, S. A., KURUVILLA, F. G., KORN, J. M., CAWLEY, S., NEMESH, J., WYSOKER, A., SHAPERO, M. H., DE BAKKER, P. I., MALLER, J. B. & KIRBY, A. J. N. G. 2008. Integrated detection and population-genetic analysis of SNPs and copy number variation. 40**,** 1166.
MCCLAY, I. & MANAL, K. 1999. Three-dimensional kinetic analysis of running: significance of secondary planes of motion. *Medicine and Science in Sports and Exercise,* 31**,** 1629-1637.
MCGINLEY, C., SHAFAT, A. & DONNELLY, A. E. 2009. Does antioxidant vitamin supplementation protect against muscle damage? *Sports Medicine,* 39**,** 1011-1032.
MCHUGH, M. P. 2003. Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scandinavian journal of medicine & science in sports,* 13**,** 88-97.
MCKAY, B. R., DE LISIO, M., JOHNSTON, A., O'REILLY, C. E., PHILLIPS, S. M., TARNOPOLSKY, M. A. & PARISE, G. 2009. Association of interleukin-6 signalling with the muscle stem cell response following muscle-lengthening contractions in humans. *PLoS one,* 4**,** e6027-e6027.
MEIJER, J., JASPERS, R., RITTWEGER, J., SEYNNES, O. R., KAMANDULIS, S., BRAZAITIS, M., SKURVYDAS, A., PIŠOT, R., ŠIMUNIČ, B. & NARICI, M. V. 2015. Single muscle fibre contractile properties differ between body-builders, power athletes and control subjects. *Experimental physiology,* 100**,** 1331-1341.
MENDIGUCHIA, J., ALENTORN-GELI, E., IDOATE, F. & MYER, G. D. 2013. Rectus femoris muscle injuries in football: a clinically relevant review of mechanisms of injury, risk factors and preventive strategies. *Br J Sports Med,* 47**,** 359-366.
MERCADO, M. L., AMENTA, A. R., HAGIWARA, H., RAFII, M. S., LECHNER, B. E., OWENS, R. T., MCQUILLAN, D. J., FROEHNER, S. C., FALLON, J. R. & MERCADO, M. L. 2006. Biglycan regulates the expression and sarcolemmal localization of dystrobrevin, syntrophin, and nNOS. *The FASEB journal,* 20**,** 1724-1726.
MILLAY, D. P., O'ROURKE, J. R., SUTHERLAND, L. B., BEZPROZVANNAYA, S., SHELTON, J. M., BASSEL-DUBY, R. & OLSON, E. N. 2013. Myomaker is a membrane activator of myoblast fusion and muscle formation. *Nature,* 499**,** 301-305.
MILLAY, D. P., SUTHERLAND, L. B., BASSEL-DUBY, R. & OLSON, E. N. 2014. Myomaker is essential for muscle regeneration. *Genes & development,* 28**,** 1641.
MILLER, B. F., OLESEN, J. L., HANSEN, M., DØSSING, S., CRAMERI, R. M., WELLING, R. J., LANGBERG, H., FLYVBJERG, A., KJAER, M. & BABRAJ, J. A. 2005. Coordinated

collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *The Journal of physiology,* 567**,** 1021-1033.

MILLS, M., YANG, N., WEINBERGER, R., VANDER WOUDE, D. L., BEGGS, A. H., EASTEAL, S. & NORTH, K. 2001. Differential expression of the actin-binding proteins, α-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. *Human Molecular Genetics,* 10**,** 1335-1346.

MIRANDA-VILELA, A. L., AKIMOTO, A. K., LORDELO, G. S., PEREIRA, L. C., GRISOLIA, C. K. & DE NAZARÉ KLAUTAU-GUIMARÃES, M. 2012. Creatine kinase MM TaqI and methylenetetrahydrofolate reductase C677T and A1298C gene polymorphisms influence exercise-induced C-reactive protein levels. *European journal of applied physiology,* 112**,** 183-192.

MITTENDORFER, B., ANDERSEN, J., PLOMGAARD, P., SALTIN, B., BABRAJ, J., SMITH, K. & RENNIE, M. 2005. Protein synthesis rates in human muscles: neither anatomical location nor fibre-type composition are major determinants. *The Journal of physiology,* 563**,** 203-211.

MIYAMOTO-MIKAMI, E., MURAKAMI, H., TSUCHIE, H., TAKAHASHI, H., OHIWA, N., MIYACHI, M., KAWAHARA, T. & FUKU, N. 2017. Lack of association between genotype score and sprint/power performance in the Japanese population. *J Sci Med Sport,* 20**,** 98-103.

MOLINA, R. & DENADAI, B. S. 2012. Dissociated time course recovery between rate of force development and peak torque after eccentric exercise. *Clinical physiology and functional imaging,* 32**,** 179-184.

MONTGOMERY, H. E., MARSHALL, R., HEMINGWAY, H., MYERSON, S., CLARKSON, P., DOLLERY, C., HAYWARD, M., HOLLIMAN, D. E., JUBB, M. & WORLD, M. 1998. Human gene for physical performance. *Nature,* 393**,** 221-222.

MOORE, B. B., KOLODSICK, J. E., THANNICKAL, V. J., COOKE, K., MOORE, T. A., HOGABOAM, C., WILKE, C. A. & TOEWS, G. B. 2005a. CCR2-mediated recruitment of fibrocytes to the alveolar space after fibrotic injury. *The American journal of pathology,* 166**,** 675-684.

MOORE, D. R., PHILLIPS, S. M., BABRAJ, J. A., SMITH, K. & RENNIE, M. J. 2005b. Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions. *American Journal of Physiology-Endocrinology and Metabolism,* 288**,** E1153-E1159.

MORAN, C. N. & PITSILADIS, Y. P. J. J. O. S. S. 2017. Tour de France Champions born or made: where do we take the genetics of performance? 35**,** 1411-1419.

MORAN, C. N., YANG, N., BAILEY, M. E. S., TSIOKANOS, A., JAMURTAS, A., MACARTHUR, D. G., NORTH, K., PITSILADIS, Y. P. & WILSON, R. H. 2007. Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. *European Journal of Human Genetics,* 15**,** 88-93.

MORGAN, D. L. 1990. New insights into the behavior of muscle during active lengthening. *Biophysical journal,* 57**,** 209-221.

MORSE, C. I., DEGENS, H. & JONES, D. A. 2007. The validity of estimating quadriceps volume from single MRI cross-sections in young men. *European journal of applied physiology,* 100**,** 267-274.

MUELLER-WOHLFAHRT, H.-W., HAENSEL, L., MITHOEFER, K., EKSTRAND, J., ENGLISH, B., MCNALLY, S., ORCHARD, J., VAN DIJK, C. N., KERKHOFFS, G. M. & SCHAMASCH, P.

2012. Terminology and classification of muscle injuries in sport: a consensus statement. *British journal of sports medicine***,** bjsports-2012-091448.
MUNAFÒ, M. R. & SMITH, G. D. 2018. Robust research needs many lines of evidence. Nature Publishing Group.
MUNZENMAIER, D. H. & GREENE, A. S. 1996. Opposing actions of angiotensin II on microvascular growth and arterial blood pressure. *Hypertension,* 27**,** 760-765.
MURASE, S., TERAZAWA, E., QUEME, F., OTA, H., MATSUDA, T., HIRATE, K., KOZAKI, Y., KATANOSAKA, K., TAGUCHI, T. & URAI, H. 2010. Bradykinin and nerve growth factor play pivotal roles in muscular mechanical hyperalgesia after exercise (delayed-onset muscle soreness). *The journal of neuroscience,* 30**,** 3752-3761.
MURPHEY, L. J., GAINER, J. V., VAUGHAN, D. E. & BROWN, N. J. 2000. Angiotensin-converting enzyme insertion/deletion polymorphism modulates the human in vivo metabolism of bradykinin. *Circulation,* 102**,** 829-32.
MURPHY, M. M., LAWSON, J. A., MATHEW, S. J., HUTCHESON, D. A. & KARDON, G. 2011. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. *Development,* 138**,** 3625-3637.
MURTON, A. J., CONSTANTIN, D. & GREENHAFF, P. L. 2008. The involvement of the ubiquitin proteasome system in human skeletal muscle remodelling and atrophy. *Biochim Biophys Acta,* 1782**,** 730-43.
MUSARÒ, A., MCCULLAGH, K., PAUL, A., HOUGHTON, L., DOBROWOLNY, G., MOLINARO, M., BARTON, E. R., SWEENEY, H. L. & ROSENTHAL, N. 2001. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nature genetics,* 27**,** 195-200.
MYERSON, S., HEMINGWAY, H., BUDGET, R., MARTIN, J., HUMPHRIES, S. & MONTGOMERY, H. 1999. Human angiotensin I-converting enzyme gene and endurance performance. *J Appl Physiol (1985),* 87**,** 1313-6.
NAKAMURA, Y. N., IWAMOTO, H., ETOH, T., SHIOTSUKA, Y., YAMAGUCHI, T., ONO, Y., TABATA, S., NISHIMURA, S. & GOTOH, T. 2007. Three-dimensional observation of connective tissue of bovine masseter muscle under concentrate-and roughage-fed conditions by using immunohistochemical/confocal laser-scanning microscopic methods. *Journal of food science,* 72**,** E375-E381.
NAKANO, T., LI, X., SUNWOO, H. & SIM, J. 1997. Immunohistochemical localization of proteoglycans in bovine skeletal muscle and adipose connective tissues. *Canadian Journal of Animal Science,* 77**,** 169-172.
NAZAROV, I. B., WOODS, D. R., MONTGOMERY, H. E., SHNEIDER, O. V., KAZAKOV, V. I., TOMILIN, N. V. & ROGOZKIN, V. A. 2001. The angiotensin converting enzyme I/D polymorphism in Russian athletes. *Eur J Hum Genet,* 9**,** 797-801.
NEDELEC, M., MCCALL, A., CARLING, C., LEGALL, F., BERTHOIN, S. & DUPONT, G. 2014. The influence of soccer playing actions on the recovery kinetics after a soccer match. *The Journal of Strength & Conditioning Research,* 28**,** 1517-1523.
NEUMANN, D. A. 2010. Kinesiology of the hip: a focus on muscular actions. *journal of orthopaedic & sports physical therapy,* 40**,** 82-94.
NEWHAM, D., JONES, D. & CLARKSON, P. 1987. Repeated high-force eccentric exercise: effects on muscle pain and damage. *Journal of applied physiology,* 63**,** 1381-1386.
NEWHAM, D., JONES, D. & EDWARDS, R. 1983. Large delayed plasma creatine kinase changes after stepping exercise. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine,* 6**,** 380-385.

NGUYEN, H. X. & TIDBALL, J. G. 2003. Interactions between neutrophils and macrophages promote macrophage killing of rat muscle cells in vitro. *The Journal of physiology,* 547**,** 125-132.

NICOL, C., AVELA, J. & KOMI, P. V. 2006. The stretch-shortening cycle. *Sports Medicine,* 36**,** 977-999.

NIELSEN, J., FARUP, J., RAHBEK, S. K., DE PAOLI, F. V. & VISSING, K. 2015. Enhanced Glycogen Storage of a Subcellular Hot Spot in Human Skeletal Muscle during Early Recovery from Eccentric Contractions. *PloS one,* 10.

NIEMAN, D. C., NEHLSEN-CANNARELLA, S. L., FAGOAGA, O. R., HENSON, D., UTTER, A., DAVIS, J. M., WILLIAMS, F. & BUTTERWORTH, D. E. 1998. Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Medicine and Science in Sports and Exercise,* 30**,** 671-678.

NIEMAN, D. C., PETERS, E. M., HENSON, D. A., NEVINES, E. I. & THOMPSON, M. M. 2000. Influence of vitamin C supplementation on cytokine changes following an ultramarathon. *Journal of Interferon & Cytokine Research,* 20**,** 1029-1035.

NIEMI, A.-K. & MAJAMAA, K. 2005. Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. *European Journal of Human Genetics,* 13**,** 965-969.

NIGRO, J. M., SCHWEINFEST, C. W., RAJKOVIC, A., PAVLOVIC, J., JAMAL, S., DOTTIN, R. P., HART, J. T., KAMARCK, M. E., RAE, P. M., CARTY, M. D. & ET AL. 1987. cDNA cloning and mapping of the human creatine kinase M gene to 19q13. *Am J Hum Genet,* 40**,** 115-25.

NORTH, K. N. & BEGGS, A. H. 1996. Deficiency of a skeletal muscle isoform of α-actinin (α-actinin-3) in merosin-positive congenital muscular dystrophy. *Neuromuscular Disorders,* 6**,** 229-235.

NORTH, K. N., YANG, N., WATTANASIRICHAIGOON, D., MILLS, M., EASTEAL, S. & BEGGS, A. H. 1999. A common nonsense mutation results in α-actinin-3 deficiency in the general population. *Nature genetics,* 21**,** 353-354.

NOSAKA, K. & CLARKSON, P. M. 1996. Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. *Int J Sports Med,* 17**,** 120-7.

NOSAKA, K., SAKAMOTO, K., NEWTON, M. & SACCO, P. 2001. How long does the protective effect on eccentric exercise-induced muscle damage last? *Medicine and science in sports and exercise,* 33**,** 1490-1495.

NOWAK, K. J., WATTANASIRICHAIGOON, D., GOEBEL, H. H., WILCE, M., PELIN, K., DONNER, K., JACOB, R. L., HÜBNER, C., OEXLE, K. & ANDERSON, J. R. 1999. Mutations in the skeletal muscle α-actin gene in patients with actin myopathy and nemaline myopathy. *Nature genetics,* 23**,** 208.

O'DELL, S. D. & DAY, I. N. 1998. Insulin-like growth factor II (IGF-II). *Int J Biochem Cell Biol,* 30**,** 767-71.

ONISHI, H., YAGI, R., OYAMA, M., AKASAKA, K., IHASHI, K. & HANDA, Y. 2002. EMG-angle relationship of the hamstring muscles during maximum knee flexion. *Journal of Electromyography and Kinesiology,* 12**,** 399-406.

OPAR, D. A., WILLIAMS, M. D. & SHIELD, A. J. 2012. Hamstring strain injuries. *Sports Medicine,* 42**,** 209-226.

ORCHARD, J. W. 2001. Intrinsic and Extrinsic Risk Factors for Muscle Strains in Australian Football. *The American Journal of Sports Medicine,* 29**,** 300-303.

OSTROWSKI, K., SCHJERLING, P. & PEDERSEN, B. K. 2000. Physical activity and plasma interleukin-6 in humans–effect of intensity of exercise. *European journal of applied physiology,* 83**,** 512-515.

OWENS, D. J., ALLISON, R. & CLOSE, G. L. 2018. Vitamin D and the Athlete: Current Perspectives and New Challenges. *Sports Med,* 48**,** 3-16.

OWENS, D. J., FRASER, W. D. & CLOSE, G. L. 2014. Vitamin D and the athlete: Emerging insights. *European journal of sport science,* 15**,** 73-84.

OWENS, D. J., SHARPLES, A. P., POLYDOROU, I., ALWAN, N., DONOVAN, T., TANG, J., FRASER, W. D., COOPER, R. G., MORTON, J. P. & STEWART, C. 2015. A systems-based investigation into vitamin D and skeletal muscle repair, regeneration, and hypertrophy. *American Journal of Physiology-Endocrinology and Metabolism,* 309**,** E1019-E1031.

PADULO, J., TILOCA, A., POWELL, D., GRANATELLI, G., BIANCO, A. & PAOLI, A. J. S. 2013. EMG amplitude of the biceps femoris during jumping compared to landing movements. 2**,** 520.

PAQUETTE, M. R., PEEL, S. A., SCHILLING, B. K., MELCHER, D. A. & BLOOMER, R. J. 2017. Soreness-related changes in three-dimensional running biomechanics following eccentric knee extensor exercise. *European Journal of Sport Science***,** 1-9.

PASCHALIS, V., GIAKAS, G., BALTZOPOULOS, V., JAMURTAS, A. Z., THEOHARIS, V., KOTZAMANIDIS, C. & KOUTEDAKIS, Y. 2007. The effects of muscle damage following eccentric exercise on gait biomechanics. *Gait & posture,* 25**,** 236-242.

PASSERIEUX, E., ROSSIGNOL, R., LETELLIER, T. & DELAGE, J. P. 2007. Physical continuity of the perimysium from myofibers to tendons: involvement in lateral force transmission in skeletal muscle. *J Struct Biol,* 159**,** 19-28.

PATEL, J. A., NAIR, S., OCHOA, E. E., HUDA, R., ROBERTS JR, N. J. & CHONMAITREE, T. 2010. Interleukin-6 -174 and tumor necrosis factor α -308 polymorphisms enhance cytokine production by human macrophages exposed to respiratory viruses. *Journal of Interferon & Cytokine Research,* 30**,** 917-921.

PAUL, A. C. & ROSENTHAL, N. 2002. Different modes of hypertrophy in skeletal muscle fibers. *The Journal of cell biology,* 156**,** 751-760.

PAULSEN, G., CRAMERI, R., BENESTAD, H. B., FJELD, J. G., MØRKRID, L., HALLEN, J. & RAASTAD, T. 2010. Time course of leukocyte accumulation in human muscle after eccentric exercise. *Medicine and science in sports and exercise,* 42**,** 75-85.

PAULSEN, G., MIKKELSEN, U. R., RAASTAD, T. & PEAKE, J. M. 2012. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise. *Exerc Immunol Rev,* 18**,** 42-97.

PEAKE, J., DELLA GATTA, P., SUZUKI, K. & NIEMAN, D. 2015. Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. *Exercise immunology review,* 21**,** 8-25.

PEAKE, J., NOSAKA, K. K. & SUZUKI, K. 2005. Characterization of inflammatory responses to eccentric exercise in humans. *Exerc Immunol Rev,* 11**,** 64-85.

PEAKE, J. & SUZUKI, K. 2004. Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. *Exerc Immunol Rev,* 10**,** 129-41.

PEDERSEN, B. K. 2011. Muscles and their myokines. *The Journal of experimental biology,* 214**,** 337-346.

PEDERSEN, B. K. & FEBBRAIO, M. A. 2008. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiological reviews,* 88**,** 1379-1406.

PEDERSEN, B. K. & FISCHER, C. P. 2007. Beneficial health effects of exercise–the role of IL-6 as a myokine. *Trends in pharmacological sciences,* 28**,** 152-156.

PEDERSEN, B. K., STEENSBERG, A., FISCHER, C., KELLER, C., KELLER, P., PLOMGAARD, P., FEBBRAIO, M. & SALTIN, B. 2003. Searching for the exercise factor: is IL-6 a candidate? *J Muscle Res Cell Motil,* 24**,** 113-9.

PEGORARO, E., HOFFMAN, E. P., PIVA, L., GAVASSINI, B. F., CAGNIN, S., ERMANI, M., BELLO, L., SORARU, G., PACCHIONI, B., BONIFATI, M. D., LANFRANCHI, G., ANGELINI, C., KESARI, A., LEE, I., GORDISH-DRESSMAN, H., DEVANEY, J. M., MCDONALD, C. M. & COOPERATIVE INTERNATIONAL NEUROMUSCULAR RESEARCH, G. 2011. SPP1 genotype is a determinant of disease severity in Duchenne muscular dystrophy. *Neurology,* 76**,** 219-26.

PEREIRA, D. S., GARCIA, D. M., NARCISO, F. M. S., SANTOS, M. L. A. S., DIAS, J. M. D., QUEIROZ, B. Z., SOUZA, E. R., NOBREGA, O. T. & PEREIRA, L. S. M. 2011. Effects of 174 G/C polymorphism in the promoter region of the interleukin-6 gene on plasma IL-6 levels and muscle strength in elderly women. *Brazilian journal of medical and biological research,* 44**,** 123-129.

PEREIRA, D. S., MATEO, E. C. C., DE QUEIROZ, B. Z., ASSUMPÇÃO, A. M., MIRANDA, A. S., FELÍCIO, D. C., ROCHA, N. P., DOS ANJOS, D. M. D. C., PEREIRA, D. A. G. & TEIXEIRA, A. L. 2013. TNF-α, IL6, and IL10 polymorphisms and the effect of physical exercise on inflammatory parameters and physical performance in elderly women. *Age,* 35**,** 2455-2463.

PETERSEN, A. M. W. & PEDERSEN, B. K. 2005. The anti-inflammatory effect of exercise. *Journal of applied physiology,* 98**,** 1154-1162.

PETERSEN, J., THORBORG, K., NIELSEN, M. B., BUDTZ-JØRGENSEN, E. & HÖLMICH, P. 2011. Preventive effect of eccentric training on acute hamstring injuries in men's soccer: a cluster-randomized controlled trial. *The American journal of sports medicine,* 39**,** 2296-2303.

PETUSHEK, E. J., SUGIMOTO, D., STOOLMILLER, M., SMITH, G. & MYER, G. D. 2018. Evidence-based best-practice guidelines for preventing anterior cruciate ligament injuries in young female athletes: a systematic review and meta-analysis. *The American journal of sports medicine***,** 0363546518782460.

PHILIPPOU, A., MARIDAKI, M., THEOS, A. & KOUTSILIERIS, M. 2012. Cytokines in muscle damage. *Advances in clinical chemistry,* 58**,** 49.

PHILLIPS, B. E., WILLIAMS, J. P., GUSTAFSSON, T., BOUCHARD, C., RANKINEN, T., KNUDSEN, S., SMITH, K., TIMMONS, J. A. & ATHERTON, P. J. 2013. Molecular networks of human muscle adaptation to exercise and age. *PLoS Genet,* 9**,** e1003389.

PHILLIPS, M. I., SPEAKMAN, E. A. & KIMURA, B. 1993. Levels of angiotensin and molecular biology of the tissue renin angiotensin systems. *Regul Pept,* 43**,** 1-20.

PICCOLO, S., DUPONT, S. & CORDENONSI, M. 2014. The biology of YAP/TAZ: hippo signaling and beyond. *Physiological reviews,* 94**,** 1287-1312.

PIMENTA, E. M., COELHO, D. B., CRUZ, I. R., MORANDI, R. F., VENEROSO, C. E., DE AZAMBUJA PUSSIELDI, G., CARVALHO, M. R. S., SILAMI-GARCIA, E. & FERNÁNDEZ, J. A. D. P. 2012. The ACTN3 genotype in soccer players in response to acute eccentric training. *European journal of applied physiology,* 112**,** 1495-1503.

PISTILLI, E. E., DEVANEY, J. M., GORDISH-DRESSMAN, H., BRADBURY, M. K., SEIP, R. L., THOMPSON, P. D., ANGELOPOULOS, T. J., CLARKSON, P. M., MOYNA, N. M., PESCATELLO, L. S., VISICH, P. S., ZOELLER, R. F., GORDON, P. M. & HOFFMAN, E. P.

2008. Interleukin-15 and interleukin-15R alpha SNPs and associations with muscle, bone, and predictors of the metabolic syndrome. *Cytokine,* 43**,** 45-53.
PLEASE SEE CHAPTER III.
PLEASE SEE CHAPTER IV.
PLEASE SEE CHAPTER V.
PLOMGAARD, P., BOUZAKRI, K., KROGH-MADSEN, R., MITTENDORFER, B., ZIERATH, J. R. & PEDERSEN, B. K. 2005. Tumor necrosis factor-α induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. *Diabetes,* 54**,** 2939-2945.
PLOUTZ-SNYDER, L. L., GIAMIS, E. L., FORMIKELL, M. & ROSENBAUM, A. E. 2001. Resistance training reduces susceptibility to eccentric exercise-induced muscle dysfunction in older women. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 56**,** B384-B390.
PORTER, J. D., KHANNA, S., KAMINSKI, H. J., RAO, J. S., MERRIAM, A. P., RICHMONDS, C. R., LEAHY, P., LI, J., GUO, W. & ANDRADE, F. H. 2002. A chronic inflammatory response dominates the skeletal muscle molecular signature in dystrophin-deficient mdx mice. *Human molecular genetics,* 11**,** 263-272.
POSTHUMUS, M., SCHWELLNUS, M. P. & COLLINS, M. 2011. The COL5A1 gene: a novel marker of endurance running performance. *Medicine and science in sports and exercise,* 43**,** 584-589.
POTTER, J. J., LIU, X., KOTEISH, A. & MEZEY, E. 2013. 1, 25-dihydroxyvitamin D3 and its nuclear receptor repress human α1 (I) collagen expression and type I collagen formation. *Liver International,* 33**,** 677-686.
POWELL, P. L., ROY, R. R., KANIM, P., BELLO, M. A. & EDGERTON, V. R. 1984. Predictability of skeletal muscle tension from architectural determinations in guinea pig hindlimbs. *Journal of Applied Physiology,* 57**,** 1715-1721.
POWERS, S. K. & JACKSON, M. J. 2008. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological reviews,* 88**,** 1243-1276.
PROSKE, U. & MORGAN, D. L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of physiology,* 537**,** 333-345.
PRUNA, R., ARTELLS, R., RIBAS, J., MONTORO, B., COS, F., MUÑOZ, C., RODAS, G. & MAFFULLI, N. 2013. Single nucleotide polymorphisms associated with non-contact soft tissue injuries in elite professional soccer players Influence on degree of injury and recovery time. *BMC musculoskeletal disorders,* 14**,** 221.
QI, L., ZHANG, C., VAN DAM, R. M. & HU, F. B. 2007. Interleukin-6 genetic variability and adiposity: associations in two prospective cohorts and systematic review in 26,944 individuals. *The Journal of Clinical Endocrinology & Metabolism,* 92**,** 3618-3625.
QUINLAN, K. G., SETO, J. T., TURNER, N., VANDEBROUCK, A., FLOETENMEYER, M., MACARTHUR, D. G., RAFTERY, J. M., LEK, M., YANG, N. & PARTON, R. G. 2010. α-Actinin-3 deficiency results in reduced glycogen phosphorylase activity and altered calcium handling in skeletal muscle. *Human molecular genetics,* 19**,** 1335-1346.
RAASTAD, T., OWE, S. G., PAULSEN, G., ENNS, D., OVERGAARD, K., CRAMERI, R., KIIL, S., BELCASTRO, A., BERGERSEN, L. & HALLEN, J. 2010. Changes in calpain activity, muscle structure, and function after eccentric exercise. *Med Sci Sports Exerc,* 42**,** 86-95.

RAFEEUDDIN, R., SHARIR, R., STAES, F., DINGENEN, B., GEORGE, K., ROBINSON, M. A. & VANRENTERGHEM, J. 2016. Mapping current research trends on neuromuscular risk factors of non-contact ACL injury. *Physical Therapy in Sport,* 22**,** 101-113.

RAMASWAMY, K. S., PALMER, M. L., VAN DER MEULEN, J. H., RENOUX, A., KOSTROMINOVA, T. Y., MICHELE, D. E. & FAULKNER, J. A. 2011. Lateral transmission of force is impaired in skeletal muscles of dystrophic mice and very old rats. *The Journal of physiology,* 589**,** 1195-1208.

RAMPININI, E., BOSIO, A., FERRARESI, I., PETRUOLO, A., MORELLI, A. & SASSI, A. 2011. Match-related fatigue in soccer players. *Medicine and science in sports and exercise,* 43**,** 2161-2170.

RANKINEN, T., WOLFARTH, B., SIMONEAU, J. A., MAIER-LENZ, D., RAURAMAA, R., RIVERA, M. A., BOULAY, M. R., CHAGNON, Y. C., PERUSSE, L., KEUL, J. & BOUCHARD, C. 2000. No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. *J Appl Physiol (1985),* 88**,** 1571-5.

RAWSON, E. S., CLARKSON, P. M. & TARNOPOLSKY, M. A. 2017. Perspectives on exertional rhabdomyolysis. *Sports Medicine,* 47**,** 33-49.

REEVES, N. D., NARICI, M. V. & MAGANARIS, C. N. 2004a. Effect of resistance training on skeletal muscle-specific force in elderly humans. *Journal of applied physiology,* 96**,** 885-892.

REEVES, N. D., NARICI, M. V. & MAGANARIS, C. N. 2004b. In vivo human muscle structure and function: adaptations to resistance training in old age. *Experimental physiology,* 89**,** 675-689.

REID, M. B. 2005. Response of the ubiquitin-proteasome pathway to changes in muscle activity. *Am J Physiol Regul Integr Comp Physiol,* 288**,** R1423-31.

REID, M. B. & LI, Y. P. 2001. Tumor necrosis factor-alpha and muscle wasting: a cellular perspective. *Respir Res,* 2**,** 269-72.

REILLY, T. & WILLIAMS, A. M. 2003. *Science and Soccer*, Routledge.

REIN, T., FÖRSTER, R., KRAUSE, A., WINNACKER, E.-L. & ZORBAS, H. 1995. Organization of the-Globin Promoter and Possible Role of Nuclear Factor I in an-Globin-inducible and in a Noninducible Cell Line. *Journal of Biological Chemistry,* 270**,** 19643-19650.

RENZI, D., PELLEGRINI, B., TONELLI, F., SURRENTI, C. & CALABRO, A. 2000. Substance P (neurokinin-1) and neurokinin A (neurokinin-2) receptor gene and protein expression in the healthy and inflamed human intestine. *The American journal of pathology,* 157**,** 1511-1522.

RIGAT, B., HUBERT, C., ALHENC-GELAS, F., CAMBIEN, F., CORVOL, P. & SOUBRIER, F. 1990. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest,* 86**,** 1343-6.

ROBINSON, M. A. & VANRENTERGHEM, J. 2012. An evaluation of anatomical and functional knee axis definition in the context of side-cutting. *Journal of biomechanics,* 45**,** 1941-1946.

ROBINSON, R., CARPENTER, D., SHAW, M. A., HALSALL, J. & HOPKINS, P. 2006. Mutations in RYR1 in malignant hyperthermia and central core disease. *Hum Mutat,* 27**,** 977-89.

RODAN, G. A. 1995. Osteopontin overview. *Annals of the New York Academy of Sciences,* 760**,** 1-5.

ROIG, M., O'BRIEN, K., KIRK, G., MURRAY, R., MCKINNON, P., SHADGAN, B. & REID, D. W. 2008. The effects of eccentric versus concentric resistance training on muscle

strength and mass in healthy adults: a systematic review with meta-analyses. *British journal of sports medicine,* 43**,** 556-568.

ROSEN, C. J., KURLAND, E. S., VEREAULT, D., ADLER, R. A., RACKOFF, P. J., CRAIG, W. Y., WITTE, S., ROGERS, J. & BILEZIKIAN, J. P. 1998. Association between serum insulin growth factor-I (IGF-I) and a simple sequence repeat in IGF-I gene: implications for genetic studies of bone mineral density. *The Journal of Clinical Endocrinology & Metabolism,* 83**,** 2286-2290.

ROTH, S. M., MARTEL, G. F., IVEY, F. M., LEMMER, J. T., TRACY, B. L., HURLBUT, D. E., METTER, E. J., HURLEY, B. F. & ROGERS, M. A. 1999. Ultrastructural muscle damage in young vs. older men after high-volume, heavy-resistance strength training. *Journal of Applied Physiology,* 86**,** 1833-1840.

ROTH, S. M., ZMUDA, J. M., CAULEY, J. A., SHEA, P. R. & FERRELL, R. E. 2004. Vitamin D receptor genotype is associated with fat-free mass and sarcopenia in elderly men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,* 59**,** B10-B15.

RUBINSTEIN, I., ABASSI, Z., COLEMAN, R., MILMAN, F., WINAVER, J. & BETTER, O. S. 1998. Involvement of nitric oxide system in experimental muscle crush injury. *Journal of Clinical Investigation,* 101**,** 1325.

RUIZ, J. R., ARTETA, D., BUXENS, A., ARTIEDA, M., GÓMEZ-GALLEGO, F., SANTIAGO, C., YVERT, T., MORÁN, M. & LUCIA, A. 2010a. Can we identify a power-oriented polygenic profile? *Journal of Applied Physiology,* 108**,** 561-566.

RUIZ, J. R., BUXENS, A., ARTIEDA, M., ARTETA, D., SANTIAGO, C., RODRÍGUEZ-ROMO, G., LAO, J. I., GÓMEZ-GALLEGO, F. & LUCIA, A. 2010b. The− 174 G/C polymorphism of the IL6 gene is associated with elite power performance. *Journal of Science and Medicine in Sport,* 13**,** 549-553.

RUIZ, J. R., GÓMEZ-GALLEGO, F., SANTIAGO, C., GONZÁLEZ-FREIRE, M., VERDE, Z., FOSTER, C. & LUCIA, A. 2009. Is there an optimum endurance polygenic profile? *The Journal of physiology,* 587**,** 1527-1534.

RUTHERFORD, O., JONES, D., NEWHAM, D. J. J. O. N., NEUROSURGERY & PSYCHIATRY 1986. Clinical and experimental application of the percutaneous twitch superimposition technique for the study of human muscle activation. 49**,** 1288-1291.

SACHECK, J. M., HYATT, J.-P. K., RAFFAELLO, A., JAGOE, R. T., ROY, R. R., EDGERTON, V. R., LECKER, S. H. & GOLDBERG, A. L. 2007. Rapid disuse and denervation atrophy involve transcriptional changes similar to those of muscle wasting during systemic diseases. *The FASEB Journal,* 21**,** 140-155.

SAKUMA, K. & YAMAGUCHI, A. 2010. The functional role of calcineurin in hypertrophy, regeneration, and disorders of skeletal muscle. *BioMed Research International,* 2010.

SANTAMARIA, L. J. & WEBSTER, K. E. 2010. The effect of fatigue on lower-limb biomechanics during single-limb landings: a systematic review. *journal of orthopaedic & sports physical therapy,* 40**,** 464-473.

SANTTILA, S., SAVINAINEN, K. & HURME, M. 1998. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol,* 47**,** 195-8.

SARGEANT, A. J., HOINVILLE, E. & YOUNG, A. J. J. O. A. P. 1981. Maximum leg force and power output during short-term dynamic exercise. 51**,** 1175-1182.

SATKUNSKIENE, D., STASIULIS, A., ZAICENKOVIENE, K., SAKALAUSKAITE, R. & RAUKTYS, D. 2015. Effect of muscle-damaging eccentric exercise on running kinematics and economy for running at different intensities. *The Journal of Strength & Conditioning Research,* 29**,** 2404-2411.

SAYERS, S. P. & CLARKSON, P. M. 2001. Force recovery after eccentric exercise in males and females. *European journal of applied physiology,* 84**,** 122-126.

SCHÄFER, R., KNAUF, U., ZWEYER, M., HÖGEMEIER, O., DE GUARRINI, F., LIU, X., EICHHORN, H. J., KOCH, F. W., MUNDEGAR, R. R. & ERZEN, I. 2006. Age dependence of the human skeletal muscle stem cell in forming muscle tissue. *Artificial organs,* 30**,** 130-140.

SCHMAIER, A. H. 2003. The kallikrein-kinin and the renin-angiotensin systems have a multilayered interaction. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 285**,** R1-R13.

SCHNEIDER, B. S. P. & TIIDUS, P. M. 2007. Neutrophil Infiltration in Exercise-Injured Skeletal Muscle. *Sports Medicine,* 37**,** 837-856.

SCHOENFELD, B. J. 2010. The mechanisms of muscle hypertrophy and their application to resistance training. *The Journal of Strength & Conditioning Research,* 24**,** 2857-2872.

SCHOENFELD, B. J. 2012. Does exercise-induced muscle damage play a role in skeletal muscle hypertrophy? *The Journal of Strength & Conditioning Research,* 26**,** 1441-1453.

SCHOENFELD, B. J., CONTRERAS, B., KRIEGER, J., GRGIC, J., DELCASTILLO, K., BELLIARD, R. & ALTO, A. 2018. Resistance Training Volume Enhances Muscle Hypertrophy. *Med Sci Sports Exerc*.

SCHUERMANS, J., VAN TIGGELEN, D., DANNEELS, L. & WITVROUW, E. 2014. Biceps femoris and semitendinosus—teammates or competitors? New insights into hamstring injury mechanisms in male football players: a muscle functional MRI study. *British journal of sports medicine,* 48**,** 1599-1606.

SCHUERMANS, J., VAN TIGGELEN, D., DANNEELS, L. & WITVROUW, E. 2016. Susceptibility to hamstring injuries in soccer: a prospective study using muscle functional magnetic resonance imaging. *The American journal of sports medicine,* 44**,** 1276-1285.

SCHWARTZ, M. H. & ROZUMALSKI, A. 2005. A new method for estimating joint parameters from motion data. *Journal of biomechanics,* 38**,** 107-116.

SCOTT, J. & HUSKISSON, E. 1979. Vertical or horizontal visual analogue scales. *Annals of the rheumatic diseases,* 38**,** 560.

SELLWOOD, K. L., BRUKNER, P., WILLIAMS, D., NICOL, A. & HINMAN, R. 2007. Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial. *British journal of sports medicine,* 41**,** 392-397.

SETO, J. T., LEK, M., QUINLAN, K. G., HOUWELING, P. J., ZHENG, X. F., GARTON, F., MACARTHUR, D. G., RAFTERY, J. M., GARVEY, S. M. & HAUSER, M. A. 2011. Deficiency of α-actinin-3 is associated with increased susceptibility to contraction-induced damage and skeletal muscle remodeling. *Human molecular genetics,* 20**,** 2914-2927.

SETO, J. T., QUINLAN, K. G., LEK, M., ZHENG, X. F., GARTON, F., MACARTHUR, D. G., HOGARTH, M. W., HOUWELING, P. J., GREGOREVIC, P. & TURNER, N. 2013. ACTN3 genotype influences muscle performance through the regulation of calcineurin signaling. *The Journal of clinical investigation,* 123**,** 4255-4263.

SEWRIGHT, K. A., HUBAL, M. J., KEARNS, A., HOLBROOK, M. T. & CLARKSON, P. M. 2008. Sex differences in response to maximal eccentric exercise. *Medicine and science in sports and exercise,* 40**,** 242-251.

SEYMORE, K. D., DOMIRE, Z. J., DEVITA, P., RIDER, P. M. & KULAS, A. S. 2017. The effect of Nordic hamstring strength training on muscle architecture, stiffness, and strength. *European journal of applied physiology,* 117**,** 943-953.

SHARIR, R., RAFEEUDDIN, R., STAES, F., DINGENEN, B., GEORGE, K., VANRENTERGHEM, J. & ROBINSON, M. A. 2016. Mapping current research trends on anterior cruciate ligament injury risk against the existing evidence: in vivo biomechanical risk factors. *Clinical Biomechanics,* 37**,** 34-43.

SHARPLES, A. P., AL-SHANTI, N., LEWIS, M. P. & STEWART, C. E. 2011. Reduction of myoblast differentiation following multiple population doublings in mouse C2C12 cells: a model to investigate ageing? *Journal of cellular biochemistry,* 112**,** 3773-3785.

SHARPLES, A. P. & STEWART, C. E. 2011. Myoblast models of skeletal muscle hypertrophy and atrophy. *Current Opinion in Clinical Nutrition & Metabolic Care,* 14**,** 230-236.

SHERWOOD, R., LAMBERT, A., NEWHAM, D., WASSIF, W. & PETERS, T. 1996. The effect of eccentric exercise on serum creatine kinase activity in different ethnic groups. *Annals of clinical biochemistry,* 33**,** 324-329.

SHIELD, A. & MURPHY, S. 2018. Preventing hamstring injuries-Part 1: Is there really an eccentric action of the hamstrings in high speed running and does it matter?

SHIMODA-MATSUBAYASHI, S., MATSUMINE, H., KOBAYASHI, T., NAKAGAWA-HATTORI, Y., SHIMIZU, Y. & MIZUNO, Y. 1996. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem Biophys Res Commun,* 226**,** 561-5.

SIEMS, W., CAPUOZZO, E., LUCANO, A., SALERNO, C. & CRIFO, C. 2003. High sensitivity of plasma membrane ion transport ATPases from human neutrophils towards 4-hydroxy-2, 3-trans-nonenal. *Life sciences,* 73**,** 2583-2590.

SIM, M.-K., WONG, Y.-C., XU, X.-G. & LOKE, W.-K. 2014. Des-aspartate-angiotensin I attenuates ICAM-1 formation in hydrogen peroxide-treated L6 skeletal muscle cells and soleus muscle of mice subjected to eccentric exercise. *Regulatory peptides,* 188**,** 40-45.

SLAVOTINEK, J. P., VERRALL, G. M. & FON, G. T. 2002. Hamstring injury in athletes: using MR imaging measurements to compare extent of muscle injury with amount of time lost from competition. *American Journal of Roentgenology,* 179**,** 1621-1628.

SMITH, C., KRUGER, M. J., SMITH, R. M. & MYBURGH, K. H. 2008. The inflammatory response to skeletal muscle injury: illuminating complexities. *Sports Med,* 38**,** 947-69.

SMITH, C. K., 2ND, JANNEY, M. J. & ALLEN, R. E. 1994. Temporal expression of myogenic regulatory genes during activation, proliferation, and differentiation of rat skeletal muscle satellite cells. *J Cell Physiol,* 159**,** 379-85.

SMITH, L. L., MCCAMMON, M., SMITH, S., CHAMNESS, M., ISRAEL, R. G. & O'BRIEN, K. F. 1989. White blood cell response to uphill walking and downhill jogging at similar metabolic loads. *Eur J Appl Physiol Occup Physiol,* 58**,** 833-7.

SMITH, L. L., MCKUNE, A. J., SEMPLE, S. J., SIBANDA, E., STEEL, H. & ANDERSON, R. 2007. Changes in serum cytokines after repeated bouts of downhill running. *Appl Physiol Nutr Metab,* 32**,** 233-40.

SOMBOONWONG, J., TRAISAENG, S. & SAGUANRUNGSIRIKUL, S. 2015. Moderate-intensity exercise training elevates serum and pancreatic zinc levels and pancreatic ZnT8 expression in streptozotocin-induced diabetic rats. *Life Sci,* 139**,** 46-51.

SONG, Y., STÅL, P. S., YU, J.-G., LORENTZON, R., BACKMAN, C. & FORSGREN, S. 2014. Inhibitors of endopeptidase and angiotensin-converting enzyme lead to an amplification of the morphological changes and an upregulation of the substance P system in a muscle overuse model. *BMC musculoskeletal disorders,* 15**,** 126.

SPROUSE, C., GORDISH-DRESSMAN, H., ORKUNOGLU-SUER, E. F., LIPOF, J. S., MOECKEL-COLE, S., PATEL, R. R., ADHAM, K., LARKIN, J. S., HUBAL, M. J., KEARNS, A. K. & DEVANEY, J. M. 2014. SLC30A8 nonsynonymous variant is associated with recovery following exercise and skeletal muscle size and strength. *Diabetes,* 63**,** 363-368.

STAFILIDIS, S. & ARAMPATZIS, A. 2007. Muscle–tendon unit mechanical and morphological properties and sprint performance. *Journal of sports sciences,* 25**,** 1035-1046.

STAIGER, H., MACHICAO, F., STEFAN, N., TSCHRITTER, O., THAMER, C., KANTARTZIS, K., SCHAFER, S. A., KIRCHHOFF, K., FRITSCHE, A. & HARING, H. U. 2007. Polymorphisms within novel risk loci for type 2 diabetes determine beta-cell function. *PLoS One,* 2**,** e832.

STANTON, P. & PURDAM, C. 1989. Hamstring injuries in sprinting—the role of eccentric exercise. *Journal of Orthopaedic & Sports Physical Therapy,* 10**,** 343-349.

STARKIE, R., OSTROWSKI, S. R., JAUFFRED, S., FEBBRAIO, M. & PEDERSEN, B. K. 2003. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. *FASEB J,* 17**,** 884-6.

STAUBER, W. T., CLARKSON, P. M., FRITZ, V. K. & EVANS, W. J. 1990. Extracellular matrix disruption and pain after eccentric muscle action. *Journal of Applied Physiology,* 69**,** 868-874.

STEENSBERG, A., FISCHER, C. P., KELLER, C., MOLLER, K. & PEDERSEN, B. K. 2003. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab,* 285**,** E433-7.

STERNLICHT, M. D., LOCHTER, A., SYMPSON, C. J., HUEY, B., ROUGIER, J.-P., GRAY, J. W., PINKEL, D., BISSELL, M. J. & WERB, Z. 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell,* 98**,** 137-146.

STEWART, C. E., JAMES, P. L., FANT, M. E. & ROTWEIN, P. 1996. Overexpression of insulin-like growth factor-II induces accelerated myoblast differentiation. *J Cell Physiol,* 169**,** 23-32.

STEWART, C. E., NEWCOMB, P. V. & HOLLY, J. M. P. 2004. Multifaceted roles of TNF-α in myoblast destruction: A multitude of signal transduction pathways. *Journal of cellular physiology,* 198**,** 237-247.

STEWART, C. E. & ROTWEIN, P. 1996a. Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. *Physiol Rev,* 76**,** 1005-26.

STEWART, C. E. & ROTWEIN, P. 1996b. Insulin-like growth factor-II is an autocrine survival factor for differentiating myoblasts. *Journal of Biological Chemistry,* 271**,** 11330-11338.

STEYAERT, A., BURSSENS, P., FORSYTH, R. & VANDERSTRAETEN, G. 2010. Qualitative analysis of substance P, NK1-receptor and nerve ingrowth in substance P-treated ruptured rat Achilles tendon. *Acta Orthop Belg,* 76**,** 387-95.

STRATOS, I., LI, Z., HERLYN, P., ROTTER, R., BEHRENDT, A.-K., MITTLMEIER, T. & VOLLMAR, B. 2013. Vitamin D increases cellular turnover and functionally restores the skeletal muscle after crush injury in rats. *The American journal of pathology,* 182**,** 895-904.

STUPKA, N., TARNOPOLSKY, M. A., YARDLEY, N. & PHILLIPS, S. M. 2001. Cellular adaptation to repeated eccentric exercise-induced muscle damage. *Journal of Applied Physiology,* 91**,** 1669-1678.

SU, A. I., COOKE, M. P., CHING, K. A., HAKAK, Y., WALKER, J. R., WILTSHIRE, T., ORTH, A. P., VEGA, R. G., SAPINOSO, L. M. & MOQRICH, A. 2002. Large-scale analysis of the human and mouse transcriptomes. *Proceedings of the National Academy of Sciences,* 99**,** 4465-4470.

SUZUKI, K., NAGANUMA, S., TOTSUKA, M., SUZUKI, K.-J., MOCHIZUKI, M., SHIRAISHI, M., NAKAJI, S. & SUGAWARA, K. 1996. Effects of exhaustive endurance exercise and its one-week daily repetition on neutrophil count and functional status in untrained men. *International journal of sports medicine,* 17**,** 205-212.

SUZUKI, K., NAKAJI, S., YAMADA, M., TOTSUKA, M., SATO, K. & SUGAWARA, K. 2002. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol Rev,* 8**,** 6-48.

SUZUKI, Y. J. & FORD, G. D. 1999. Redox regulation of signal transduction in cardiac and smooth muscle. *Journal of molecular and cellular cardiology,* 31**,** 345-353.

SWEENEY, H. L., BOWMAN, B. F. & STULL, J. T. 1993. Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. *American Journal of Physiology,* 264**,** C1085-C1085.

SZCZESNA, D., ZHAO, J., JONES, M., ZHI, G., STULL, J. & POTTER, J. D. 2002. Phosphorylation of the regulatory light chains of myosin affects Ca2+ sensitivity of skeletal muscle contraction. *J Appl Physiol (1985),* 92**,** 1661-70.

TARPEY, P. S., BEHJATI, S., COOKE, S. L., VAN LOO, P., WEDGE, D. C., PILLAY, N., MARSHALL, J., O'MEARA, S., DAVIES, H., NIK-ZAINAL, S., BEARE, D., BUTLER, A., GAMBLE, J., HARDY, C., HINTON, J., JIA, M. M., JAYAKUMAR, A., JONES, D., LATIMER, C., MADDISON, M., MARTIN, S., MCLAREN, S., MENZIES, A., MUDIE, L., RAINE, K., TEAGUE, J. W., TUBIO, J. M., HALAI, D., TIRABOSCO, R., AMARY, F., CAMPBELL, P. J., STRATTON, M. R., FLANAGAN, A. M. & FUTREAL, P. A. 2013. Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma. *Nat Genet,* 45**,** 923-6.

TAYLOR, A., HUMPHRIES, B., SMITH, P. & BRONKS, R. 1997. Electrophoretic separation of myosin heavy chain isoforms in the human m. vastus lateralis: references to reproducibility and relationships with force, electromechanical delay, fibre conduction velocity, endurance and electromyography. *Archives of physiology and biochemistry,* 105**,** 10-18.

TAYLOR, J., SUN, Y. V., CHU, J., MOSLEY, T. H. & KARDIA, S. L. 2008. Interactions between metallopeptidase 3 polymorphism rs679620 and BMI in predicting blood pressure in African–American women with hypertension. *Journal of hypertension,* 26**,** 2312.

TEDESCO, F. S., DELLAVALLE, A., DIAZ-MANERA, J., MESSINA, G. & COSSU, G. 2010. Repairing skeletal muscle: regenerative potential of skeletal muscle stem cells. *J Clin Invest,* 120**,** 11-9.

TERRY, E. E., ZHANG, X., HOFFMANN, C., HUGHES, L. D., LEWIS, S. A., LI, J., WALLACE, M. J., RILEY, L. A., DOUGLAS, C. M. & GUTIERREZ-MONREAL, M. A. 2018. Transcriptional profiling reveals extraordinary diversity among skeletal muscle tissues. *eLife,* 7.

THELEN, D. G., CHUMANOV, E. S., HOERTH, D. M., BEST, T. M., SWANSON, S. C., LI, L., YOUNG, M. & HEIDERSCHEIT, B. C. 2005. Hamstring muscle kinematics during treadmill sprinting. *Med Sci Sports Exerc,* 37**,** 108-114.

THIEBAUD, R. S. 2012. Exercise-induced muscle damage: is it detrimental or beneficial. *J Trainol,* 1**,** 36-44.

THOMAS, K., DENT, J., HOWATSON, G. & GOODALL, S. 2017. Etiology and Recovery of Neuromuscular Fatigue after Simulated Soccer Match Play. *Medicine and science in sports and exercise,* 49**,** 955-964.

THOMIS, M., BEUNEN, G., LEEMPUTTE, M. V., MAES, H., BLIMKIE, C., CLAESSENS, A., MARCHAL, G., WILLEMS, E. & VLIETINCK, R. 1998. Inheritance of static and dynamic arm strength and some of its determinants. *Acta Physiologica Scandinavica,* 163**,** 59-71.

THOMPSON, H. S., HYATT, J.-P., DE SOUZA, M. J. & CLARKSON, P. M. 1997. The effects of oral contraceptives on delayed onset muscle soreness following exercise. *Contraception,* 56**,** 59-65.

THOMPSON, W. R. & BINDER-MACLEOD, S. A. 2006. Association of genetic factors with selected measures of physical performance. *Physical therapy,* 86**,** 585-591.

TIAINEN, K., THINGGAARD, M., JYLHÄ, M., BLADBJERG, E., CHRISTENSEN, K. & CHRISTIANSEN, L. 2012. Associations between inflammatory markers, candidate polymorphisms and physical performance in older Danish twins. *Experimental gerontology,* 47**,** 109-115.

TIDBALL, J. G. 1991. Force transmission across muscle cell membranes. *Journal of biomechanics,* 24**,** 43-52.

TIDBALL, J. G. 2005. Inflammatory processes in muscle injury and repair. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 288**,** R345-R353.

TIDBALL, J. G. 2011. Mechanisms of muscle injury, repair, and regeneration. *Comprehensive Physiology,* 1**,** 2029-2062.

TIDBALL, J. G. & VILLALTA, S. A. 2010. Regulatory interactions between muscle and the immune system during muscle regeneration. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 298**,** R1173-R1187.

TIMMINS, R. G., BOURNE, M. N., SHIELD, A. J., WILLIAMS, M. D., LORENZEN, C. & OPAR, D. A. 2016. Short biceps femoris fascicles and eccentric knee flexor weakness increase the risk of hamstring injury in elite football (soccer): a prospective cohort study. *Br J Sports Med,* 50**,** 1524-1535.

TIMMINS, R. G., OPAR, D. A., WILLIAMS, M. D., SCHACHE, A. G., DEAR, N. M. & SHIELD, A. J. 2014. Reduced biceps femoris myoelectrical activity influences eccentric knee flexor weakness after repeat sprint running. *Scandinavian journal of medicine & science in sports,* 24**,** e299-e305.

TIMMINS, R. G., SHIELD, A. J., WILLIAMS, M. D., LORENZEN, C. & OPAR, D. A. 2015. Biceps femoris long head architecture: a reliability and retrospective injury study. *Medicine and science in sports and exercise,* 47**,** 905-913.

TOFT, A. D., JENSEN, L. B., BRUUNSGAARD, H., IBFELT, T., HALKJÆR-KRISTENSEN, J., FEBBRAIO, M. & PEDERSEN, B. K. 2002. Cytokine response to eccentric exercise in

young and elderly humans. *American Journal of Physiology-Cell Physiology,* 283**,** C289-C295.

TORRENTE, Y., EL FAHIME, E., CARON, N. J., DEL BO, R., BELICCHI, M., PISATI, F., TREMBLAY, J. P. & BRESOLIN, N. 2003. Tumor necrosis factor-alpha (TNF-alpha) stimulates chemotactic response in mouse myogenic cells. *Cell Transplant,* 12**,** 91-100.

TOTH, K. G., MCKAY, B. R., DE LISIO, M., LITTLE, J. P., TARNOPOLSKY, M. A. & PARISE, G. 2011. IL-6 induced STAT3 signalling is associated with the proliferation of human muscle satellite cells following acute muscle damage. *PLoS one,* 6**,** e17392.

TOUMI, H., F'GUYER, S. & BEST, T. M. 2006. The role of neutrophils in injury and repair following muscle stretch. *Journal of anatomy,* 208**,** 459-470.

TRAPPE, T. A., CARRITHERS, J. A., WHITE, F., LAMBERT, C. P., EVANS, W. J. & DENNIS, R. A. 2002. Titin and nebulin content in human skeletal muscle following eccentric resistance exercise. *Muscle & nerve,* 25**,** 289-292.

TSAOPOULOS, D. E., BALTZOPOULOS, V. & MAGANARIS, C. N. J. C. B. 2006. Human patellar tendon moment arm length: measurement considerations and clinical implications for joint loading assessment. 21**,** 657-667.

TSATALAS, T., GIAKAS, G., SPYROPOULOS, G., SIDERIS, V., LAZARIDIS, S., KOTZAMANIDIS, C. & KOUTEDAKIS, Y. 2013. The effects of eccentric exercise-induced muscle damage on running kinematics at different speeds. *Journal of sports sciences,* 31**,** 288-298.

TURRINA, A., MARTÍNEZ-GONZÁLEZ, M. A. & STECCO, C. 2013. The muscular force transmission system: role of the intramuscular connective tissue. *Journal of bodywork and movement therapies,* 17**,** 95-102.

VAESSEN, N., HEUTINK, P., JANSSEN, J. A., WITTEMAN, J. C., TESTERS, L., HOFMAN, A., LAMBERTS, S. W., OOSTRA, B. A., POLS, H. A. & VAN DUIJN, C. M. 2001. A polymorphism in the gene for IGF-I functional properties and risk for type 2 diabetes and myocardial infarction. *Diabetes,* 50**,** 637-642.

VAN DER HORST, N., SMITS, D.-W., PETERSEN, J., GOEDHART, E. A. & BACKX, F. J. 2015. The Preventive Effect of the Nordic Hamstring Exercise on Hamstring Injuries in Amateur Soccer Players A Randomized Controlled Trial. *The American journal of sports medicine***,** 0363546515574057.

VAN DYK, N., BAHR, R., WHITELEY, R., TOL, J. L., KUMAR, B. D., HAMILTON, B., FAROOQ, A. & WITVROUW, E. 2016. Hamstring and Quadriceps Isokinetic Strength Deficits Are Weak Risk Factors for Hamstring Strain Injuries A 4-Year Cohort Study. *The American journal of sports medicine***,** 0363546516632526.

VAN HOOREN, B. & BOSCH, F. 2018. Preventing hamstring injuries-Part 2: There is possibly an isometric action of the hamstrings in high-speed running and it does matter.

VAN POTTELBERGH, I., GOEMAERE, S., NUYTINCK, L., DE PAEPE, A. & KAUFMAN, J. 2001. Association of the type I collagen alpha1 Sp1 polymorphism, bone density and upper limb muscle strength in community-dwelling elderly men. *Osteoporosis International,* 12**,** 895-901.

VANGSTED, A. J., KLAUSEN, T. W., ABILDGAARD, N., ANDERSEN, N. F., GIMSING, P., GREGERSEN, H., NEXO, B. A. & VOGEL, U. 2011. Single nucleotide polymorphisms in the promoter region of the IL1B gene influence outcome in multiple myeloma patients treated with high-dose chemotherapy independently of relapse treatment with thalidomide and bortezomib. *Ann Hematol,* 90**,** 1173-81.

VAUGHAN, D., HUBER-ABEL, F. A., GRABER, F., HOPPELER, H. & FLÜCK, M. 2013. The angiotensin converting enzyme insertion/deletion polymorphism alters the response of muscle energy supply lines to exercise. *European journal of applied physiology,* 113**,** 1719-1729.

VENCKUNAS, T., SKURVYDAS, A., BRAZAITIS, M., KAMANDULIS, S., SNIECKUS, A. & MORAN, C. N. 2012. Human alpha-actinin-3 genotype association with exercise-induced muscle damage and the repeated-bout effect. *Applied Physiology, Nutrition, and Metabolism,* 37**,** 1038-1046.

VERBRUGGE, S. A. J., SCHONFELDER, M., BECKER, L., YAGHOOB NEZHAD, F., HRABE DE ANGELIS, M. & WACKERHAGE, H. 2018. Genes Whose Gain or Loss-Of-Function Increases Skeletal Muscle Mass in Mice: A Systematic Literature Review. *Front Physiol,* 9**,** 553.

VERHEUL, J., CLANSEY, A. C. & LAKE, M. J. 2017. Adjustments with running speed reveal neuromuscular adaptations during landing associated with high mileage running training. *J Appl Physiol (1985),* 122**,** 653-665.

VERMA, S., MOIZ, J., SHAREEF, M. & HUSAIN, M. 2015. Physical performance and markers of muscle damage following sports specific sprints in male collegiate soccer players: repeated bout effect. *The Journal of sports medicine and physical fitness*.

VINCENT, B., DE BOCK, K., RAMAEKERS, M., VAN DEN EEDE, E., VAN LEEMPUTTE, M., HESPEL, P. & THOMIS, M. A. 2007. ACTN3 (R577X) genotype is associated with fiber type distribution. *Physiol Genomics,* 32**,** 58-63.

VINCENT, B., WINDELINCKX, A., NIELENS, H., RAMAEKERS, M., VAN LEEMPUTTE, M., HESPEL, P. & THOMIS, M. A. 2010. Protective role of α-actinin-3 in the response to an acute eccentric exercise bout. *Journal of Applied Physiology,* 109**,** 564-573.

VOLAKLIS, K. A., HALLE, M. & MEISINGER, C. 2015. Muscular strength as a strong predictor of mortality: a narrative review. *European journal of internal medicine,* 26**,** 303-310.

VRIEZE, S. I., MCGUE, M., MILLER, M. B., HICKS, B. M. & IACONO, W. G. 2013. Three mutually informative ways to understand the genetic relationships among behavioral disinhibition, alcohol use, drug use, nicotine use/dependence, and their co-occurrence: twin biometry, GCTA, and genome-wide scoring. *Behavior genetics,* 43**,** 97-107.

WAHL, P., BRIXIUS, K. & BLOCH, W. 2008. Exercise-induced stem cell activation and its implication for cardiovascular and skeletal muscle regeneration. *Minimally Invasive Therapy & Allied Technologies,* 17**,** 91-99.

WALDÉN, M., HÄGGLUND, M., MAGNUSSON, H. & EKSTRAND, J. 2016. ACL injuries in men's professional football: a 15-year prospective study on time trends and return-to-play rates reveals only 65% of players still play at the top level 3 years after ACL rupture. *Br J Sports Med,* 50**,** 744-750.

WALLACE, J. L. & NORTON, K. I. 2014. Evolution of World Cup soccer final games 1966–2010: Game structure, speed and play patterns. *Journal of Science and Medicine in Sport,* 17**,** 223-228.

WALLIMANN, T., WYSS, M., BRDICZKA, D., NICOLAY, K. & EPPENBERGER, H. M. 1992. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem J,* 281 (Pt 1)**,** 21-40.

WALSH, S., LIU, D., METTER, E. J., FERRUCCI, L. & ROTH, S. M. 2008. ACTN3 genotype is associated with muscle phenotypes in women across the adult age span. *Journal of applied physiology,* 105**,** 1486-1491.

WALSTON, J., ARKING, D. E., FALLIN, D., LI, T., BEAMER, B., XUE, Q., FERRUCCI, L., FRIED, L. P. & CHAKRAVARTI, A. 2005. IL-6 gene variation is not associated with increased serum levels of IL-6, muscle, weakness, or frailty in older women. *Experimental gerontology,* 40**,** 344-352.

WANG, Y.-C., HU, Y.-W., SHA, Y.-H., GAO, J.-J., MA, X., LI, S.-F., ZHAO, J.-Y., QIU, Y.-R., LU, J.-B. & HUANG, C. 2015. Ox-LDL Upregulates IL-6 Expression by Enhancing NF-κB in an IGF2-Dependent Manner in THP-1 Macrophages. *Inflammation,* 38**,** 1-8.

WARREN, G. L., HULDERMAN, T., JENSEN, N., MCKINSTRY, M., MISHRA, M., LUSTER, M. I. & SIMEONOVA, P. P. 2002a. Physiological role of tumor necrosis factor alpha in traumatic muscle injury. *FASEB J,* 16**,** 1630-2.

WARREN, G. L., HULDERMAN, T., MISHRA, D., GAO, X., MILLECCHIA, L., O'FARRELL, L., KUZIEL, W. A. & SIMEONOVA, P. P. 2005. Chemokine receptor CCR2 involvement in skeletal muscle regeneration. *The FASEB journal,* 19**,** 413-415.

WARREN, G. L., LOWE, D. A. & ARMSTRONG, R. B. 1999. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Medicine,* 27**,** 43-59.

WARREN, G. L., LOWE, D. A., HAYES, D. A., KARWOSKI, C. J., PRIOR, B. M. & ARMSTRONG, R. 1993. Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *The Journal of Physiology,* 468**,** 487-499.

WARREN, J. D., BLUMBERGS, P. C. & THOMPSON, P. D. 2002b. Rhabdomyolysis: a review. *Muscle Nerve,* 25**,** 332-47.

WATANABE, K., KOUZAKI, M. & MORITANI, T. 2016. Effect of Electrode Location on Task-Dependent Electromyography Responses Within the Human Biceps Femoris Muscle. *Journal of applied biomechanics,* 32.

WEBSTER, K. E. & HEWETT, T. E. 2018. Meta-analysis of meta-analyses of anterior cruciate ligament injury reduction training programs. *Journal of Orthopaedic Research*.

WEI, W., FAREED, M. U., EVENSON, A., MENCONI, M. J., YANG, H., PETKOVA, V. & HASSELGREN, P. O. 2005. Sepsis stimulates calpain activity in skeletal muscle by decreasing calpastatin activity but does not activate caspase-3. *Am J Physiol Regul Integr Comp Physiol,* 288**,** R580-90.

WIEWELHOVE, T., FERNANDEZ-FERNANDEZ, J., RAEDER, C., KAPPENSTEIN, J., MEYER, T., KELLMANN, M., PFEIFFER, M. & FERRAUTI, A. 2015. Acute responses and muscle damage in different high-intensity interval running protocols. *The Journal of sports medicine and physical fitness*.

WILLEMS, S. M., WRIGHT, D. J., DAY, F. R., TRAJANOSKA, K., JOSHI, P. K., MORRIS, J. A., MATTEINI, A. M., GARTON, F. C., GRARUP, N. & OSKOLKOV, N. 2017. Large-scale GWAS identifies multiple loci for hand grip strength providing biological insights into muscular fitness. *Nature communications,* 8**,** 16015.

WILLIAMS, A. G., DAY, S. H., FOLLAND, J. P., GOHLKE, P., DHAMRAIT, S. & MONTGOMERY, H. E. 2005. Circulating angiotensin converting enzyme activity is correlated with muscle strength. *Med Sci Sports Exerc,* 37**,** 944-948.

WILLIAMS, A. G. & FOLLAND, J. P. 2008. Similarity of polygenic profiles limits the potential for elite human physical performance. *The journal of physiology,* 586**,** 113-121.

WILLOUGHBY, D., MCFARLIN, B. & BOIS, C. 2003. Interleukin-6 expression after repeated bouts of eccentric exercise. *International journal of sports medicine,* 24**,** 15-21.

WILSON, A. G., SYMONS, J. A., MCDOWELL, T. L., MCDEVITT, H. O. & DUFF, G. W. 1997. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. *Proceedings of the National Academy of Sciences,* 94**,** 3195-3199.

WILSON, E. M., HSIEH, M. M. & ROTWEIN, P. 2003. Autocrine growth factor signaling by insulin-like growth factor-II mediates MyoD-stimulated myocyte maturation. *Journal of Biological Chemistry,* 278**,** 41109-41113.

WILSON, G. J., WOOD, G. A. & ELLIOTT, B. C. 1991. Optimal stiffness of series elastic component in a stretch-shorten cycle activity. *Journal of applied physiology,* 70**,** 825-833.

WILSON, I. A., BRINDLE, K. M. & FULTON, A. 1995. Differential localization of the mRNA of the M and B isoforms of creatine kinase in myoblasts. *Biochem. J,* 308**,** 599-605.

WINDELINCKX, A., DE MARS, G., BEUNEN, G., AERSSENS, J., DELECLUSE, C., LEFEVRE, J. & THOMIS, M. 2007. Polymorphisms in the vitamin D receptor gene are associated with muscle strength in men and women. *Osteoporosis International,* 18**,** 1235-1242.

WISDOM, K. M., DELP, S. L. & KUHL, E. 2015. Use it or lose it: multiscale skeletal muscle adaptation to mechanical stimuli. *Biomechanics and modeling in mechanobiology,* 14**,** 195-215.

WOODS, C., HAWKINS, R., MALTBY, S., HULSE, M., THOMAS, A. & HODSON, A. 2004. The Football Association Medical Research Programme: an audit of injuries in professional football—analysis of hamstring injuries. *British journal of sports medicine,* 38**,** 36-41.

WOODS, D., HICKMAN, M., JAMSHIDI, Y., BRULL, D., VASSILIOU, V., JONES, A., HUMPHRIES, S. & MONTGOMERY, H. 2001. Elite swimmers and the D allele of the ACE I/D polymorphism. *Hum Genet,* 108**,** 230-2.

WOOLFSON, A. M., HEATLEY, R. V. & ALLISON, S. P. 1979. Insulin to inhibit protein catabolism after injury. *New England Journal of Medicine,* 300**,** 14-17.

WOZNIAK, A. C., KONG, J., BOCK, E., PILIPOWICZ, O. & ANDERSON, J. E. 2005. Signaling satellite-cell activation in skeletal muscle: markers, models, stretch, and potential alternate pathways. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine,* 31**,** 283-300.

WU, Y., ERDŐDI, F., MURÁNYI, A., NULLMEYER, K. D., LYNCH, R. M. & HARTSHORNE, D. J. 2003. Myosin phosphatase and myosin phosphorylation in differentiating C2C12 cells. *Journal of Muscle Research & Cell Motility,* 24**,** 499-511.

WÜST, R. C., MORSE, C. I., DE HAAN, A., JONES, D. A. & DEGENS, H. 2008. Sex differences in contractile properties and fatigue resistance of human skeletal muscle. *Experimental physiology,* 93**,** 843-850.

YAHIAOUI, L., GVOZDIC, D., DANIALOU, G., MACK, M. & PETROF, B. J. 2008. CC family chemokines directly regulate myoblast responses to skeletal muscle injury. *J Physiol,* 586**,** 3991-4004.

YAMIN, C. 2009. Reply to "IL6 genotype and creatine kinase response to exercise". *European journal of applied physiology,* 107**,** 375-375.

YAMIN, C., AMIR, O., SAGIV, M., ATTIAS, E., MECKEL, Y., EYNON, N., SAGIV, M. & AMIR, R. E. 2007. ACE ID genotype affects blood creatine kinase response to eccentric exercise. *Journal of Applied Physiology,* 103**,** 2057-2061.

YAMIN, C., DUARTE, J. A. R., OLIVEIRA, J. M. F., AMIR, O., SAGIV, M., EYNON, N., SAGIV, M. & AMIR, R. E. 2008. IL6 (-174) and TNFA (-308) promoter polymorphisms are

associated with systemic creatine kinase response to eccentric exercise. *European journal of applied physiology,* 104**,** 579-586.

YAMIN, C., OLIVEIRA, J., MECKEL, Y., EYNON, N., SAGIV, M., AYALON, M., ALVES, A. J. & DUARTE, J. A. 2010. CK-MM gene polymorphism does not influence the blood CK activity levels after exhaustive eccentric exercise. *Int J Sports Med,* 31**,** 213-7.

YANG, J., BAKSHI, A., ZHU, Z., HEMANI, G., VINKHUYZEN, A. A., LEE, S. H., ROBINSON, M. R., PERRY, J. R., NOLTE, I. M. & VAN VLIET-OSTAPTCHOUK, J. V. 2015. Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. *Nature genetics*.

YANG, J. & XU, X. 2012. alpha-Actinin2 is required for the lateral alignment of Z discs and ventricular chamber enlargement during zebrafish cardiogenesis. *FASEB J,* 26**,** 4230-42.

YANG, M., RAMACHANDRAN, A., YAN, H. M., WOOLBRIGHT, B. L., COPPLE, B. L., FICKERT, P., TRAUNER, M. & JAESCHKE, H. 2014. Osteopontin is an initial mediator of inflammation and liver injury during obstructive cholestasis after bile duct ligation in mice. *Toxicol Lett,* 224**,** 186-95.

YANG, N., MACARTHUR, D. G., GULBIN, J. P., HAHN, A. G., BEGGS, A. H., EASTEAL, S. & NORTH, K. 2003. ACTN3 Genotype Is Associated with Human Elite Athletic Performance. *The American Journal of Human Genetics,* 73**,** 627-631.

YANG, S. Y. & GOLDSPINK, G. 2002. Different roles of the IGF-I Ec peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. *FEBS letters,* 522**,** 156-160.

YANG, W. & HU, P. 2018. Skeletal muscle regeneration is modulated by inflammation. *Journal of orthopaedic translation*.

YE, S. 2006. Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. *Cardiovascular research,* 69**,** 636-645.

YIN, H., PRICE, F. & RUDNICKI, M. A. 2013. Satellite cells and the muscle stem cell niche. *Physiological reviews,* 93**,** 23-67.

YOO, J., KIM, B.-H., KIM, S.-H., KIM, Y. & YIM, S.-V. 2016. Genetic polymorphisms to predict gains in maximal O 2 uptake and knee peak torque after a high intensity training program in humans. *European journal of applied physiology,* 116**,** 947-957.

YOSHIDA, T., HUQ, T. S. & DELAFONTAINE, P. 2014. Angiotensin type 2 receptor signaling in satellite cells potentiates skeletal muscle regeneration. *Journal of Biological Chemistry***,** jbc. M114. 585521.

YU, Z. B. 2013. Tetanic contraction induces enhancement of fatigability and sarcomeric damage in atrophic skeletal muscle and its underlying molecular mechanisms. *Zhongguo ying yong sheng li xue za zhi= Zhongguo yingyong shenglixue zazhi= Chinese journal of applied physiology,* 29**,** 525-533.

ZALIN, R. 1972. Creatine kinase activity in cultures of differentiating myoblasts. *Biochem. J,* 130.

ZAMMIT, P. S. 2008. All muscle satellite cells are equal, but are some more equal than others? *J Cell Sci,* 121**,** 2975-82.

ZANOTTI, S., GIBERTINI, S., DI BLASI, C., CAPPELLETTI, C., BERNASCONI, P., MANTEGAZZA, R., MORANDI, L. & MORA, M. 2011. Osteopontin is highly expressed in severely dystrophic muscle and seems to play a role in muscle regeneration and fibrosis. *Histopathology,* 59**,** 1215-1228.

ZANOU, N. & GAILLY, P. 2013. Skeletal muscle hypertrophy and regeneration: interplay between the myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways. *Cellular and Molecular Life Sciences,* 70**,** 4117-4130.

ZHAN, M., JIN, B., CHEN, S.-E., REECY, J. M. & LI, Y.-P. 2007. TACE release of TNF-α mediates mechanotransduction-induced activation of p38 MAPK and myogenesis. *J Cell Sci,* 120**,** 692-701.

ZHANG, B.-T., YEUNG, S. S., ALLEN, D. G., QIN, L. & YEUNG, E. W. 2008. Role of the calcium-calpain pathway in cytoskeletal damage after eccentric contractions. *Journal of Applied Physiology,* 105**,** 352-357.

ZHANG, G., YOUNG, B., EZURA, Y., FAVATA, M., SOSLOWSKY, L., CHAKRAVARTI, S. & BIRK, D. E. 2005. Development of tendon structure and function: regulation of collagen fibrillogenesis. *J Musculoskelet Neuronal Interact,* 5**,** 5-21.

ZHAO, Z., LIU, B., LI, B., SONG, C., DIAO, H., GUO, Z., LI, Z. & ZHANG, J. 2017. Inhibition of long noncoding RNA IGF2AS promotes angiogenesis in type 2 diabetes. *Biomedicine & Pharmacotherapy,* 92**,** 445-450.

ZILLIKENS, M. C., DEMISSIE, S., HSU, Y.-H., YERGES-ARMSTRONG, L. M., CHOU, W.-C., STOLK, L., LIVSHITS, G., BROER, L., JOHNSON, T. & KOLLER, D. L. 2017. Large meta-analysis of genome-wide association studies identifies five loci for lean body mass. *Nature communications,* 8**,** 80.