Sex-based differences in the androgen receptor response to exercise

Sex-specific changes to androgen receptor content following exercise and its influence on skeletal muscle adaptions

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TITLE: Sex-specific changes to androgen receptor content following exercise and its influence on skeletal muscle adaptions

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LAY ABSTRACT

Skeletal muscle is crucial for proper function and activities of daily living. Many factors can regulate the amount and quality of skeletal muscle such as the expression of a protein known as the androgen receptor (AR). The AR plays a role in many cellular pathways that can ultimately dictate the growth and size of a particular tissue like skeletal muscle. There is currently minimal research about AR during skeletal muscle damage and in female skeletal muscle. Understanding how exercise increases AR content in males and females could progress our knowledge of how muscle adapts differently to exercise between sexes. Therefore, the purpose of this study was to understand how the AR behaves in the muscle, in males and females, after a single session of exercise that damages the muscle or after long term resistance exercise (RE). We observed that the AR gene is more abundant in females than males at rest and following damaging exercise. Furthermore, we show that AR protein content increases in both sexes following a single session of damaging exercise and after chronic RE. Muscle stem cells are a component of the muscle that helps to heal muscle after exercise has been performed. In the current study, we demonstrate that AR has a closer relationship to muscle stem cells in males relative to females. Further, AR seems to be more closely linked to muscle growth in males than females. Altogether, AR is a component of the muscle that adapts to exercise differently in males and females. This study may, in part, explain how skeletal muscle responds differently between sexes after exercise.

ABSTRACT

The androgen hormone is responsible for the growth of secondary sex characteristics in humans, such as skeletal muscle. Upon an exercise stimulus, the androgen receptor (AR) plays a crucial role in transmitting the androgenic signal to the nucleus which upregulates transcription of target genes related to growth of skeletal muscle. AR content has been implicated in the hypertrophic response between high and low responders following resistance exercise training (RET) in males. Little is known of the impact of AR expression on acute skeletal muscle damage and whether AR may influence the adaptive response to RET in females. This study aimed to investigate acute changes to AR content following a single bout of muscle damage-inducing exercise as well as sex differences during skeletal muscle repair and hypertrophy. A skeletal muscle biopsy from the *vastus lateralis* was obtained from 26 healthy, young males (n=13) and females (n=13) at baseline and 48 hours following a single bout of 300 eccentric contractions of the quadriceps. Subsequently, participants performed whole-body RET 4 times a week for 10 weeks, followed by a final skeletal muscle biopsy under resting conditions. Females had greater AR mRNA than males at baseline (~53%) and post-damage (~126%; $p<0.05$) while AR protein content increased in both sexes similarly following a single bout of eccentric exercise ($p<0.05$). Damage- and RET-induced satellite cell response was associated with percent change in AR protein content in a sex-specific manner ($p<0.05$). RET-induced percent change in nuclear AR content was ~17% greater in males compared to females ($p<0.05$). Interestingly, following RET, males experiencing the highest percent change in myofibre cross sectional area (CSA) exhibited greater changes in nuclear-associated AR protein content compared to females with the highest percent change in CSA. Collectively, AR protein content is elevated following acute eccentric

exercise and 10 weeks of RET. Findings from this study suggests that males are more reliant on AR-related mechanisms than females to induce skeletal muscle hypertrophy following RET.

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Abbreviations

ANOVA – Analysis of variance

BCA – Bicinchoninic acid

BME – β-mercaptoethanol

BSA – Bovine serum albumin

cDNA – Complimentary deoxyribonucleic acid

CSA – Cross sectional area

DAPI – 4,6-diamidino-2-phenylindole

DHT – Dihydrotestosterone

DTA – Diphtheria toxin fragment A

DXA – Dual-energy X-ray absorptiometry

GAPDH – Glyceraldehyde 3-phosphate dehydrogenase

IF – Immunofluorescence

IGF-1 – Insulin like growth factor-1

KO – Knockout

MHCII – Myosin heavy chain II

mKO – Muscle-specific knockout

MPS – Muscle protein synthesis

MRF – Myogenic regulatory factor

MRF4 – Myogenic regulatory factor 4

MRI – Magnetic resonance imaging

mTORC1 – Mammalian target of rapamycin complex 1

MYF5 – Myogenic Factor 5

Mylk4 – Myosin light chain kinase family member 4

MyoD – Myoblast determination protein 1

NSAIDS – Nonsteroidal anti-inflammatory drugs

OCT – Optimal cutting temperature

Pax7 – Paired box transcription factor-7

qPCR – Quantitative polymerase chain reaction

RE – Resistance exercise

RET – Resistance exercise training

RIPA – Radio-immunoprecipitation assay

RM – Repetition maximum

RNA – Ribonucleic Acid

ROI – Region of interest

SC – Satellite cells

TBST – Tris buffered saline and tween

WT – Wild-type

LITERATURE REVIEW

Introduction

Skeletal muscle is a crucial organ that accounts for ~60% of total body mass and plays a pivotal role in breathing and locomotion as well as maintaining metabolic homeostasis. The amount of skeletal muscle can vary depending on internal variables such as genetic variation as well as external stimuli such as exercise or lack thereof, which could result in skeletal muscle growth (i.e., hypertrophy) or loss (i.e., atrophy), respectively. More specifically, total muscle mass is determined by the balance between muscle protein synthesis (MPS) and breakdown (Chesley et al., 1992). Skeletal muscle hypertrophy occurs when the rates of synthesis exceed breakdown which can be achieved with chronic resistance exercise training (RET), along with proper nutritional supplementation (Delorme, 1945; Phillips, 2014). In contrast, a consistent negative protein balance, through excessive sedentary behaviour and inadequate nutrition, can lead to skeletal muscle breakdown and subsequent atrophy as seen in conditions such as age-related muscle loss, hospital admissions and androgen deficiencies. Skeletal muscle growth in adults occurs through hypertrophy of individual myofibres, which can be directly measured via fibre cross-sectional area (CSA) obtained from a skeletal muscle biopsy. Other non-invasive methods are also commonly used to measure skeletal muscle mass and area such as magnetic resonance imaging (MRI) and ultrasonography (Haun et al., 2019). Furthermore, dual x-ray absorptiometry is a common measure of fat-free mass, which is primarily skeletal muscle in the limbs.

Satellite cells (SC) are muscle stem cells that contribute to hypertrophy following RET by donating myonuclei to existing myofibres (Snijders et al., 2015). They are necessary for adequate skeletal muscle regeneration and repair following an acute bout of damage (Bentzinger et al., 2012; H. Yin et al., 2013). SC function is highly dependent on their interaction with circulating signalling molecules, hormones, and growth factors that are distributed via capillaries in an autocrine and

endocrine manner (Nederveen et al., 2016, 2018). Androgens are circulating hormones that impact SC proliferation (Sinha-Hikim, Roth, Lee, Bhasin, et al., 2003). The androgen hormone must bind to the androgen receptor (AR) to induce skeletal muscle adaptations (Davey & Grossmann, 2016). AR content is an effective modifier of skeletal muscle repair and hypertrophy due to its ability to drive androgenic transcriptional changes in the cell. As such, higher AR expression is associated with increased number of SC and improved muscle repair, as well as hypertrophy (Kim et al., 2016; Mackrell et al., 2015). Interestingly, the hypertrophic response in young healthy males was positively correlated to AR content but not circulating testosterone (Morton et al., 2018). Nevertheless, the AR response in humans following definitive skeletal muscle repair remains to be examined. Additionally, studies examining changes in AR content with exercise in healthy females are scarce. Therefore, the purpose of this review is to 1) outline the AR function and its role in skeletal muscle, 2) discuss the AR response and its relation to SC content following exercise, and 3) summarize the evidence regarding skeletal muscle AR expression and sex-based differences at rest and after exercise.

Androgens

Sex hormones, produced primarily by reproductive organs, facilitate the development of primary and secondary sex characteristics of numerous tissues including skeletal muscle (Davey & Grossmann, 2016; Wierman, 2007). Androgens (testosterone and dihydrotestosterone [DHT]) are steroid hormones that are mainly produced in the testis in males and are ligands of AR. Although testosterone is the most abundant androgen in circulation, it cannot effectively bind to AR in its true form and therefore, must be converted into DHT via 5α-reductase to enhance binding affinity (Burd, 2006).

Early investigation on the effects of androgens on skeletal muscle mass were performed in the 1960s on castrated mice (Koller, 1960), where they noted that anabolic steroids influence skeletal muscle adaptation such as myofibre size and SC content. Supraphysiological doses of androgens have also been shown to increase skeletal muscle mass and strength in humans (Bhasin et al., 2001). Sinha-Hikim and colleagues (2003, 2004, 2006) demonstrated that testosterone administration induced skeletal muscle hypertrophy concomitant with an increase in myonuclei and SC number. Additionally, they found a dose-dependent relationship between testosterone administration and skeletal muscle growth in young men (Sinha-hikim et al., 2004; Sinha-Hikim, Roth, Lee, & Bhasin, 2003). However, exogenous testosterone supplementation for muscle growth has various deleterious off-target effects such as excess hair growth, prostate cancer, and pathological cardiac hypertrophy (Davey & Grossmann, 2016; Basaria, 2011; Tan et al., 2015; Yeh et al., 1998). Although the direct impact of testosterone on skeletal muscle mass has yet to be fully elucidated, recent work suggests that intramuscular AR, and not circulating endogenous androgens, may augment muscle growth in human skeletal muscle (MacLean et al., 2008; Morton et al., 2018; West et al., 2010, 2012). This theory, which contradicts much of the previous androgen literature, could uncover a novel approach to understanding how AR impacts skeletal muscle growth and repair from exercise.

Androgen receptor structure and function

The AR is a member of the nuclear receptor family and is required for androgen hormone signalling in target cells and tissues. The AR protein is approximately 100-120 kDa and is made up of 4 domains, consisting of an N-terminal domain, DNA binding domain, and 2 ligand binding domains (Zaręba & Sidorkiewicz, 2017). AR can impact cellular growth through genomic or non-

genomic pathways (Davey & Grossmann, 2016). Genomic signalling requires androgens, most commonly DHT, to bind AR in the cytosol causing a conformational change to the receptor which involves discarding heat shock proteins that are typically bound to AR when inactive (La Montagna et al., 2012; Zaręba & Sidorkiewicz, 2017). Once bound, the AR is actively transported through the 60-90 kDa nuclear pore complex (R. Wang & Brattain, 2007) and binds to androgen response elements (Lu et al., 2021). This elicits a signalling cascade to upregulate the transcription of several genes including insulin-like growth factor-1 (IGF-1), myosin light chain kinase family member 4 (Mylk4) and myostatin, all of which are known to regulate skeletal muscle mass.

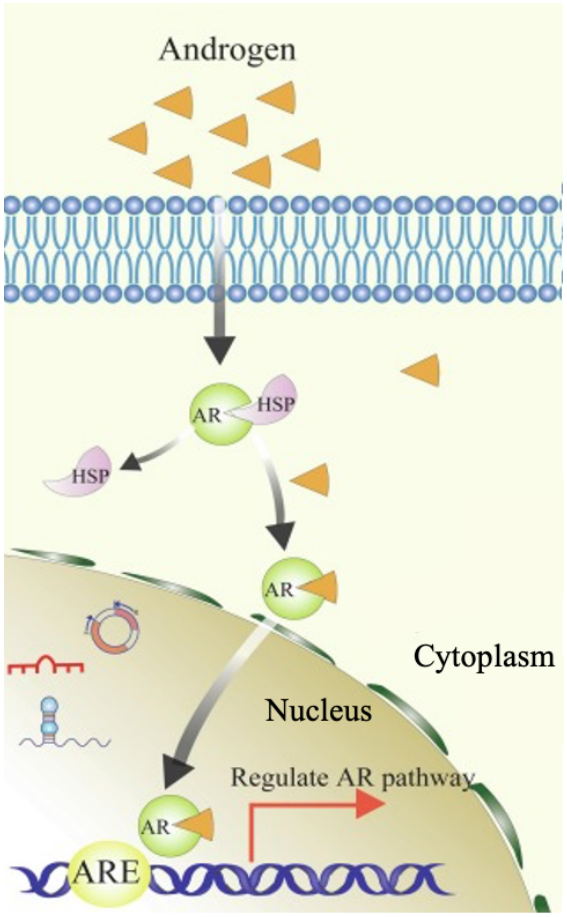


Figure 1: A schematic of AR dynamics in the cell. AR floats freely in the cytosol in an inactive form, bound by heat shock proteins (HSP). Androgens diffuse through the membrane of the cell to bind AR which dislodges HSP. The androgen-AR complex then translocates through the nuclear pores and into the nucleus. AR binds to androgen response elements which triggers transcriptional regulation of downstream AR targets. Adapted from Lu et al. (2021).

Following the release of the androgen from its receptor, DNA binding is terminated to return the AR to its inactive form and enable nuclear export to the cytosol where it can be reused or degraded (Saporita et al., 2003; Tyagi et al., 2000). AR is the only nuclear receptor that can be

recycled, meaning that the protein can undergo a cyclical nuclear import/export pattern up to 4 times before becoming unstable and breaking down in the cytosol (Jaworski, 2006; Tyagi et al., 2000) through upregulation of growth factors and cytokines triggering proteasomal degradation (Ikezoe et al., 2004; Lee & Chang, 2003). Interestingly, declines in AR protein content has been observed in human skeletal muscle within minutes to hours following a single bout of RE (Ratamess et al., 2005; Spiering et al., 2009; Vingren et al., 2009). This rapid AR response following acute RE is likely due to enhanced proteasome activity. Briefly, some studies have demonstrated that AR protein content decreases acutely upon ligand binding and AR protein is spared from degradation once reaching the nucleus, where it can upregulate itself (Gaughan et al., 2005; Jaworski, 2006). This acute AR regulatory mechanism might explain the rapid AR protein decline because the RE would rapidly increase blood androgen concentration which would elicit more androgens binding AR in the skeletal muscle. Measuring AR protein content immediately after exercise would therefore result in a decline in AR content since androgens are just beginning to bind AR in the cytosol which is a period of fragility for AR as it is still exposed to degradation in the cytosol before translocating to the safety of the nucleus.

AR in skeletal muscle and changes with exercise

Examining AR regulation in skeletal muscle

The cascade of transcriptional events that AR creates is expansive and complex. There are certain target transcripts of AR within skeletal muscle that are worth highlighting that impact skeletal muscle growth and repair. Notably, IGF-1 plays a role in regulating mammalian target of rapamycin complex 1 (mTORC1) which upregulates several elements of MPS (Chambon et al., 2010; Chen et al., 2005; X. Wang & Proud, 2006; L. Yin et al., 2020). Of late, Mylk4 has been

characterized as a potential target of AR that can improve skeletal muscle adaptation by increasing skeletal muscle strength (Miyamoto-Mikami et al., 2018; Sakakibara et al., 2021; Tumasian et al., 2021). AR knockout (KO) mouse models (SC-specific or gobal) express less myostatin mRNA than control (Dubois et al., 2014). Moreover, androgen treatment induced greater muscle hypertrophy of gonadectomized myostatin KO mice than WT control that naturally expressed myostatin. Since myostatin is a negative regulator of muscle hypertrophy, this work by Dubois et al. (2014) suggests that AR upregulates myostatin as a control mechanism to limit excess muscle growth.

Genetic manipulation of pre-clinical models has been frequently used to characterize the role of AR within skeletal muscle. Mice lacking AR within skeletal muscle (AR mKO) display an increased number of type-I fibres, and lower type-II fibres compared to wild-type (WT) (Ophoff et al., 2009) suggesting that the presence of AR may alter fibre type composition. Moreover, AR mKO animals have lower amounts of lean body mass relative to control. In contrast, others have demonstrated that, irrespective of AR content, androgens regulate limb muscle mass in mice (Chambon et al., 2010). Chambon et al. (2010) showed that AR mKO mice experienced type-I and type-II fibre atrophy to the same degree as the non-KO control mouse, indicating that AR does not influence fibre type distribution or skeletal muscle hypertrophy (Chambon et al., 2010). Altogether, Ophoff et al., (2009) and Chambon et al., (2010) both report no change in mass of most leg muscles in AR KO mice, yet they report opposing findings for lean body mass and fibre type distribution. These discrepancies could be a result of differing AR ablation methods in the mutant mouse models which could impact how skeletal muscle adapts during murine development. The literature surrounding AR regulation in skeletal muscle is unclear and often conflicting. Understanding the intervention behind each study may explain some of the variability to do with

AR research in skeletal muscle. Considering the methodology underlying each study may justify some of the inconsistencies regarding AR's impact on skeletal muscle adaptations.

Preliminary work in young healthy men revealed that AR protein content increases in response to exogenous testosterone (30 or 100nM) administration (Sinha-hikim et al., 2004). More recently, Morton and colleagues (2018) found that young men with the most hypertrophy following RET had greater baseline AR protein content relative to those with least hypertrophy. It is worth highlighting that these participants were trained males prior to the study commencement whereas many other pertinent studies examine AR in untrained individuals. The participant training history could have a notable impact on AR outcomes as 2 bouts of heavy RE is sufficient to increase AR mRNA and protein in untrained men (Willoughby & Taylor, 2004). Therefore, it is inappropriate to compare AR content of untrained participants to those of Morton et al., 2018 since they recruited young men performing 3 to 6 bouts of RE a week. Overall, it is important to account for differences in participant characteristics (i.e., sex, age, training status etc.) and the type of training conducted when comparing between studies. Other studies examining AR have observed tremendous inter-individual variability (Ahtiainen et al., 2011; Mitchell et al., 2013). Classifying participants as low or high responders to hypertrophy appears to, in part, explain these vast differences in AR content (Morton et al., 2018). Overall, AR is debated to enhance skeletal muscle growth but variability within studies and between individuals hinders reliability. Large discrepancies in the literature make it difficult to draw conclusions around AR's impact on skeletal muscle adaptions following exercise.

AR and acute exercise

Inoue and colleagues (1993) were the first to investigate changes in AR protein content following acute exercise. Gastrocnemius muscles from WT mice were electrically stimulated every other day for 3, 5, 13 and 27 days to emulate exercise. It was found that AR protein content peaked around the third day, which is similar to findings in later studies examining RE-induced AR content. Two studies examined AR mRNA in untrained, young males following 3 sequential bouts of heavy RE. Both protocols were similar, including 48 hours of rest between each bout of RE. However, one study showed no change in AR mRNA (Poole, Oberts, Albo, & Underland, 2011) whereas the other observed an increase in AR mRNA 48 hours after the second bout of RE (Willoughby & Taylor, 2004). An important difference between these studies is how they familiarized participants prior to the RE protocol. Specifically, Poole et al., (2011) had subjects perform 3 familiarization sessions within a week to determine 1 repetition maximum (RM) testing and to acclimate to the protocol. After the third familiarization session participants rested for a week prior to the RE protocol. Willoughby et al., (2004) had subjects perform no more than 5 trials of 1RM testing on a single day. They purposefully designated 12 days of rest after 1RM tests to ensure that any upregulation in mRNA or protein returned to baseline. Taken together, it appears that RE familiarization sessions prior to 3 bouts of heavy RE can blunt AR mRNA expression in untrained, young males indicating that AR gene expression increased following subsequent RE bouts (Poole et al., 2011b; Willoughby & Taylor, 2004). The increase in AR mRNA expression, shown by Willoughby et al., (2004) was hypothesized to sensitize the skeletal muscle to androgens for an enhanced skeletal muscle hypertrophy response.

Studies have shown that AR protein content increases following RE of the upper (Spiering et al., 2009) and lower (Willoughby & Taylor, 2004) body with intensities sufficient to increase

circulating androgen hormone concentrations. When analyzing the acute AR protein response, it seems that AR content is transiently downregulated immediately post-RE (at ~1 hour), followed by an increase at ~48 hours post-RE (Bamman et al., 2001; Ratamess et al., 2005; Spiering et al., 2009; Vingren et al., 2009). Acute exercise activates many pathways, including proteasomal degradation, to clear unwanted debris (Ikezoe et al., 2004; Lee & Chang, 2003). The short-term downregulation of AR protein following acute exercise is likely due to proteasomal degradation of AR in the cytoplasm. Muscle damage and myofibrillar protein synthetic rate (i.e., an indicator of hypertrophy) are greatest following an initial bout of RE in untrained young men (Damas et al., 2016). Once training began, myofibrillar protein synthesis rates, following the latter 2 bouts of RE, were correlated with muscle hypertrophy. This indicated that trained muscle became "fine-tuned" and demonstrated that an acute response was representative of long-term adaptations (Damas et al., 2016). This evidence further suggests that untrained individuals respond differently to acute RE relative to trained individuals. More work is necessary to uncover the acute AR response following exercise.

Eccentric contraction-induced muscle damage

Eccentric contractions are an effective and commonly used method to induce skeletal muscle damage in order to study the mechanisms underlying human skeletal muscle repair (Mahoney et al., 2008). A commonly utilized protocol consists of 300 eccentric contractions of the knee extensors on an isokinetic dynamometer to elicit damage in the *vastus lateralis* (Mahoney et al., 2008; Nederveen et al., 2018). Our laboratory, and others, reported that this method successfully increases central nucleation, satellite cell activity and myogenic gene expression, all of which are indicators of muscle repair (Fortino et al., 2022; Nederveen et al., 2018). To date, no

studies have investigated changes in AR expression and signalling following 300 eccentric contractions. Bamman and colleagues (2001) utilized an alternative mechanism to elicit acute muscular damage. Using a crossover experimental design, untrained males and females were instructed to perform 64 concentric or eccentric squats and AR mRNA expression was subsequently measures. The authors reported that 48 hours following exercise, there was an increase in AR mRNA independent of the type of contraction. Future work should focus on utilizing a traditional eccentric damage protocol (300 contractions) to comprehensively understand the role of AR signalling during skeletal muscle repair as 64 eccentric squats has not been shown to sufficiently induce repair.

AR and resistance exercise training

AR content is increased 48 hours post-RE, therefore, undergoing repeated bouts of RE for several weeks (i.e., RET) should, theoretically, increase AR content as well. Twelve weeks of RET downregulates AR protein content, where myonuclear and SC content are upregulated in untrained males, regardless of low, medium, or high responder ranking (Mobley et al., 2018). This may have to do with the lower intensity of RE, which was enough to induce a SC response and hypertrophy but did not provide enough stimulus to maintain an elevated AR protein content throughout the twelve weeks of RET. Based on previous findings, AR protein content is highly heterogenous between individuals, which has limited some studies from determining changes with RET in untrained males (Ahtiainen et al., 2011; Mitchell et al., 2013). The same studies have demonstrated a significant correlation between intramuscular AR content and hypertrophy following RET. Ahtiainen et al., (2009) reported that exercise training modality (strength, endurance or combined) did not affect AR protein or mRNA expression either before or after each 7-week training program.

These findings from Ahtiainen et al., (2011) and Mitchel et al., (2013) were extended when resistance-exercise-trained males were divided into high and low responders to RET (greatest and least hypertrophy), which was implemented to help understand the large variability found between individuals in skeletal muscle mass following RET (Hubal et al., 2005). High responders to hypertrophy had greater AR content post-RET relative to the low hypertrophic responders, there was also no increase in AR protein content from baseline to post-RET in either group (Morton et al., 2018). Interestingly, there was no difference in testosterone concentration between the high and low hypertrophy groups, although AR protein content was greater in the high responders. These results suggest that intramuscular AR protein content is associated with an increased hypertrophy response that is independent of circulating testosterone concentrations. Overall, previous work reports mixed results when investigating the AR protein response to several weeks of RET, which highlights the inter-individual variability with AR following exercise. As satellite cells have been shown to be important for skeletal muscle repair and hypertrophy, SC interplay with AR has been shown to augment skeletal muscle repair and growth after exercise.

Satellite cells and skeletal muscle

Mauro et al. (1961) first characterized SC as being situated between the basal lamina and the sarcolemma of the skeletal muscle fibre (Mauro, 1961). SCs in adult muscle donate myonuclei to existing skeletal muscle fibres to aid in repair, regeneration and remodelling following exercise, injury or disease (Sambasivan et al., 2011), or can fuse to form myotubes under circumstances of extreme muscle damage (E. Schultz, D. Jaryszak, 1985). Typically in a quiescent state, SCs express paired box transcription factor 7 (Pax7) which is required for replenishment of the SC pool (Seale et al., 2000). Pax7 KO mice experience reduced skeletal muscle hypertrophy, greater

atrophy, and impaired muscle repair (Kuang et al., 2006; Seale et al., 2000) suggesting that Pax7+ SC are essential for healthy regeneration of adult skeletal muscle.

Myogenic regulatory factors and SC characteristics

Upon muscle injury of varying degrees, myogenic regulatory factor (MRF) expression is upregulated in SC, which coordinates a myogenic response to aid in muscle fibre repair (Buckingham & Rigby, 2014). MRFs are muscle-specific proteins composed of myoblast determination protein 1 (MyoD), myogenic factor 5 (MYF5), myogenin and myogenic regulatory factor 4 (MRF4). Differentiation of SC necessitates the expression Myf5 to allow for early myogenic commitment. This is followed by the upregulation of MyoD which denotes "activated" SCs. Upon a stimulus, such as exercise, SCs activate by co-expressing MyoD and Pax7 which is indicative of early myogenic commitment (Grounds et al., 1992; Snijders et al., 2015). Once activated, SCs can downregulate MyoD, which instigates proliferation to replenish the SC pool, alternatively, SCs reduce Pax7 to differentiate and donate myonuclei to existing myofibres as part of skeletal muscle repair (Bentzinger et al., 2012; H. Yin et al., 2013). Immunofluorescence (IF) allows for the visualization and quantification of SC content and activity within skeletal muscle cross-sections by identifying transcription factors such as Pax7 and MyoD. Using this method, exercise biologists can examine the SC response to various external factors including changes in hormones, growth factors and oxygen, which are essential for the proper functioning of the cell (Timmerman et al., 2010). Parabiosis experiments revealed that SC from an old mouse exposed to the systemic environment of a young mouse enhanced SC activation (Conboy et al., 2005). In other words, factors associated with the SC environment impairs function, not the SC itself. The AR is one of many components of the SC environment that could ultimately influence SC behaviour.

This relationship involves AR being situated directly in SC as well as the AR situated in the muscle microenvironment which could influence SC via downstream pathways which are triggered by AR.

The influence of exercise on SC

SCs have demonstrated a contraction-dependent upregulation in content and activity following exercise. A muscle-damaging bout of eccentric exercise has been shown to trigger a pronounced activation and expansion of the SC pool (Fortino et al., 2022; Nederveen et al., 2018). In a study comparing acute bouts of eccentric and concentric exercise, matched for intensity and volume, only eccentric exercise induced SC activation 24 hours post-exercise (Hyldahl & Hubal, 2014). Relative to concentric contractions, eccentric contractions induced greater muscle damage, which is likely responsible for an enhanced SC response (Mahoney et al., 2008). Although there is evidence that SC have an influence on skeletal muscle hypertrophy following resistance training, it has been a debated topic in the literature. Work in SC depleted mice has shown an increase in hypertrophy, suggesting that SC are not essential for skeletal muscle hypertrophy (Fry et al., 2015; Mccarthy et al., 2011). Conversely, others have demonstrated a relationship between SC and hypertrophy in human studies (Bellamy et al., 2014; Mackey et al., 2011; Petrella et al., 2006; Verdijk et al., 2009). These opposing conclusions are a result of different research questions as Fry et al. (2015) and McCarthy et al. (2011) utilized a Pax7-Diphtheria toxin fragment A (DTA) mouse model ablating greater than 90% of SC to determine whether SC are necessary for hypertrophy. Other work, in human and mouse models, would indicate that SC have an important function in skeletal muscle hypertrophy following exercise (Bellamy et al., 2014; Mackey et al., 2011; Petrella et al., 2006; Verdijk et al., 2009), regardless of their necessity (Fry et al., 2015; Mccarthy et al., 2011). Recently, the soleus and plantaris of mice were depleted of SC where the

muscle adapted to exercise but to a lesser extent than the mice that underwent SC repletion (Englund et al., 2020). These authors also demonstrate that SC depletion affected the muscle transcriptome which highlights various gene networks that are impacted by presence of SC. Altogether, these findings suggest SC are important for skeletal muscle hypertrophy in ways that are more complex than once thought.

Androgen signalling and its influence on SC

Experiments administering testosterone to mice or treating cells, *in vitro,* first demonstrated that AR protein is expressed in both quiescent and activated SC (Doumitt, Cooks, & Merkel, 1996; Joubert & Tobin, 1995). Genetic profiling revealed an increase in MRF expression with androgen hormone treatment following acute skeletal muscle injury in mice, indicating the importance of androgens in the acute SC response to skeletal muscle damage (Mackrell et al., 2015). Kim et al. (2016) supplemented DHT in orchiectomized mice which resulted in an increase in CSA and a 3-fold expansion of the SC population following an acute skeletal muscle injury. The AR response during skeletal muscle regeneration appears to influence gene expression related to SC activity, which has been shown in cell and murine models to ultimately change the behaviour of SC upon a drastic androgenic stimulus.

Although many studies have reported a relationship between AR content and SC, Englund and colleagues (2019) demonstrated that giving excess testosterone increased hypertrophy independent of SC content. This suggests that SC do not play a role in AR's impacts on hypertrophy. Notably, these experiments were performed using female mice which begs the question of how this experiment impacts male mice. Although this study utilized a SC depleted mouse model (which may not be a physiologically representative model), female mouse skeletal

muscle may respond differently to a hypertrophic stimulus compared to a male mouse where males could potential be relying more on AR's influence on SC during skeletal muscle hypertrophy as compared to females. More work is needed to understand AR's impact on SC in skeletal muscle following damage or hypertrophy, particularly in females.

Sex differences and AR in skeletal muscle

AR and skeletal muscle in males and females

Androgens are important for the development of male sex characteristics, including muscle mass (Davey & Grossmann, 2016). Sex differences in human skeletal muscle following exercise are apparent in various physiological conditions. Understanding the relationship between AR and muscle between males and females could help to explain some of the differential responses observed in skeletal muscle following exercise. Baseline AR protein content was reported to be higher in males than females (Nicoll et al., 2019), although, this study did not report activity levels of female participants. Specifically, males were reported to have least 2 years of RET leading up to the study while no exercise history was noted for females. This oversight on female training status could impact interpretation of the results related to baseline AR content. Maclean and colleagues (2008) examined the effect of AR ablation on skeletal muscle mass in male and female mice. Males had up to 20% lower skeletal muscle mass relative to WT counterparts. Conversely, in the female AR knockout had no effect on skeletal muscle content in female suggesting that AR may be dispensable for maintaining skeletal muscle mass in females. This AR-mediated support of muscle mass in males has been driven by an AR association with slow-twitch oxidative fibres (Altuwaijri et al., 2004; Hulmi et al., 2008). There are a lack of studies examining sex-based

difference in AR content. The existing literature suggests that females do not require AR to maintain skeletal muscle mass.

AR, sex-based differences and resistance exercise

The acute timeline of AR mRNA and protein expression is sensitive and different between sexes. AR mRNA and protein are increased in female rats immediately following exercise relative to non-exercised controls, but there is no difference in males following exercise (Aizawa et al., 2010). The exercise stimulus for this experiment was 30 minutes of treadmill running at 30 metres per minute. This exercise protocol was likely too low of an intensity and volume to drive an increase in AR mRNA in male mice considering that mice are naturally highly active animals. Additionally, these authors collected tissue immediately following exercise which could change the outcome drastically relative to collecting in the hours and days following exercise. In males, it has been proposed that AR is initially downregulated and subsequently increased beyond baseline levels following RE (Kraemer & Ratamess, 2005). Furthermore, female AR protein content has been shown to decrease following a bout of RE after 10 minutes of recovery, whereas male AR protein decreases after 70 minutes (Vingren et al., 2009). This suggests that AR may remain in the nucleus for longer in males than females. This is supported by the mechanism that more androgens circulate and binds to AR in males, retaining AR in the nucleus and protecting it from degradation. Following a bout of RE, in untrained individuals, AR protein content in males was greater overall than in females (West et al., 2012). This study also suggests that change in circulating testosterone with exercise is not necessary for MPS. It appears that intramuscular mechanisms, such as the androgen receptor, are mainly driving sex-based changes in MPS.

Objectives and hypotheses

The purpose of this study was to determine how eccentric damage and RET impact AR content and whether the responses are different between sexes. There are inconclusive findings surrounding the acute response of AR gene and protein expression; to this end, no study has explored AR content 48 hours following eccentric damage to understand its abundance in skeletal muscle repair. Additionally, there is a lack of research on AR regulation following RET in males and females. The objectives of this study were to 1) investigate changes in AR content during skeletal muscle repair and 2) characterize AR-mediated sex-based differences following RET.

We hypothesized that 1) AR gene and protein content would increase during skeletal muscle repair with an association to SC content and 2) AR content would be associated with hypertrophy in males following RET since AR has been frequently shown to be associated with hypertrophy in males while skeletal muscle in female mice has been unaffected by AR knockout.

METHODS

Participants

Twenty-six healthy, young males (n=13) and females (n=13) were recruited to participate in this study. This project was part of a previous study investigating the effects of a multi-ingredient supplement on resistance training outcomes (Wageh et al., 2021). For the purposes of this study, the multi-ingredient supplement was disregarded. All participants were recreationally active with no structured exercise in the previous year. Exclusion criteria included smoking, diabetes, the use of nonsteroidal anti-inflammatory drugs (NSAIDs), and/or statins, and history of respiratory disease and/or any major orthopaedic disability. The study conformed to the guidelines outlined in the Declaration of Helsinki. The participants gave their informed written consent before their inclusion in the study.

TABLE 1. Baseline participant characteristics.

	Female (n=13)	Male (n=13)
Age, (y)	22.1 ± 0.6	21.8 ± 0.6
Height (m)	162.4 ± 2.0	178.9 ± 1.9#
Body Mass (kg)	62.4 ± 2.9	74.7 ± 1.7#
Leg Lean Mass (kg)	6.5 ± 5.6	9.6 ± 2.2#
Body Fat %	33.7 ± 2.0	24.2 ± 1.6#

Values are means ± SEM. # Significant difference between sex.

Experimental outline

On the initial assessment day, participants visited the laboratory at approximately 0800, underwent anthropometric measurements (weight, height), body composition (dual-energy X-ray absorptiometry [DXA] scan), and a muscle biopsy was collected. Then, participants performed 300 maximal eccentric contractions on the biopsied leg, a protocol that we have used previously (McKay et al., 2010; Nederveen et al., 2018; O'Reilly et al., 2008). Forty-eight hours following

the damaging exercise bout, participants visited the laboratory to have a second muscle biopsy taken from the same leg. Participants underwent 10 weeks of supervised RE sessions 4 times per week. Each resistance training session included a variety of exercises consisting of 3-5 sets and 8-12 reps per set, to target the entire body. Training lasted roughly 1 hour in duration each day. Participants performed exercises at 80% of their individual 1RM.

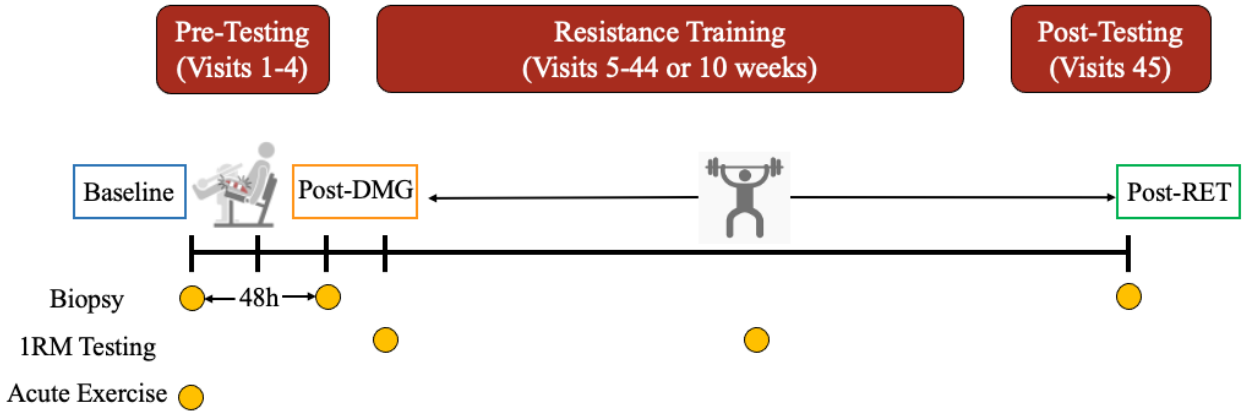


Figure 2: Outline of experimental design. Muscle biopsies were taken at baseline, 48 hours after acute damaging exercise bout of 300 eccentric contractions, and 96 hours following 10 weeks of RET. Strength measurements were taken after the second biopsy and at the midpoint of RET.

Maximal strength testing

One-repetition maximum strength tests adhered to the guidelines established by the National Strength and Conditioning Association. Participants first cycled for 5 minutes on a cycle ergometer, then performed 5-7 repetitions at ~50% of their predicted maximal strength. Following 3 minutes of rest, participants attempted 1 repetition at their predicted 1RM load. If the attempt was successful, the load was increased 5-15% until 1RM was achieved. Three minutes of rest was given between each attempt. Maximal isometric torque was measured using the Biodex dynamometer (Biodex-System 3, Biodex Medical Systems, Inc., Shirley, NY, USA), and 1RM

strength was calculated as the maximum amount of mass (kg) pushed for one repetition on a leg press.

Eccentric damage

Unaccustomed eccentric exercise is a common technique that induces a significant level of skeletal muscle damage, evidenced by extensive z-band streaming, desmin disruption (Beaton et al., 2002; Proske & Morgan, 2001), a significant increase in plasma creatine kinase (Farup et al., 2014), and upregulation of myogenic regulatory factors (McKay et al., 2008). Following a familiarization session, maximal isokinetic unilateral muscle-lengthening contractions of the quadriceps femoris were performed on a Biodex. Subjects performed 30 sets of 10 maximal muscle-lengthening contractions with 60 seconds of rest between sets for a total of 300 lengthening contractions.

Body composition

Whole-body lean soft tissue mass (fat-free and bone-free mass), leg lean mass, fat mass, body fat percentage, and bone mineral content were measured and analyzed using DXA scanner, after a 10- to 12-h overnight fast. Ultrasound measures were also performed on the bicep and thigh for measures of muscle thickness.

Muscle biopsy sampling

Three muscle biopsies were taken from the mid-portion of the vastus lateralis under local anesthetic using a 5-mm Bergstrom needle adapted for manual suction. Biopsies were performed on a single leg, which was randomized for each participant. One muscle biopsy was obtained at rest (baseline), another 48 hours following eccentric damage (post-damage) and lastly, 72-96 hours

following the final bout of resistance exercise training (post-RET). Approximately 150 mg of muscle tissue was collected from each biopsy. Following collection of the muscle sample, the muscle was dissected free of adipose and connective tissue and flash-frozen in liquid nitrogen, then stored at −80°C for later analysis. For immunohistochemistry, a fresh piece of muscle (approximately 40mg) was sectioned from the biopsies, orientated in cross-section, mounted in optimal cutting temperature (OCT) compound, and frozen in isopentane cooled with liquid nitrogen. The embedded samples were stored at −80°C and then sectioned (7 μm) at −20°C using a cryostat microtome. The cross-sections were mounted on slides and stored at −80°C for immunohistochemical analysis.

RNA isolation

Muscle samples were homogenized with 1mL of TRIzol Reagent (Life Technologies, Burlington, ON, Canada), in Lysing Maxtrix D tubes (MP Biomedicals, Solon, OH), with the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals) for a duration of 40 s, at 6 m/s. Following centrifugation, samples were incubated for 5 minutes to permit complete disassociation of the nucleoprotein complex. Then, 200 uL of chloroform (Sigma-Aldrich, Oakville, ON, Canada) was added, mixed for 10 seconds, incubated at room temperature for 3 minutes, and centrifuged for 15 min at 12,000 g at 4°C. The aqueous phase was transferred into a new tube. RNA was precipitated by adding 500uL of isopropanol, incubated at room temperature for 10 min, and centrifuged for 10 min at 12,000 g at 4°C. The resultant RNA pellet was resuspended and washed in 1mL of 75% ethanol (Commercial Alcohols, Brampton, ON, Canada), centrifuged for 5 min at 7500 g at 4°C, and the supernatant was discarded. Finally, the RNA pellet was resuspended and diluted in 50uL of RNase-free water. RNA concentration and purity (260/280) were determined with a Nano-Drop

1000 Spectrophotometer (Thermo Fisher Scientific, Rockville, MD, USA). The average RNA purity (260/280) was 1.8 ± 0.1 (SEM).

Reverse transcription

Samples were reverse transcribed using a high-capacity complimentary deoxyribonucleic acid (cDNA) reverse transcription kit (Applied Biosystems, Foster City, CA, USA), 1000ng of RNA was diluted in 20 µL reaction solution, as per manufacturer's instructions. SimpliAmp Thermal Cycler (Thermo Fisher Scientific) was used to obtain cDNA for gene expression analysis.

Quantitative real-time RT-qPCR

Quantitative polymerase chain reaction (qPCR) using TaqMan™ methodology was performed to determine AR (Hs00171172_m1, ThermoFisher) and Pax7 (Hs00242962_m1, ThermoFisher) gene expression. Commercially available gene assays and primers were stored at -20°C prior to use. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Hs02786624_g1, Thermofisher) was used as the reference gene. For each assay, qPCR was performed using 10ng of cDNA in TaqMan™ Fast-Advanced master mix (ThermoFisher, cat. #4444556), and Taqman™ gene expression assays (ThermoFisher) in a QuantStudio 5 – 384-Well Block (Applied Biosystems, Thermo Fisher Scientific) Real-Time qPCR machine. Samples were run in triplicate. TaqMan™ gene expression assays were conjugated to FAM reporter dye, NFQ-MGB quencher and ROX as a passive reference. The expression of the housekeeping gene GAPDH was not affected by the intervention. Samples were normalized to ΔC_t of TaqMan™ GAPDH. mRNA expression was calculated by using the $2^{-\Delta\Delta}C_t$ method (Livak & Schmittgen, 2001). Values were expressed as fold change from Pre for graphical purposes.

Immunohistochemistry

Skeletal muscle cross-sections (7 μm) were prepared from tissue embedded in OCT compound, air-dried for 30 min, and stored at -80°C. Slides were then stained separately to identify 1) SC content and 2) SC activity using antibodies against Pax7 (neat), MyoD (anti-MyoD1, 1:100), A4.951 [myosin heavy chain (MHC) type I, slow isoform, 1:1], and MHC-II (fast isoform, 1:1000; ab91506). Secondary antibodies used were Pax7 (Alexa Fluor 488 or 594, 1:500), MyoD (biotinylated secondary antibody, 1:200; and streptavidin-594 fluorochrome, 1:200), A4.951 (Alexa Fluor 488, 1:500), MHC-II (Alexa Fluor 647, 1:500), and laminin (Wheat Germ Agglutinin 488 or 647, 1:200). SC activity status was determined via the colocalization of Pax7, MyoD, and DAPI (i.e., Pax7+/MyoD+). An immunofluorescent stain was optimized to determine AR intensity associated with skeletal muscle fibres (Polanco et al., 2016). Slides were stained with antibodies against myosin heavy chain II (MHCII) (fast isoform, 1:500; ab91506; Abcam) and AR (1:100). Secondary antibodies utilized were Alexa Fluor 647-Rabbit (1:500) and Alexa Fluor 594 (1:200) for MHCII and AR, respectively. Nuclei were labelled with 4,6-diamidino-2-phenylindole (DAPI; 1:20,000; Sigma-Aldrich) before applying a coverslip with fluorescent mounting media (Dako) to the stained slide. Samples were imaged with a Nikon Eclipse Ti Microscope, equipped with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera. Images were captured and analyzed using Nikon NIS Elements AR 3.2 software. All images were captured through 20X objective. Negative controls (no primary antibody control, no secondary antibody control) were used to validate staining intensity and omit autofluorescence during quantification. Samples were blinded for both subject and timepoint during analysis. Images were analyzed using sum and mean nuclear intensity.

Sum and nuclear intensity

Regions of interest (ROIs) were selected for each image to reliably quantify AR intensity within the perimeters of the skeletal muscle section since the samples fluoresced greater around the perimeter of the fibre. Each region of interest (ROI) was set to a 500 A.U. circle. Two to six ROIs were applied per image to maximize quantifiable area on each sample. Areas of substantial gaps between fibres and adipose or fibrotic infiltrate were omitted when positioning the ROIs to determine AR content most associated with the muscle fibres **(Figure 3)**.

The ROI statistics tool in NIS was utilized to quantify sum AR fluorescence intensity of each ROI. The mean of all ROIs on a section was calculated to determine the final sum intensity for the selected sample, which is interpreted to reflect total AR protein content in a muscle cross-section.

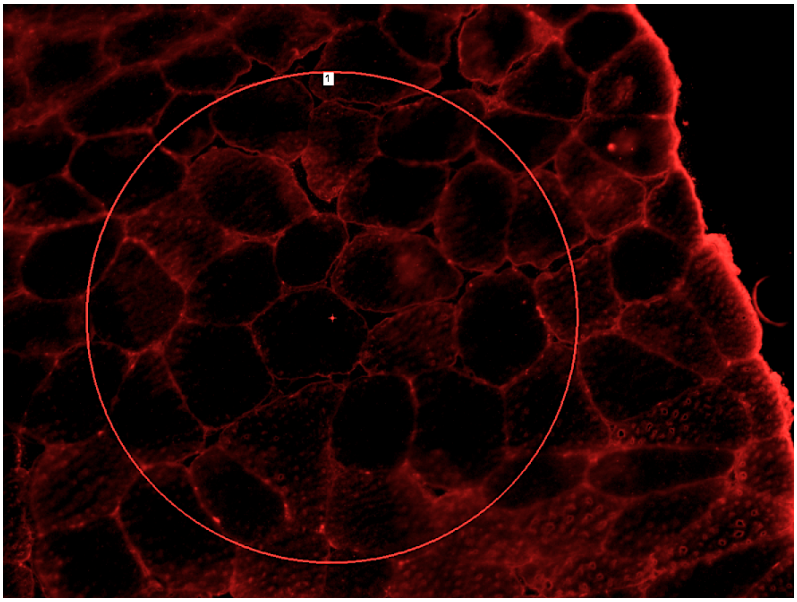


Figure 3: ROI (large red circle) positioned in an optimal region of the muscle fibre section containing AR through the TRITC (red) channel.

The DAPI channel was then selected to threshold nuclei. On NIS, the "Pick threshold from image" tool was selected to identify one of the smallest DAPI punctate in the ROI. As such, the

thresholding technique identifies DAPI punctate of that variety or larger so that the program identifies and thresholds each nucleus in the ROI **(Figure 4)**.

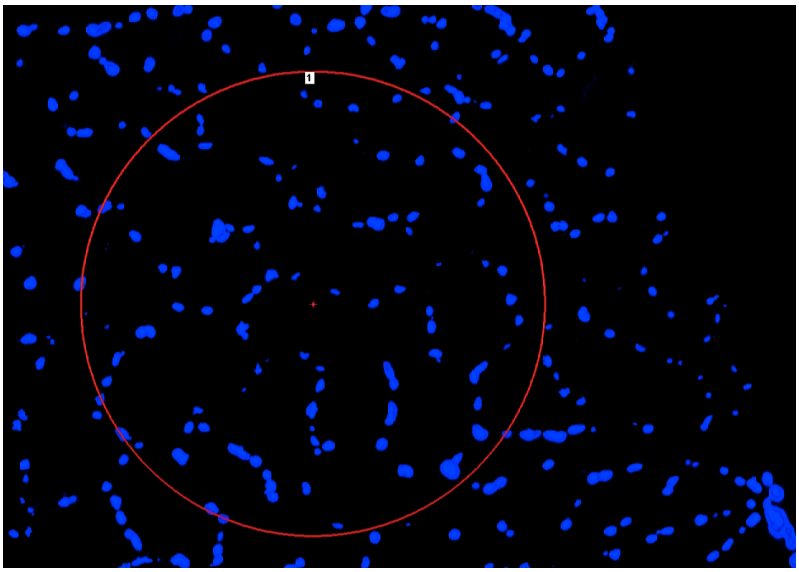


Figure 4: Same ROI as **Figure 3**. DAPI (blue), identifying nuclei are highlighted via thresholding to then utilize binary (thresholded DAPI areas) data in ROI statistics.

The binary area from thresholding DAPI was calculated via ROI statistics to determine mean intensity of TRITC (AR) in the same binary area as DAPI (nuclei) **(Figure 5)**. Thresholding was not applied to the TRITC channel, only DAPI to determine the nuclear area of the ROI. As such, the mean intensity of AR content localized to each nucleus in the ROI was determined. Similar to sum intensity, the nuclear mean intensity from each ROI was collected and averaged as part of the nuclear intensity for the entire sample, which is interpreted to reflect nuclear-associated AR protein content.

TRITC | Export | MR MS MC

Feature	ROI	Binary
Area [µm²]	196581.12	7452.79
Mean Intensity	434.22	572.73
Min Intensity	255.00	301.00
Max Intensity	2415.00	2338.00
Sum Intensity	814028017.00	40705434.00
StDev Intensity	102.17	139.59
Signal/Background	3.84 : 1	5.52 : 1

Figure 5: Sample data illustrating ROI statistics. By selecting TRITC with only the area of nuclei being highlighted in the ROI, AR nuclear intensity is therefore determined by "binary mean intensity" of TRITC within the "binary area" of DAPI.

Protein isolation

Protein was isolated using the TRIzol® manufacterer's protocol with simple modifications. Due to technical difficulties with protein samples, the protein had to be further lysed using Radio-immunoprecipitation assay (RIPA) buffer for maximal protein yield then re-homogenized using MP biomedicals FastPrep™ instrument and were resuspended 1:1 SDS:8M urea solution. Protein content was determined using Peirce™ Bicinchoninic acid (BCA) Protein Assay Kit (microplate procedure) with a sample to working reagent ratio of 1:5 and absorbance measured using a plate reader.

Western blotting

Isolated proteins were prepared at 1 µL/µg and put in 4x Laemmeli buffer with 10% β-mercaptoethanol (BME) (BioRad, Mississauga, ON, Cat. #1610747). Samples were boiled for 5 minutes, spun down the tubes to reincorporate water then stored in -80°C freezer until running the western. To begin the western blot, pre-cast gels (10, 15, or 26-well; depending on amount of protein necessary to add) were used. 4ul Precision Plus Protein™ Kaleidoscope™ Prestained Ladder (BioRad, Cat. #1610375) along with 10ul of a control sample (pooled from samples) were added to each gel. Western blot protein preps were added to each well at a volume of 12ul per well. The gel was run at 74V for the first 10 minutes then increased to 200V for 45 minutes. Transfer was performed using Turbo-blot transfer machine (Trans-blot® Turbo™ System) and buffer using a 1x transfer buffer (100ml of 10x transfer buffer, [10x Tris/Glycine/SDS Buffer (Cat. # 1610732) BioRad, Made in USA], 100ml of 100% of ethanol, 800ml of water). Filter paper (Mini Trans-Blot ® Filter Paper, Cat. # 1703932, BioRad, Made in USA) and nitrocellulose membrane (0.45um, Cat. # 1620115, BioRad Made in Germany) were cut to match the according

gel size. On biorad turboblot machine, mixed molecular weight was chosen and run for 10 minutes. Stain free ponceau was performed where the membrane was imaged on the chemidoc imaging system (BioRad) before ponceau staining, along with imaging the gel itself using the "stain-free" setting. The membrane was also imaged once immersed in ponceau for 3 minutes. Membranes were cut at appropriate areas for the molecular weight of interest (~110 kDa for AR). Ponceau staining was removed using a 5-minute tris buffer saline and tween (TBST) wash performed 3 consecutive times, until ponceau was removed from membrane. A 5% bovine serum albumin (BSA) (Bioshop Canada, Cat. #ALB007.500) block was applied to the membrane on a rocker for 1 hour. Antibodies were prepared in 5% BSA at a concentration of 1:100. Following 1-hour BSA block, antibodies were added to the membrane and placed in a cold room on a rocker overnight (~16 hours). Following overnight incubation with primary, the antibodies were removed, and membranes underwent 3, 5-minute TBST wash followed by a secondary antibody incubation for 1 hour (made with 5% BSA at a concentration of 1:10 000). Following secondary incubation, a 5-minute wash 3 times was performed and ECL was placed on the membrane for 3 minutes. Clarity™ Western ECL Substrates were mixed at a ratio of 1:1 (Cat. # 170-5060, BioRad Made in United States). Membranes were imaged using the biorad imager via the chemiluminescence setting and autoexposure was adjusted accordingly.

Statistical analyses

Prism 8.0 was used for data analysis and graphical illustration. Two-way analysis of variance (ANOVA) multiple comparisons was applied to sex specific analysis of high and low groups. This included high and low groups of mixed, type-I, or type-II fibre-associated SC, and changes in fibre CSA (mixed, type-I or type-II). Post-Hoc analysis consisted of Tukey's test when appropriate.

Unpaired T-tests were applied for analysis of AR protein content (IF/WB) and percent changes between sexes.

Responder analyses are beneficial to identify participants that exhibit a robust response to exercise that is otherwise unnoticed in a highly variable data set. We sought to understand the AR using high and low response comparisons as AR content has been observed to be highly variable in human skeletal muscle (Ahtiainen et al., 2011; Mitchell et al., 2013). High and low responses were determined by ranking participants for each selected outcome (% change mixed fibre CSA, % change mixed fibre SC etc). The entire group was then divided into thirds such that a high and low group was analyzed, while the third group was removed to ensure no overlap between high and low groups. This resulted in an n=4 for each subgroup (i.e. low responder, female). In the current study, response analysis was applicable for CSA, Pax7 gene expression as well as satellite cell content and activity, in relation to AR content.

RESULTS

Females have more AR mRNA than males at baseline

Untrained females had greater baseline AR mRNA (1.33 ± 0.34-fold) than untrained males (0.87 ± 0.55-fold) ($p<0.05$). Following eccentric damage, females had greater AR mRNA (1.77 ± 1.26-fold) than males (0.78 ± 0.31-fold) ($p<0.05$) **(Figure 6)**. There was no change in AR gene expression following eccentric damage for either males or females.

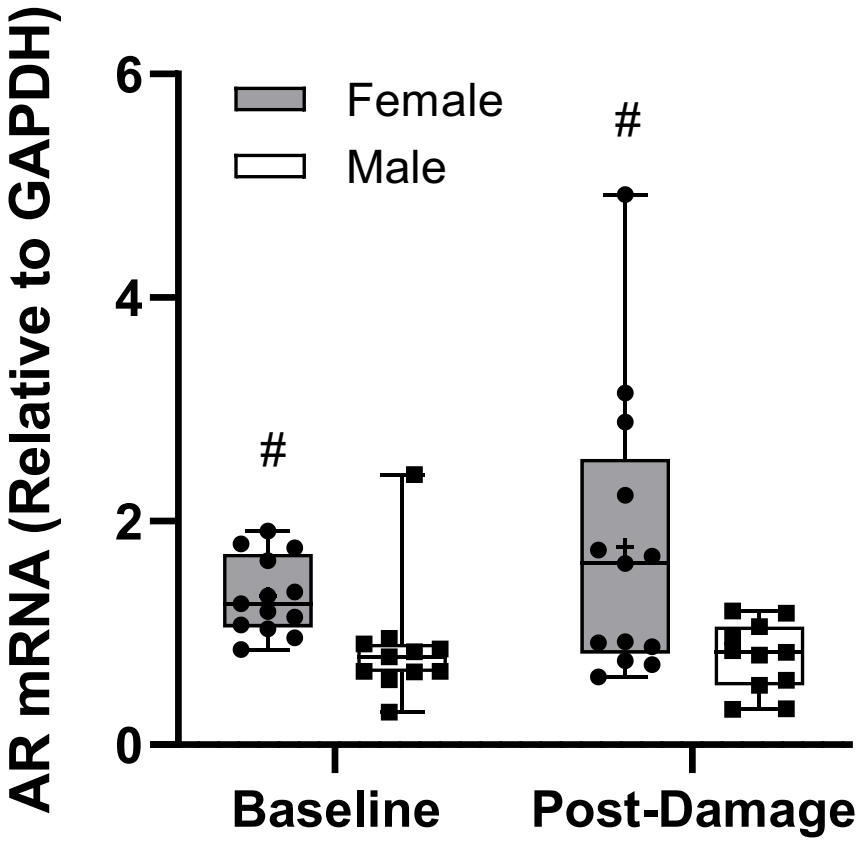


Figure 6: Sex differences in AR gene expression. AR mRNA expression in females (n=13) in grey boxes and males (n=11) in white boxes at baseline and post-damage (relative to GAPDH). Values are presented as box and whisker plots with individual data points. # refers to a significant difference relative to the opposite sex at a timepoint ($p<0.05$).

Eccentric damage increases AR protein content

A single bout of eccentric damage increased total AR protein content via IF in females from baseline (1.15 ± 0.06-fold; $p<0.05$) and in males (1.25 ± 0.09-fold; $p<0.05$) with no effect of sex **(Figure 7 A, B and E)**. There was a trend to increase nuclear-associated AR protein content via IF in females from baseline to post-damage (1.13 ± 0.09-fold; $p=0.07$) and a significant increase in males (1.25 ± 0.11-fold; $p<0.05$) with no effect of sex **(Figure 7 C, D, and F)**. There was no main effect of time or sex for percent change in total or nuclear AR intensity following damage **(Figure 7G)**.

To further examine AR content, western blot was performed to measure total AR protein content in skeletal muscle **(Figure 8A)**. There was a trend for males showing an increase in AR protein content from baseline to post-damage, relative to female baseline (0.70A.U. ± 0.11A.U. to 1.14A.U. ± 0.08A.U.; $p=0.07$). Females had greater baseline AR protein content (1.22A.U. ± 0.11A.U.) than males (0.70A.U. ± 0.11A.U.) ($p<0.05$) **(Figure 8B)**. Males had a greater percent change in AR protein (80.69% ± 28.13%) than females (-22.59% ± 11.43%) ($p<0.05$) **(Figure 8C)**.

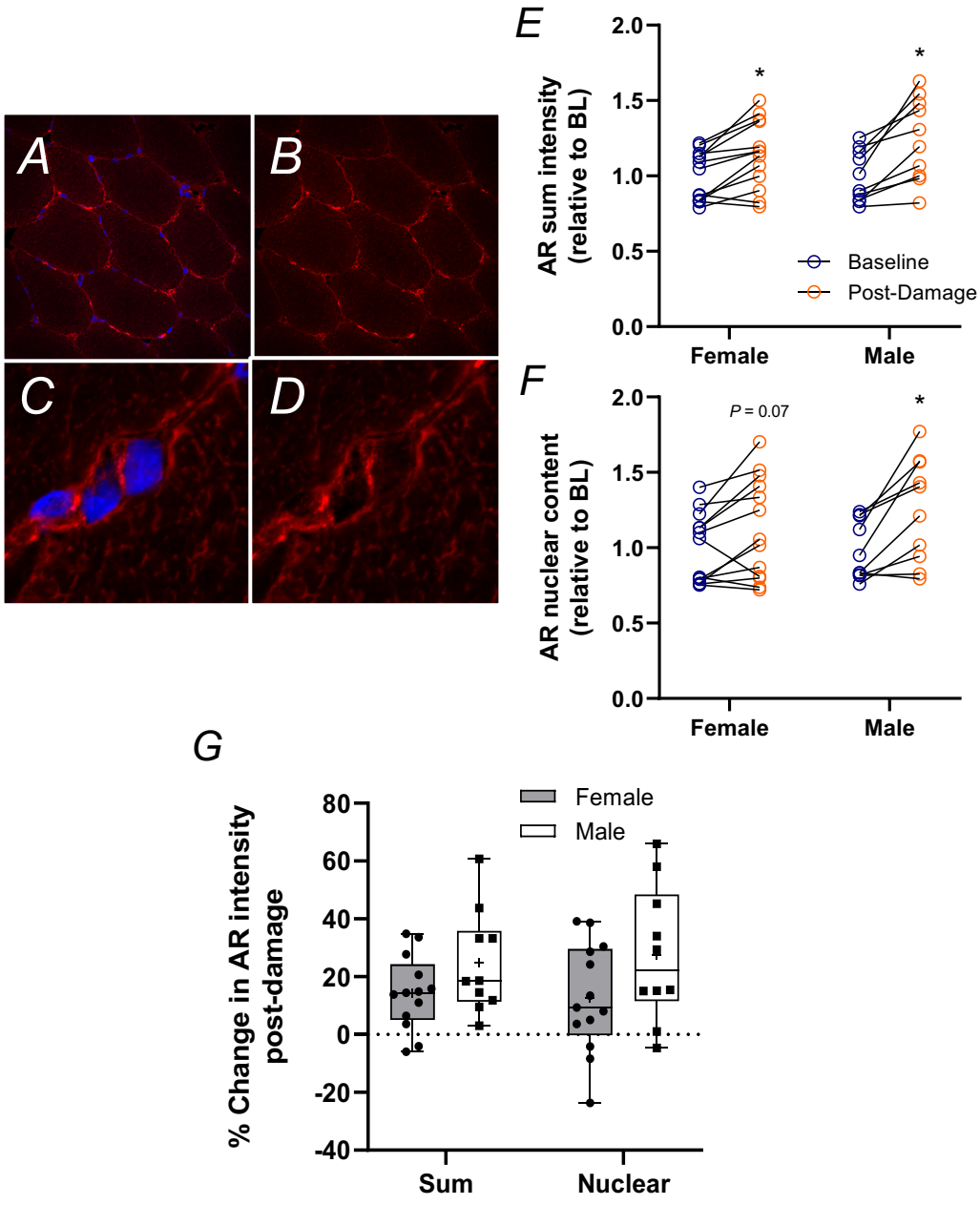


Figure 7: AR protein response following acute damage via immunofluorescence. Representative images of an immunofluorescent stain containing *A)* AR (red) and DAPI, marking nuclei (blue), *B)* AR only. Nuclear localization of AR represented in *C)* AR+DAPI and *D)* AR only. AR content at baseline and post-damage in female (n=13) and male (n=10) participants measured (relative to mean of female baseline) via *E)* sum intensity and *F)* nuclear content. *G)* Percent change (baseline to post-damage) of sum and nuclear intensities in female (n= 13) and male (n=10) participants. Values are expressed as individual data points or box and whisker plots with individual data points. * refers to a significant difference from baseline ($p<0.05$).

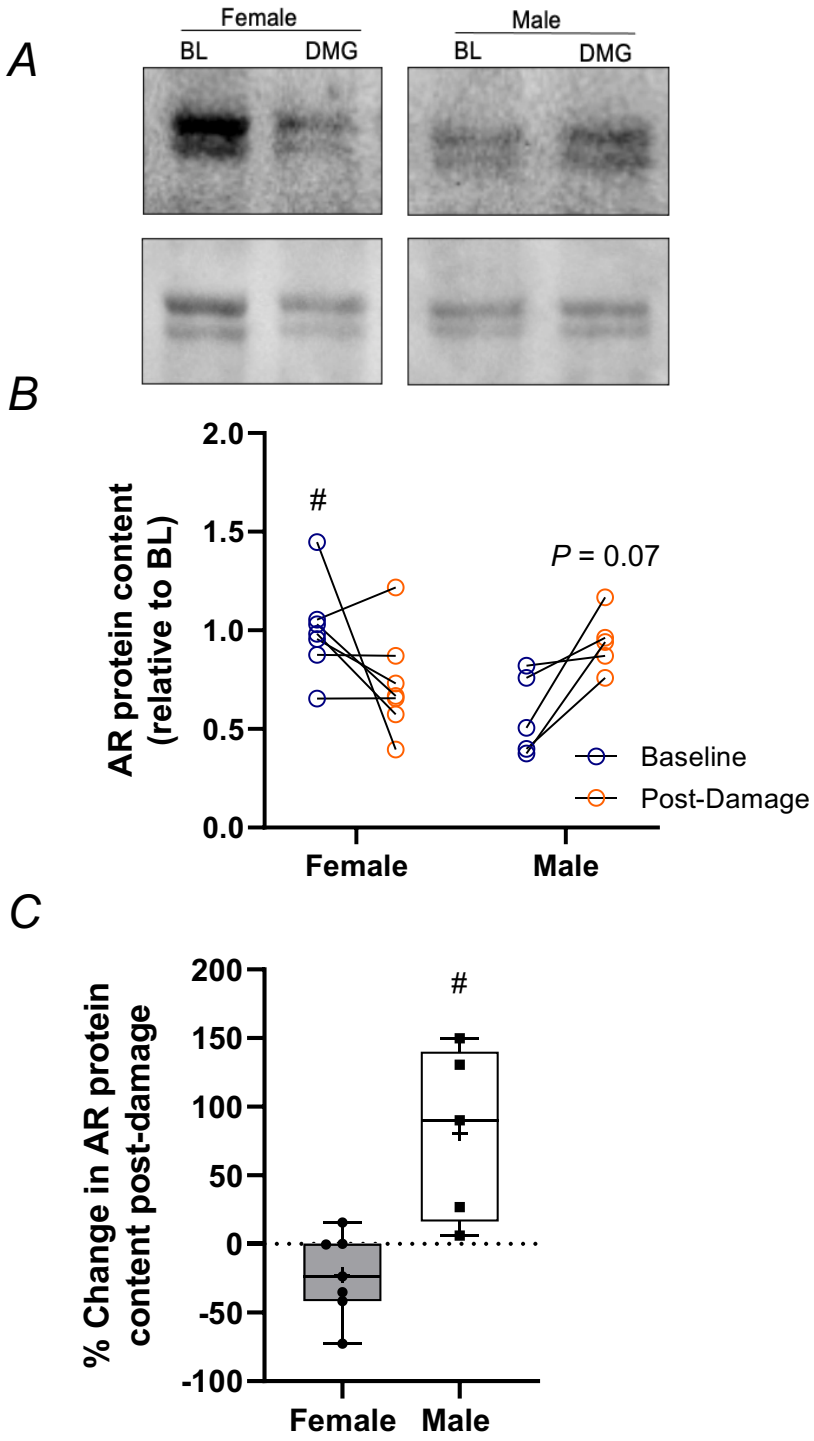


Figure 8: AR protein response following acute damage via western blot. *A)* Representative image of an immunoblot in male and female participants, at baseline and post-damage. *B)* AR protein content via western blot in baseline and post-damage (relative to mean of female baseline) in female (n=7) and male (n=5) participants. *C)* Percent change from baseline to post-damage in female (n=7) and male (n=5) participants. Values are expressed as individual data points or in conjunction with box and whisker plots with individual data points. # refers to a significant difference relative to opposite sex ($p<0.05$).

AR content is associated with SC content in males following eccentric damage

To understand the relationship between AR and SC, an IF stain was performed that identified SC content and activity **(Figure 9 A-D).** Participants were grouped by sex and high or low change in SC (mixed, type-I, or type-I fibre activated) from baseline to post-damage. There was a trend for a main effect of sex with males having greater percent change in total AR content (19.95% ± 8.59% and 34.17% ± 10.46%) as compared to females (3.798% ± 3.6% and 16.31% ± 8.08%) ($p=0.06$), irrespective of high or low change in mixed fibre SC content. Males had greater percentage of AR nuclear content associated with mixed fibre SC content (27.46% ± 7.42% and 42.13% ± 11.93%) following damage relative to females (0.71% ± 8.36% and 16.69% ± 9.34%) ($p<0.05$) (**Figure 9E)**. AR content is shown to be more abundant in type-I fibres than type-II fibres (Altuwaijri et al., 2004; Hulmi et al., 2008), so we chose to focus our analysis on type-I fibre-associated SC. There was no effect of sex or of high or low responder for percent change in AR total or nuclear content associated with change in type-I fibre associated SC content post-damage **(Figure 9F)**. There was a trend for a main effect of sex showing males to have greater percent change in total AR content associated with change in activated SC (30.48% ± 11.20% and 27.55% ± 6.73%) as compared to females (11.71% ± 5.43% and 17.49% ± 6.08%) ($p=0.08$). There was a trend for an interaction between high and low responders for change in activated type-I fibre SC post-damage and percent change in nuclear AR content between sexes ($p=0.09$) **(Figure 9G)**.

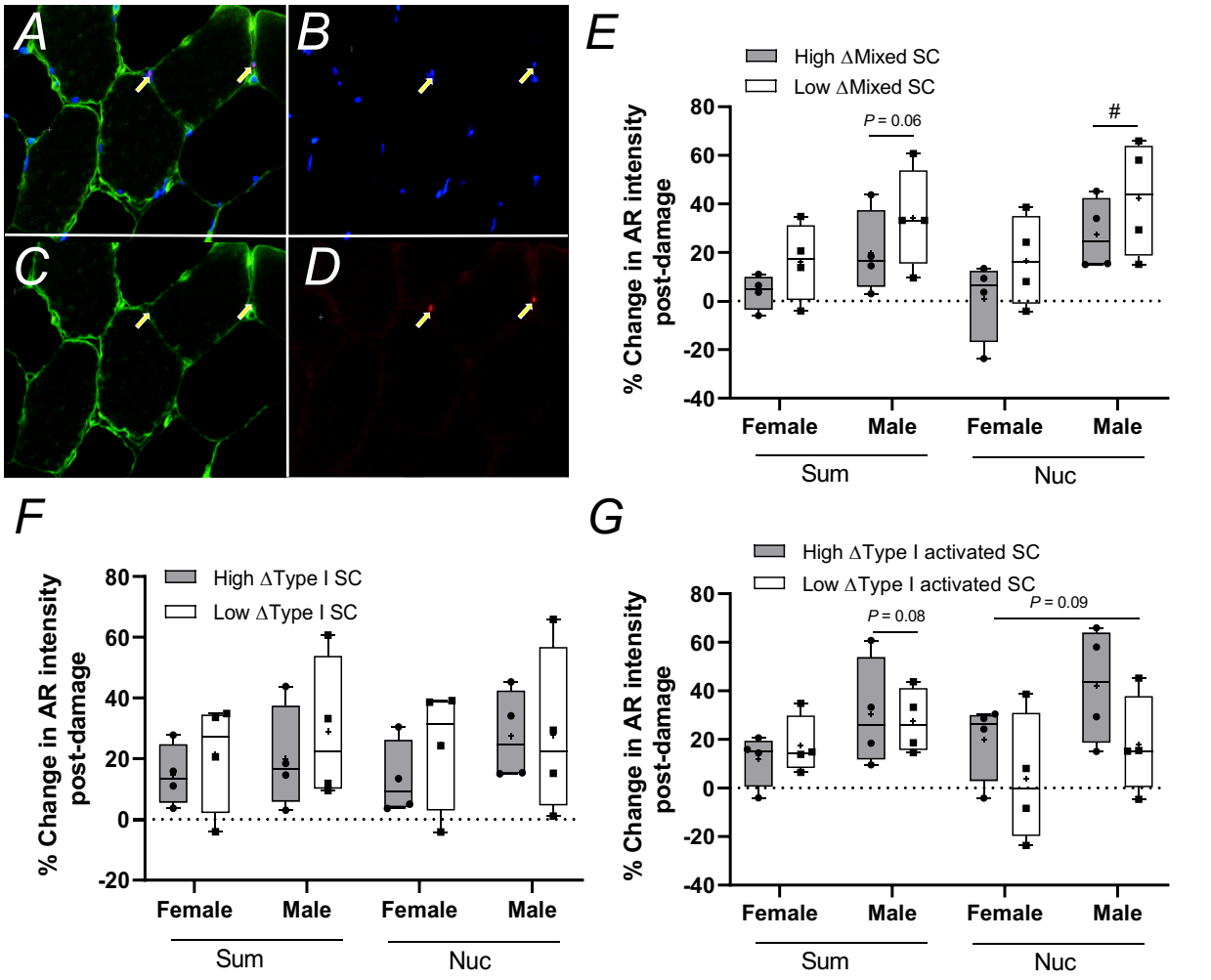


Figure 9: AR protein and SC following acute damage. *A)* Representative image of an immunofluorescent stain containing *A)* DAPI, marking nuclei (blue), Laminin marking basal lamina (green) and Pax7 denoting $Pax7^+$ cells (red), SC are denoted by yellow arrows. Single channel views of *B)* DAPI, *C)* Laminin and *D)* Pax7. Percent change from baseline to post-damage in sum and nuclear AR intensities sorted by high or low *E)* ΔMixed fibre $Pax7^+$ cells per 100 myofibres, *F)* ΔType-I fibre $Pax7^+$ cells per 100 myofibres or *G)* ΔType-I fibre $MyoD^+/Pax7^+$ cells per 100 myofibres, from baseline to post-damage in female (n=4) or male (n=4) participants. Values are expressed as box and whisker plots with individual values. # refers to a significant difference relative to opposite sex ($p<0.05$).

AR protein content increased following RET while AR nuclear-associated content exclusively increased in males

Ten weeks of resistance-exercise training increased total AR content in females from baseline to post-RET (1.09 ± 0.06-fold; $p<0.05$) and in males (1.164 ± 0.08-fold; $p<0.05$) with no group interaction **(Figure 10A)**. There was a trend for an increase in nuclear-associated AR protein content in females following RET (1.112 ± 0.08-fold; $p=0.09$) and an increase in males (1.27 ± 0.13-fold; $p<0.05$) (**Figure 10B)**. Males had a greater percent change in AR nuclear content following RET (27.67% ± 7.32%) as compared to females (10.44% ± 2.96%) ($p<0.05$) **(Figure 10C)**.

We then wanted to quantify AR post-RET in whole muscle protein using western blot **(Figure 10D)**. There was a main effect of sex for AR protein content from baseline to post-RET ($p<0.05$). RET increased AR protein content in males, relative to female control, from baseline (0.69 ± 0.11-fold) to post-RET (1.23 ± 0.21-fold) ($p<0.05$) **(Figure 10E)**. Males trended to have a higher percent change in AR protein content following RET (83.81% ± 35.78%) relative to females (22.49%± 8.28%) ($p=0.08$) **(Figure 10F).**

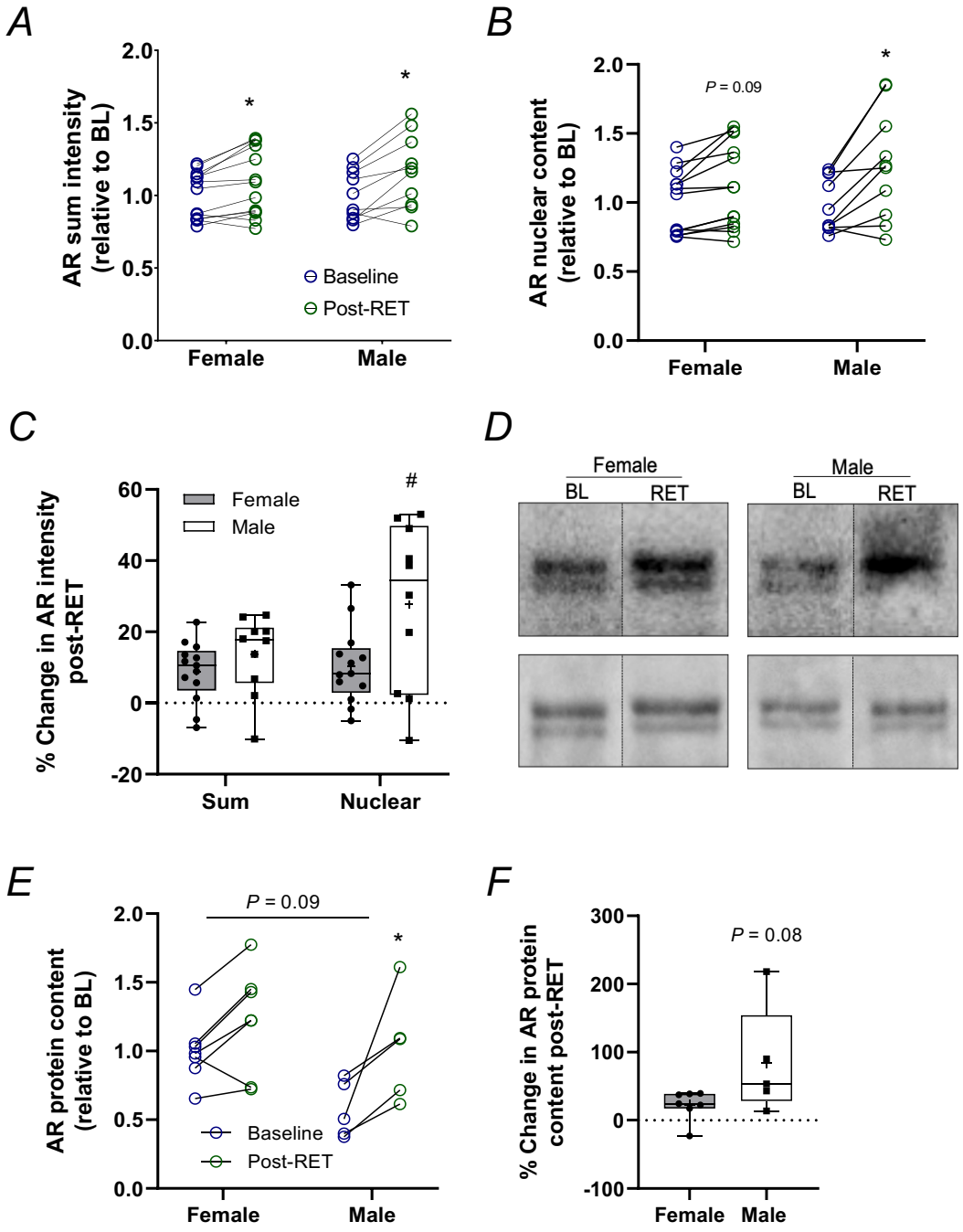


Figure 10: AR protein following resistance training. AR content expressed in female (n=13) and male (n=10) participants from baseline to post-RET via *A)* sum intensity (n=23), *B)* nuclear content, data expressed relative to mean of female baseline. *C)* Percent change from baseline to post-damage in sum or nuclear AR content in female (n=13) and male (n=10) participants. *D)* Representative images of an immunoblot in males and females, at baseline and post-RET. *E)* AR protein content expressed relative to female baseline mean in females (n=7) and males (n=5). *F)* Percent change, relative to baseline, in AR protein content in female (n=7) and male (n=5) participants. Values are expressed as individual data points or box and whisker plots with individual data points. * refers to a significant difference from baseline ($p<0.05$). # refers to a significant difference relative to opposite sex ($p<0.05$).

Sex-specific responses of AR content associated with SC post-RET

Males and females were grouped by high and low responses to pax7 gene expression. Baseline AR mRNA, relative to GAPDH was greater in females exhibiting the highest baseline Pax7 gene expression (1.49 ± 0.14-fold) relative to females exhibiting the least baseline Pax7 gene expression (1.081 ± 0.64-fold) ($p<0.05$) (**Figure 11A)**. Baseline Pax7 mRNA was positively correlated to baseline AR mRNA in females ($R^2 = 0.73$; $p = 0.007$) **(Figure 11B)**. There was a trend for an interaction between sexes for percent change in total AR content sorted by high and low change in mixed fibre SC content following RET **(Figure 11C)**. Males with the greatest change in Type-I fibre associated SC content following RET had more nuclear-associated AR content (38.81%± 12.54%) as compared to female counterparts (9.14% ± 3.53%) ($p<0.05$) **(Figure 11D)**. There was a trend for a main effect of high and low type-I fibre associated activated SC, when measuring percent change in total AR content ($p=0.08$). Females with the most change in type-I fibre associated activated SC trended to exhibit greater percent change in total AR content as compared to females with the least type-I fibre associated activated SC ($p=0.06$). There was a trend for a main effect of males expressing more total AR content associated with type I fibre activated SC than females post-RET ($p=0.07$). Males with the least increase in type-I fibre associated activated SC content had higher AR nuclear content following RET (33.47% ± 11.34%) as compared to female counterparts (0.57% ± 2.74%) ($p<0.05$) **(Figure 11E)**. Change in type-I fibre associated activated SC content and percent change in total AR content post-RET was positively correlated in females ($R^2 = 0.51$; $p = 0.05$) **(Figure 11F)**.

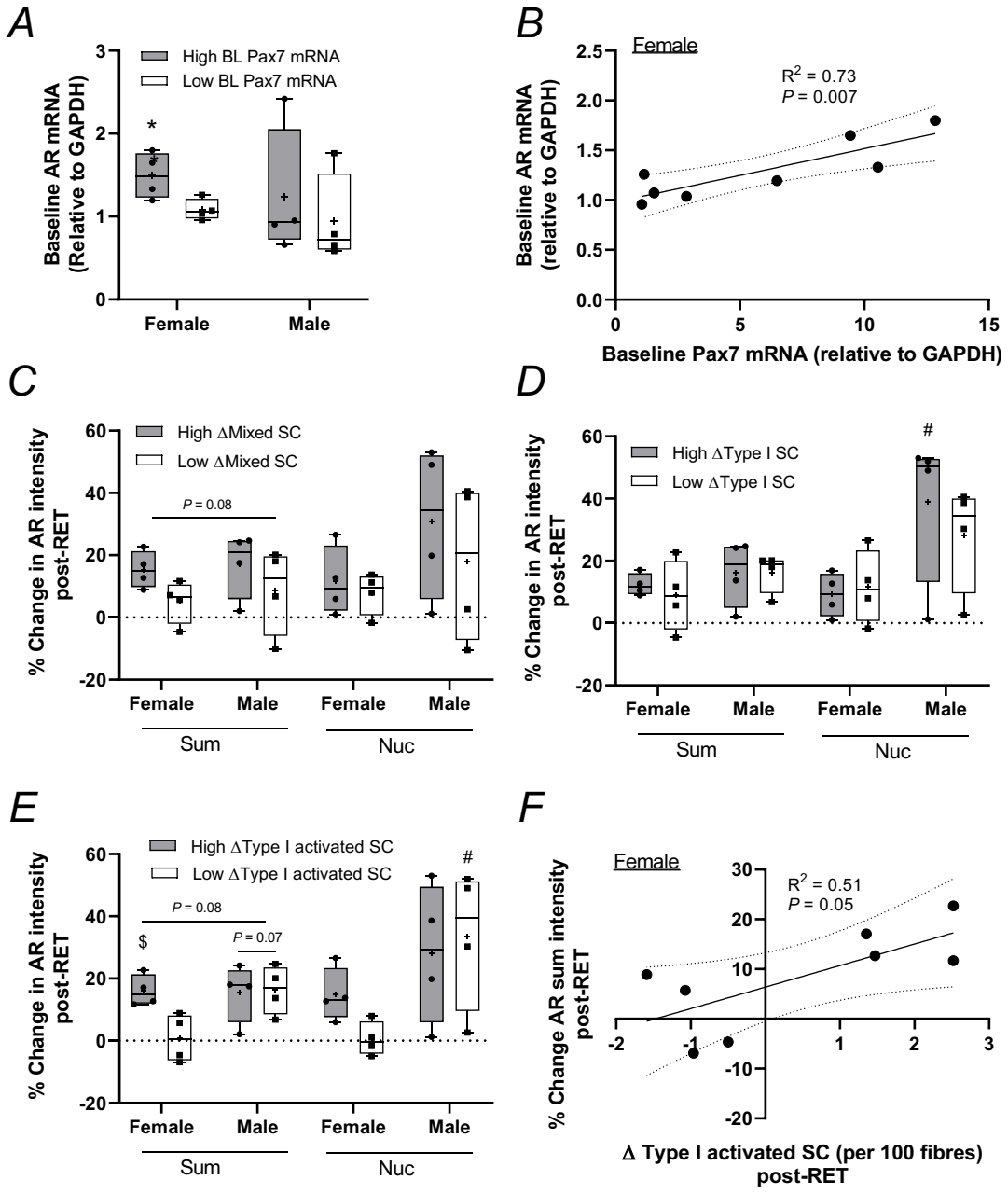


Figure 11: AR protein and SC following resistance training. *A)* Baseline AR mRNA sorted by high and low baseline Pax7 mRNA (relative to GAPDH) in males (n=4) and females (n=4). *B)* Pearson's correlation between high and low baseline Pax7 mRNA (relative to GAPDH) and corresponding baseline AR mRNA (relative to GAPDH). Percent change from baseline to post-RET in sum and nuclear AR intensities sorted by high or low *C)* ΔMixed fibre $Pax7^+$ cells per 100 myofibres, *D)* ΔType-I fibre $Pax7^+$ cells per 100 myofibres or *E)* ΔType-I fibre $MyoD^+/Pax7^+$ cells per 100 myofibres, from baseline to post-RET in female (n=4) or male (n=4) participants. *F)* Pearson's correlation between high and low Δ Type-I $Pax7^+/MyoD^+$ cells per 100 myofibres and percent change in AR sum intensity post-RET. Values are expressed as box and whisker plots with individual values. * refers to a significant difference relative between high and corresponding low ($p<0.05$). # refers to a significant difference relative to the opposite sex ($p<0.05$). $ refers to a trend between high and low within sex ($p=0.06$).

CSA is associated with higher nuclear AR content in males than females post-RET

High and low responders were determined based on percent change in skeletal muscle fibre CSA either at baseline or following RET. Males with the highest baseline mixed fibre CSA had greater percent change in AR-associated nuclear content post-RET (48.15% ± 3.29%) than female counterparts (8.49% ± 3.51%) ($p<0.05$) and compared to males with the lowest baseline mixed fibre CSA (10.58% ± 9.04%) ($p<0.05$). There was a trend for a main effect of males expressing more percent change in total AR content relative to females post-RET in association with baseline mixed fibre CSA ($p=0.08$) **(Figure 12A).** Baseline mixed fibre CSA was positively correlated to percent change in AR nuclear-associated content, in males, post-RET ($R^2 = 0.68$; $p = 0.01$) (**Figure 12B)**. Males (70.03% ± 6.75%) and females (51.63% ± 5.27%) had greater percent change in mixed fibre CSA in the high group relative to their respective low group (18.89% ± 3.37% and 8.16% ± 2.88%) ($P<0.05$). Males had greater percent change in mixed fibre CSA in the HIGH group (70.03% ± 6.75%) compared to the female counterparts (51.63% ± 5.27%) **(Figure 12C)**. Males with the highest percent change in mixed fibre CSA had greater nuclear-associated AR content (42.47% ± 4.963%) than female counterparts, post-RET (5.22% ± 4.70%) ($p<0.05$). There was a trend for an effect of sex in percent change of total AR content when sorted by high versus low percent change in mixed fibre CSA, post-RET ($p=0.06$) **(Figure 12D)**. Males with the greatest change in type-I fibre CSA post-RET had greater percent change in total (19.13% ± 2.29%) and nuclear-associated (42.47% ± 4.96%) AR content than female counterparts (4.57% ± 3.95% and 5.22% ± 4.70%) ($p<0.05$). **(Figure 12E)**. Males with the greatest increase in type-II fibre CSA post-RET had greater percent change of nuclear-associated AR content (39.86% ± 7.25%) relative to the respective female group (5.22% ± 4.70%) ($p<0.05$). There was a trend for an effect of sex

in percent change of total AR content when sorted by high versus low percent change in type-II fibre CSA (p=0.06) **(Figure 12F)**.

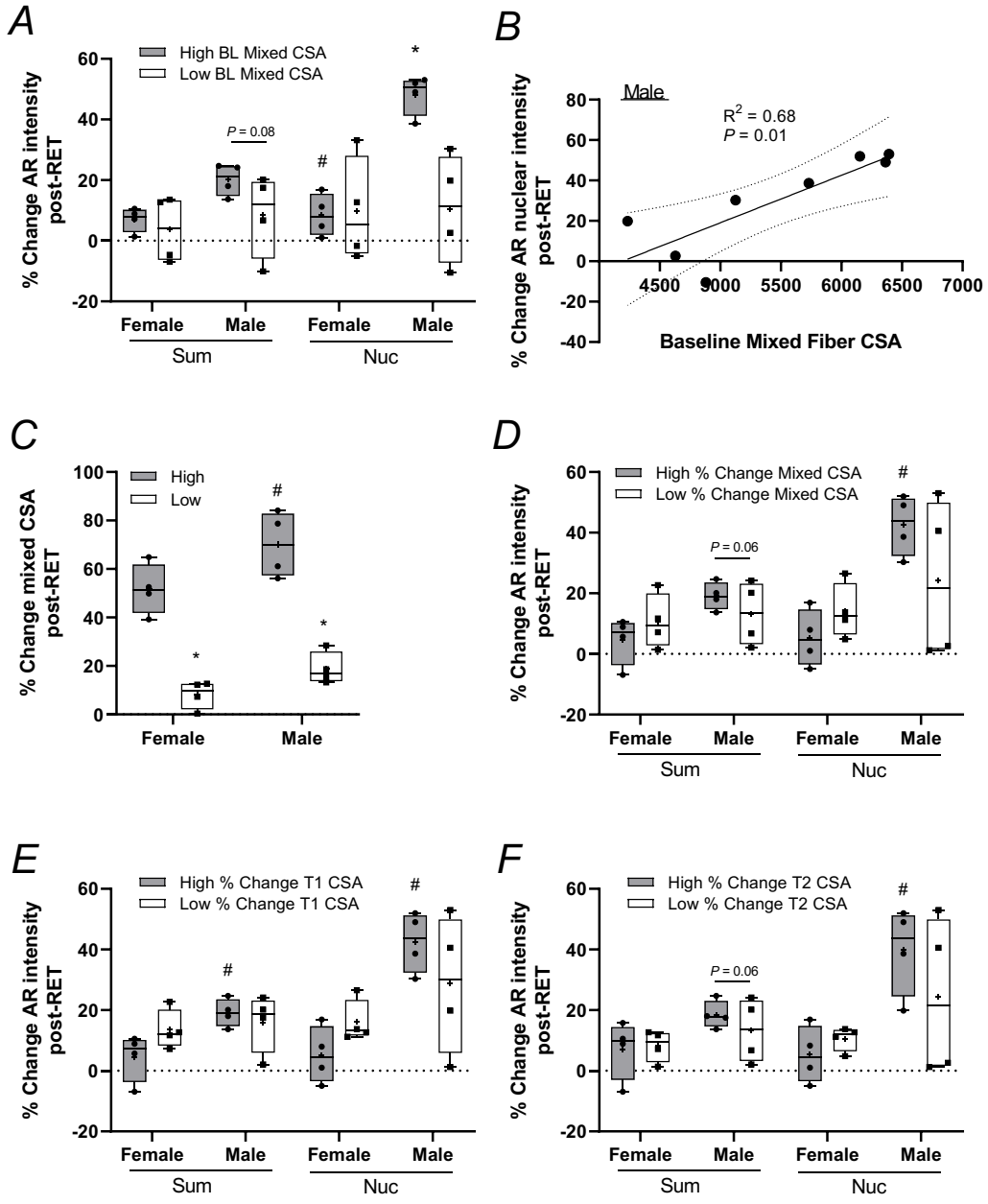


Figure 12: AR protein and CSA. *A)* Percent change in total or nuclear AR content from baseline to post-RET grouped by sex and baseline mixed fibre CSA (high/low; n=4). *B)* Pearson's correlation between baseline mixed fiber CSA responses and percent change in AR nuclear content post-RET (n=8). *C)* high or low percent change in mixed fibre CSA post-RET in males and females (n=4 per group). Percent change in AR sum or nuclear content from baseline to post-RET grouped by sex and high or low percent change in *D)* mixed, *E)* type-I, or *F)* type-II fibre CSA (n=4 per group). Values are expressed as box and whisker plots with individual values. * refers to a significant difference between high and corresponding low ($p<0.05$). # refers to a significant difference relative to the opposite sex ($p<0.05$).

DISCUSSION

This study investigated how exercise influences AR content in male and female skeletal muscle. The purpose of the present study was to 1) investigate changes in AR content during skeletal muscle repair and RET and 2) characterize AR-mediated sex-based differences following RET. We hypothesized that AR gene expression and protein content would increase during skeletal muscle repair and that it would be associated with hypertrophy in males following RET. We found that AR gene expression did not increase with exercise and that AR mRNA was greater in females than males at baseline, which was positively associated with baseline Pax7 mRNA in females. AR protein content increased in both sexes during skeletal muscle repair, with AR being more positively associated with SC content in males, post-damage. Nuclear-associated AR protein content increased following RET, to a greater extent in males than females. AR protein content was also related to CSA in males and SC activity in females, post-RET. Collectively, AR protein is elevated following damage and RET, and AR impacts skeletal muscle during repair and growth in a sex-specific manner.

Sex-based differences in androgen receptor content

Human trials examining AR content are sparse, particularly as it relates to sex-based differences. Some studies have reported no differences between human male and female baseline AR gene expression (Vingren et al., 2009; West et al., 2012), however, we have observed that untrained females have greater AR mRNA than untrained males **(Figure 6)**. The discrepancy between our findings and previous reports are most likely due previously resistance trained participants in Vingren et al. (2009) and West et al. (2012) while participants in the current investigation were untrained when this observation was made. RE has been shown to increase AR mRNA expression (Spiering et al., 2009; Willoughby & Taylor, 2004), therefore, previous training

may conceal baseline sex-based differences and account for discrepancies between the current results and those in the published literature (Vingren et al., 2009; West et al., 2012). No sex-differences at baseline were observed for total or nuclear-associated AR content **(Figure 7E and F)**. We report that in a subset of participants (7 females, and 5 males), using western blot analysis, that females had a greater baseline AR protein content as compared to males **(Figure 8B)**. It may be that AR protein content in skeletal muscle is not of sufficient abundance (Dart et al., 2013) for immunofluorescent staining analysis to detect modest differences at baseline. The limited presence of AR protein content at baseline limits us from making conclusions as changes between participants at this timepoint are minimal.

Androgen receptor content and skeletal muscle repair

To our knowledge, we are the first to assess changes in AR content during skeletal muscle repair following a single bout of damaging eccentric exercise. We did not observe an increase in AR gene expression during skeletal muscle repair **(Figure 6),** in contrast, work by Bamman et al. (2001), found that AR mRNA was upregulated 48 hours following 64 eccentric squats. Our eccentric damage protocol induces muscle damage as denoted by z-band streaming, greater creatine kinase and force output decrements (Mahoney et al., 2008). The intensity of the current protocol may have limited AR gene expression due to increased proteolysis during skeletal muscle repair (Ikezoe et al., 2004; Lee & Chang, 2003; Ratamess et al., 2005). AR protein upregulates itself within the nucleus so drastically decreasing AR protein content from proteolysis during skeletal muscle damage could limit its self-expression, thus limiting AR mRNA expression (Ikezoe et al., 2004; Lee & Chang, 2003; Ratamess et al., 2005). Alternatively, it is possible that the eccentric contraction protocol performed in our study may have shifted the period where AR gene expression is upregulated upon skeletal muscle repair, relative to a lower intensity stimulus. We

report that females express greater AR mRNA than males following damage **(Figure 6)**. There is a paucity of literature characterizing the acute AR gene expression in males and females. Our data suggest that females upregulate AR gene expression at baseline and 48 hours post-damage to compensate for the low circulating androgen ligand, relative to males. Contraction-induced muscle damage is a novel intervention that seems to elicit unique gene expression responses in males and females.

We detected an increase in AR protein content from baseline to post-damage using immunofluorescence **(Figure 7, E and F)**. Willoughby et. al. (2004) observed an increase in AR protein expression 48 hours after the second and third RE bouts in untrained males. These authors demonstrate that 2 bouts of heavy RE increase AR protein expression, whereas 300 eccentric contractions in our study elicits an upregulation of AR protein after one bout, likely owing to a more potent muscle-damaging stimulus. Ahtiainen et al. (2011) and Hulmi et al. (2008) did not observe an increase in AR protein expression post-exercise, likely because both studies utilized a very low volume and intensity of exercise. Instead, Willoughby et al. (2004) reported an AR protein response likely because they applied a higher RE volume and ran participants through the protocol again 48 hours following the first RE bout. Altogether, these data indicate that a high-intensity (or volume) exercise stimulus is required to upregulate AR protein content and suggests that AR protein is upregulated during skeletal muscle repair but not following a single bout of non-damaging RE. AR protein increased in both sexes and to a greater extent in males following damage **(Figure 7E and F; Figure 8B and C)**, which aligns, in part, with West et al. (2012) who reported acute increases in AR protein content that were greater overall in males than females. There are significant differences in study design between West et al. (2012) and the current work, including participant training status and time course following exercise, so these results cannot be

directly compared. Our findings, in agreement with West et al. (2012), demonstrate that AR protein content increases, to a greater degree in males, upon a high volume of RE. Furthermore, our data suggests that a single bout of contraction-induced skeletal muscle damage induces a response in the skeletal muscle strong enough to upregulate AR protein content acutely in both males and females.

Androgen receptor protein and SC response during skeletal muscle repair

Immunofluorescence imaging techniques commonly performed in our laboratory (Fortino et al., 2022; Nederveen et al., 2018) were utilized to examine the influence of androgen receptors on the acute SC response during skeletal muscle repair. In the current study, we observed AR protein associated with mixed fibre SC content in males more than females during repair **(Figure 9E)**. Interestingly, there was a trend for males to have more AR protein content coupled with activated type-I fibre-associated SC than females **(Figure 9G)**. These observations support work by Mackrell et al. (2015) who injected gonadectomized mice with a cardiotoxin to induce skeletal muscle damage, with or without injection of an AR agonist. Mice treated with the AR agonist had greater Pax7 and MyoD mRNA expression as compared to the untreated injured mice, providing further evidence for the role of AR on SC activation following skeletal muscle damage. These findings suggest that AR is associated with SC during enhanced proliferation and activation, and our study indicates that this association is more relevant in males than in females. The positive association between AR and SC may also explain, in part, the robust SC response we see in males post-damage relative to females (Diel et al., 2008; Doumitt et al., 1996; Fortino et al., 2022; Kim et al., 2016).

Androgen receptor content and skeletal muscle following RET

We report an increase in total and nuclear-associated AR protein content following 10 weeks of RET in previously untrained males and females **(Figure 10A and B)**. Morton et al. (2018) reported no increase in whole muscle AR protein content following RET in a population of trained men. Since these participants were already performing consistent RE, AR content did not change likely because they were accustomed to this stimulus and therefore would not have increased following the RET intervention of the study. Mobley et al. (2018) observed a downregulation of AR following 12 weeks of RET in untrained males. This result could be related to the RE volume insufficiently stimulating AR over the course of the training period. For instance, in the present study participants performed 14 lower body exercises per week in contrast to 6 lower body exercises per week in the study by Mobley et al. (2018). The RET protocol in the study by Mobley et al. (2018) was sufficient to induce skeletal muscle hypertrophy, but the lack of RE volume localized to the lower body may have led to AR protein decreasing over the 12-week period. Our novel finding that percent change in nuclear-associated AR content is greater in males than females following RT **(Figure 10C)** indicates that more AR exists at the nucleus in males post-RET, which is critical for the impact of AR on the cell (Davey & Grossmann, 2016; Tyagi et al., 2000). Specifically, exercise raises circulating androgen concentrations to facilitate androgen binding to AR in the cytosol. The androgen binding AR initiates nuclear translocation where AR can modify downstream targets that trigger cellular adaptations related to skeletal muscle growth and regeneration. Together, it seems RE volume before or during the exercise intervention affects AR protein content following RET and our work observed that males have more relative AR situated at the nucleus as compared to females following RET. This suggests that females may utilize alternative signalling mechanisms to drive changes in CSA. For example, estrogen has been shown

to be protective from skeletal muscle damage. It is likely that females use different receptors and downstream pathways following exercise to ultimately elicit a similar adaptation to the muscle cell.

Androgen receptor protein, satellite cells and hypertrophy following RET

To our knowledge, this study is the first to examine the relationship between SC and AR content in human skeletal muscle in males compared to females. Females with the highest baseline Pax7 expression also had the highest baseline AR mRNA, suggesting an interplay between AR protein and SC in untrained females but not males **(Figure 11A)**. We targeted type-I fibre-mediated SC adaptations to skeletal muscle repair because AR is more abundant in type-I fibres than type-II fibres (Altuwaijri et al., 2004; Hulmi et al., 2008). Therefore, we sought to examine the impact of AR on SCs associated with type-I skeletal muscle fibres. Males with the greatest increase in type-I fibre quiescent SC following RET had more nuclear-associated AR content relative to females **(Figure 11D)**. This finding further speaks to the importance of nuclear translocation for the regulation of skeletal muscle-related adaptations in males following exercise. Our study demonstrates that females with the greatest increase in activated type-I fibre-associated SC post-RET content had greater total AR content post-RET relative to females with the least type-I fibre-associated activated SC **(Figure 11E and F)**, suggesting a relationship between whole-muscle AR and SC activation in females, post-RET. Overall, whole-muscle AR protein appears to influence SC regulation in females post-RET while males demonstrate a greater dependence on nuclear-associated AR content.

AR protein content and hypertrophy have been shown to positively correlate despite remarkable inter-individual variability in AR content following training (Ahtiainen et al., 2011; Mitchell et al., 2013). High and low responder analyses were utilized to understand the impact of

the large variation in AR content, between participants. In accordance with Morton et al. (2018), our results demonstrate a relationship between CSA and AR protein content in males **(Figure 12A and B).** To build upon findings from Morton et al. (2018), we report that only percent change in nuclear-associated AR content post-RET (but not total) was associated with baseline CSA in males. Nuclear-associated AR content is correlated to baseline myofibre size in untrained males likely because the nuclear-associated AR content is impacting function of the nucleus. In our study, males with the greatest increase in CSA (mixed fibre, type-I fibre, type-II fibre) showed no difference in AR protein content relative to males with the lowest CSA gain, post-RET **(Figure D-F)**. This contrasts with Morton et al. (2018) where they report a difference in AR content between high and low hypertrophy responders post-RET. Discrepant findings between our study and Morton et al. (2018) are a product of variations in data expression. We chose to express AR and CSA changes as 'percent change', relative to baseline, as this accounts for each participants baseline outcomes, which allows for comparison between sexes because males and females are inherently different at baseline. Analyzing AR content using percent change results in the data being expressed differently than calculating absolute differences between timepoints (pre and post-RET) as seen in Morton et al. (2018). Intriguingly, we show that the positive correlation between change in AR protein content and CSA, in males, observed in our study and Morton et al. (2018), was not apparent in females **(Appendix B)**. Moreover, males with the greatest increase in CSA following RET had more nuclear-associated AR content relative to females with the greatest increase in CSA (mixed, type-I, type-II fibre CSA) **(Figure 12 D-F)**. The high CSA responders in males exhibited a 37% greater percent change in mixed fibre CSA than the high CSA responders in females, following RET **(Figure 12C)**. There was no difference between sexes in the low responders to percent change in mixed fibre CSA, post-RET. These findings suggests that nuclear-

associated AR protein content could be a driving factor of high CSA responders in males exhibiting more percent change in CSA compared to high CSA responders in females. Work by Maclean et al. (2008) is one of a few studies to examine AR impact on muscle mass, between sexes, albeit in mice. By investigating male and female AR KO mice, they found that muscle mass decreased in AR KO males but not females. Taken together with our findings, this suggests that females do not rely on AR content to maintain or increase skeletal muscle mass. Overall, our observations indicate that nuclear AR content is more closely associated with SC and hypertrophy in males than females following RET. This relationship in males may highlight a potential mechanism for sex-based skeletal muscle adaptations following exercise.

Conclusions

In conclusion, the results of the current study demonstrate that a single bout of eccentric damage is sufficient to increase AR protein expression in both males and females. Ten weeks of RET increased AR protein content in untrained individuals with males expressing greater nuclear-associated AR protein content relative to females. During skeletal muscle repair or following RET, AR appears to be associated with SC in males more so than females. Here, we show that nuclear-associated AR protein content is advantageous for skeletal muscle CSA gains in males more than females. AR content during skeletal muscle repair or hypertrophy is associated with sex-specific skeletal muscle adaptations.

Limitations and future directions

The tissue utilized in this investigation was collected previously (Fortino et al., 2022; Wageh et al., 2021), and tissue availability limited the sample size. Human studies generally limit our ability to establish cause and effect relationships that could be ascertained in mice or *in vitro* models. Our study did not track the menstrual cycle which could have further accounted for

differences in our sex-based comparisons. The differences in circulating testosterone during the menstrual cycle are minimal relative to the diurnal variation seen in males. However, considering this was the first study to examine sex differences in AR content within the context of human skeletal muscle repair and hypertrophy, we are not fully aware of the implications of hormone fluctuations influencing AR's impact on muscle repair.

Future work should aim to determine AR's direct impact on skeletal muscle following exercise. Identifying molecular targets of AR activity following skeletal muscle damage versus RET would clarify many unanswered questions surrounding AR mechanisms. The influence of AR on the nucleus in human skeletal muscle should be examined further as its impact on the cell is substantial. We have just begun to scratch the surface of sex-based differences within the context of AR regulation and exercise. Future studies should also examine other pathways that identify how female skeletal muscle adapts to exercise without the same reliance on AR, as exhibited by males.

REFERENCES

Ahtiainen, J. P., Hulmi, J. J., Kraemer, W. J., Lehti, M., Nyman, K., Selänne, H., Alen, M., Pakarinen, A., Komulainen, J., Kovanen, V., Mero, A. A., & Häkkinen, K. (2011). Heavy resistance exercise training and skeletal muscle androgen receptor expression in younger and older men. *Steroids*, *76*, 183–192. https://doi.org/10.1016/j.steroids.2010.10.012

Aizawa, K., Iemitsu, M., Maeda, S., Otsuki, T., Sato, K., Ushida, T., Mesaki, N., & Akimoto, T. (2010). Acute exercise activates local bioactive androgen metabolism in skeletal muscle. *Steroids*, *75*(3), 219–223. https://doi.org/10.1016/j.steroids.2009.12.002

Altuwaijri, S., Lee, D. K., Chuang, K., Ting, H., Yang, Z., Xu, Q., Tsai, M., Yeh, S., Hanchett, L. A., & Chang, H. (2004). of Skeletal Muscle – Specific Proteins and Muscle Cell Types. *October*, *25*(1), 27–32.

Bamman, M. M., Shipp, J. R., Jiang, J., Gower, B. A., Hunter, G. R., Goodman, A., McLafferty, C. L., & Urban, R. J. (2001). Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. *American Journal of Physiology - Endocrinology and Metabolism*, *280*(3 43-3). https://doi.org/10.1152/ajpendo.2001.280.3.e383

Beaton, L. J., Tarnopolsky, M. A., & Phillips, S. M. (2002). Variability in Estimating Eccentric Contraction-Induced Muscle Damage and Inflammation in Humans. *Rapid Communication*, *27*(5), 516–526.

Bellamy, L. M., Joanisse, S., Grubb, A., Mitchell, C. J., McKay, B. R., Phillips, S. M., Baker, S., & Parise, G. (2014). The acute satellite cell response and skeletal muscle hypertrophy following resistance training. *PLoS ONE*, *9*(10), 17–21. https://doi.org/10.1371/journal.pone.0109739

Bentzinger, C. F., Wang, Y. X., & Rudnicki, M. A. (2012). Building muscle: molecular regulation of myogenesis. *Cold Spring Harbor Perspectives in Biology*, *4*(2). https://doi.org/10.1101/cshperspect.a008342

Bhasin, S., Woodhouse, L., Casaburi, R., Singh, A. B., Bhasin, D., Berman, N., Chen, X., Yarasheski, K. E., Magliano, L., Dzekov, C., Dzekov, J., Bross, R., Phillips, J., Sinha-Hikim, I., Shen, R., & Storer, T. W. (2001). Testosterone dose-response relationships in healthy young men. *American Journal of Physiology - Endocrinology and Metabolism*, *281*(6 44-6), 1172–1181. https://doi.org/10.1152/ajpendo.2001.281.6.e1172

Buckingham, M., & Rigby, P. W. J. (2014). Gene Regulatory Networks and Transcriptional Mechanisms that Control Myogenesis. *Developmental Cell*, *28*(3), 225–238. https://doi.org/10.1016/j.devcel.2013.12.020

Chambon, C., Duteil, D., Vignaud, A., Ferry, A., Messaddeq, N., Malivindi, R., Kato, S., Chambon, P., Metzger, D., & Rende, di. (n.d.). *Myocytic androgen receptor controls the strength but not the mass of limb muscles*. https://doi.org/10.1073/pnas.1009536107

Chen, Y., Zajac, J. D., & MacLean, H. E. (2005). Androgen regulation of satellite cell function. *Journal of Endocrinology*, *186*(1), 21–31. https://doi.org/10.1677/joe.1.05976

Chesley, A., MacDougall, J. D., Tarnopolsky, M. A., Atkinson, S. A., & Smith, K. (1992). Changes in human muscle protein synthesis after resistance exercise. *Journal of Applied Physiology*, *73*(4), 1383–1388. https://doi.org/10.1152/jappl.1992.73.4.1383

Conboy, I. M., Conboy, M. J., Wagers, A. J., Girma, E. R., Weismann, I. L., & Rando, T. A. (2005). Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature*, *433*(7027), 760–764. https://doi.org/10.1038/nature03260

Damas, F., Phillips, S. M., Libardi, C. A., Vechin, F. C., Lixandr, M. E., Jannig, P. R., Costa, L. A. R., Bacurau, A. V, Snijders, T., Parise, G., Tricoli, V., Roschel, H., & Ugrinowitsch, C. (2016). *Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage*. *18*, 5209–5222. https://doi.org/10.1113/JP272472

Davey, R. A., & Grossmann, M. (2016). Androgen Receptor Structure, Function and Biology: From Bench to Bedside. *The Clinical Biochemist. Reviews*, *37*(1), 3–15. http://www.ncbi.nlm.nih.gov/pubmed/27057074%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4810760

Delorme, L. (1945). Restoration of muscle. *The Journal of Bone and Joint Surgery*, *27*(4), 645–667.

Diel, P., Baadners, D., Schlü, K., Velders, M., & Schwarz, J. P. (2008). C2C12 myoblastoma cell differentiation and proliferation is stimulated by androgens and associated with a modulation of myostatin and Pax7 expression. *Journal of Molecular Endocrinology*, *40*, 231–241. https://doi.org/10.1677/JME-07-0175

Doumitt, M. E., Cook$, D. R., & Merkel, R. A. (1996). *Testosterone Up-Regulates Androgen Receptors and Decreases Differentiation of Porcine Myogenic Satellite Cells in Vitro**. https://academic.oup.com/endo/article/137/4/1385/3037346

Dubois, V., Laurent, M. R., Sinnesael, M., Cielen, N., Helsen, C., Clinckemalie, L., Spans, L., Gayan-Ramirez, G., Deldicque, L., Hespel, P., Carmeliet, G., Vanderschueren, D., & Claessens, F. (2014). A satellite cell-specific knockout of the androgen receptor reveals myostatin as a direct androgen target in skeletal muscle. *FASEB Journal*, *28*(7), 2979–2994. https://doi.org/10.1096/fj.14-249748

E. Schultz, D. Jaryszak, C. V. (1985). *Response of Satellite Cells To*. 217–222.

Englund, D. A., Figueiredo, V. C., Dungan, C. M., Murach, K. A., Peck, B. D., Petrosino, J. M., Brightwell, C. R., Dupont, A. M., Neal, A. C., Fry, C. S., Accornero, F., McCarthy, J. J., & Peterson, C. A. (2020). Satellite Cell Depletion Disrupts Transcriptional Coordination and Muscle Adaptation to Exercise. *Function*, *2*(1), 1–18. https://doi.org/10.1093/function/zqaa033

Farup, J., Rahbek, S. K., Knudsen, I. S., De Paoli, F., Mackey, A. L., & Vissing, K. (2014). Whey protein supplementation accelerates satellite cell proliferation during recovery from eccentric exercise. *Amino Acids*, *46*(11), 2503–2516. https://doi.org/10.1007/s00726-014-1810-3

Fortino, S. A., Wageh, M., Pontello, R., McGlory, C., Kumbhare, D., Phillips, S. M., & Parise, G. (2022). Sex-Based Differences in the Myogenic Response and Inflammatory Gene Expression Following Eccentric Contractions in Humans. *Frontiers in Physiology*, *13*(April), 1–10. https://doi.org/10.3389/fphys.2022.880625

Fry, C. S., Lee, J. D., Mula, J., Kirby, T. J., Jackson, J. R., Liu, F., Yang, L., Mendias, C. L., Dupont-Versteegden, E. E., McCarthy, J. J., & Peterson, C. A. (2015). Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia. *Nature Medicine*, *21*(1), 76–80. https://doi.org/10.1038/nm.3710

Grounds, M. D., Garrett, K. L., Lai, M. C., Wright, W. E., & Beilharz, M. W. (1992). Identification of skeletal muscle precursor cells in vivo by use of MyoD1 and myogenin probes. *Cell & Tissue Research*, *267*(1), 99–104. https://doi.org/10.1007/BF00318695

Haun, C. T., Vann, C. G., Roberts, B. M., Vigotsky, A. D., Schoenfeld, B. J., & Roberts, M. D. (2019). A critical evaluation of the biological construct skeletal muscle hypertrophy: Size

matters but so does the measurement. *Frontiers in Physiology*, *10*(MAR), 1–23. https://doi.org/10.3389/fphys.2019.00247

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., Zoeller, R. F., Seip, R. L., & Clarkson, P. M. (2005). Variability in muscle size and strength gain after unilateral resistance training. *Medicine and Science in Sports and Exercise*, *37*(6), 964–972. https://doi.org/10.1016/s0162-0908(08)70354-4

Hulmi, J. J., Ahtiainen, J. P., Selänne, H., Volek, J. S., Häkkinen, K., Kovanen, V., & Mero, A. A. (2008). Androgen receptors and testosterone in men-Effects of protein ingestion, resistance exercise and fiber type. *Journal of Steroid Biochemistry and Molecular Biology*, *110*(1–2), 130–137. https://doi.org/10.1016/j.jsbmb.2008.03.030

Hyldahl, R. D., & Hubal, M. J. (2014). Lengthening our perspective: Morphological, cellular, and molecular responses to eccentric exercise. *Muscle and Nerve*, *49*(2), 155–170. https://doi.org/10.1002/mus.24077

Ikezoe, T., Yang, Y., Saito, T., Koeffler, H. P., & Taguchi, H. (2004). Proteasome inhibitor PS-341 down-regulates prostate-specific antigen (PSA) and induces growth arrest and apoptosis of androgen-dependent human prostate cancer LNCaP cells. *Cancer Science*, *95*(3), 271–275. https://doi.org/10.1111/j.1349-7006.2004.tb02215.x

Joubert, Y., & Tobin, C. (1995). Testosterone treatment results in quiescent satellite cells being activated and recruited into cell cycle in rat levator ani muscle. In *Developmental Biology* (Vol. 169, Issue 1, pp. 286–294). https://doi.org/10.1006/dbio.1995.1144

Kim, J. H., Han, G. C., Seo, J. Y., Park, I., Park, W., Jeong, H. W., Lee, S. H., Bae, S. H., Seong, J., Yum, M. K., Hann, S. H., Kwon, Y. G., Seo, D., Choi, M. H., & Kong, Y. Y. (2016). Sex hormones establish a reserve pool of adult muscle stem cells. *Nature Cell Biology*, *18*(9), 930–940. https://doi.org/10.1038/ncb3401

KOLLER, M. (1960). [Weight changes in the anterior tibial muscle of the prepuberal rat in normal conditions, after castration and after treatment with 2 anabolic steroids]. *Bollettino della Societa italiana di biologia sperimentale*, *36*, 756–759.

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Medicine*, *35*(4), 339–361. https://doi.org/10.2165/00007256-200535040-00004

Kuang, S., Chargé, S. B., Seale, P., Huh, M., & Rudnicki, M. A. (2006). Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. *Journal of Cell Biology*, *172*(1), 103–113. https://doi.org/10.1083/jcb.200508001

La Montagna, R., Caligiuri, I., Maranta, P., Lucchetti, C., Esposito, L., Paggi, M. G., Toffoli, G., Rizzolio, F., & Giordano, A. (2012). Androgen receptor serine 81 mediates Pin1 interaction and activity. *Cell Cycle*, *11*(18), 3415–3420. https://doi.org/10.4161/cc.21730

Lee, D. K., & Chang, C. (2003). Endocrine mechanisms of disease: Expression and degradation of androgen receptor: Mechanism and clinical implication. *Journal of Clinical Endocrinology and Metabolism*, *88*(9), 4043–4054. https://doi.org/10.1210/jc.2003-030261

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Lu, K., Yu, M., & Chen, Y. (2021). Non-coding RNAs regulating androgen receptor signaling pathways in prostate cancer. *Clinica Chimica Acta*, *513*(68), 57–63. https://doi.org/10.1016/j.cca.2020.11.027

Mackey, A. L., Andersen, L. L., Frandsen, U., & Sjøgaard, G. (2011). Strength training increases the size of the satellite cell pool in type I and II fibres of chronically painful trapezius muscle in females. *Journal of Physiology*, *589*(22), 5503–5515. https://doi.org/10.1113/jphysiol.2011.217885

Mackrell, J. G., Yaden, B. C., Bullock, H., Chen, K., Shetler, P., Bryant, H. U., & Krishnan, V. (2015). *Androgen targets characterizing muscle regeneration Nuclear Receptor Signaling | www Molecular targets of androgen signaling that characterize skeletal muscle recovery and regeneration*. *13*. https://doi.org/10.1621/nrs.13005

MacLean, H. E., Chiu, W. S. M., Notini, A. J., Axell, A.-M., Davey, R. A., McManus, J. F., Ma, C., Plant, D. R., Lynch, G. S., & Zajac, J. D. (2008). Impaired skeletal muscle development and function in male, but not female, genomic androgen receptor knockout mice . *The FASEB Journal*, *22*(8), 2676–2689. https://doi.org/10.1096/fj.08-105726

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*(6). https://doi.org/10.1152/ajpregu.00847.2007

MAURO, A. (1961). Satellite cell of skeletal muscle fibers. *The Journal of Biophysical and Biochemical Cytology*, *9*, 493–495. https://doi.org/10.1083/jcb.9.2.493

Mccarthy, J. J., Mula, J., Miyazaki, M., Erfani, R., Garrison, K., Farooqui, A. B., Srikuea, R., Lawson, B. A., Grimes, B., Keller, C., Van Zant, G., Campbell, K. S., Esser, K. A., Dupont-Versteegden, E. E., & Peterson, C. A. (2011). Effective fiber hypertrophy in satellite cell-depleted skeletal muscle. *Development*, *138*(17), 3657–3666. https://doi.org/10.1242/dev.068858

McKay, B. R., O'Reilly, C. E., Phillips, S. M., Tarnopolsky, M. A., & Parise, G. (2008). Co-expression of IGF-1 family members with myogenic regulatory factors following acute damaging muscle-lengthening contractions in humans. *Journal of Physiology*, *586*(22), 5549–5560. https://doi.org/10.1113/jphysiol.2008.160176

McKay, B. R., Toth, K. G., Tarnopolsky, M. A., & Parise, G. (2010). Satellite cell number and cell cycle kinetics in response to acute myotrauma in humans: Immunohistochemistry versus flow cytometry. *Journal of Physiology*, *588*(17), 3307–3320. https://doi.org/10.1113/jphysiol.2010.190876

Mitchell, C. J., Churchward-Venne, T. A., Bellamy, L., Parise, G., & Baker, S. K. (2013). Muscular and Systemic Correlates of Resistance Training-Induced Muscle Hypertrophy. *PLoS ONE*, *8*(10), 78636. https://doi.org/10.1371/journal.pone.0078636

Miyamoto-Mikami, E., Tsuji, K., Horii, N., Hasegawa, N., Fujie, S., Homma, T., Uchida, M., Hamaoka, T., Kanehisa, H., Tabata, I., & Iemitsu, M. (2018). Gene expression profile of muscle adaptation to high-intensity intermittent exercise training in young men. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-35115-x

Mobley, C. B., Haun, C. T., Roberson, P. A., Mumford, P. W., Kephart, W. C., Romero, M. A., Osburn, S. C., Vann, C. G., Young, K. C., Beck, D. T., Martin, J. S., Lockwood, C. M., & Roberts, M. D. (2018). *Biomarkers associated with low, moderate, and high vastus lateralis muscle hypertrophy following 12 weeks of resistance training*. https://doi.org/10.1371/journal.pone.0195203

Morton, R. W., Sato, K., Gallaugher, M. P. B., Oikawa, S. Y., Mcnicholas, P. D., Fujita, S., Phillips, S. M., & Reidy, P. T. (2018). Muscle Androgen Receptor Content but Not Systemic Hormones Is Associated With Resistance Training-Induced Skeletal Muscle

Hypertrophy in Healthy , Young Men. *Frontiers in Physiology*, *9*(October), 1–11. https://doi.org/10.3389/fphys.2018.01373

Nederveen, J. P., Joanisse, S., Snijders, T., Ivankovic, V., Baker, S. K., Phillips, S. M., & Parise, G. (2016). Skeletal muscle satellite cells are located at a closer proximity to capillaries in healthy young compared with older men. *Journal of Cachexia, Sarcopenia and Muscle*, *7*(5), 547–554. https://doi.org/10.1002/jcsm.12105

Nederveen, J. P., Joanisse, S., Snijders, T., Thomas, A. C. Q., Kumbhare, D., Parise, G., Hogan, M., & Hornberger, T. (2018). *The influence of capillarization on satellite cell pool expansion and activation following exercise-induced muscle damage in healthy young men*. *6*, 1063–1078. https://doi.org/10.1113/JP275155

Nicoll, J. X., Fry, A. C., & Mosier, E. M. (2019). Sex-based differences in resting MAPK, androgen, and glucocorticoid receptor phosphorylation in human skeletal muscle. *Steroids*, *141*(November 2018), 23–29. https://doi.org/10.1016/j.steroids.2018.11.004

O'Reilly, C., McKay, B., Phillips, S., Tarnopolsky, M., & Parise, G. (2008). Hepatocyte growth factor (HGF) and the satellite cell response following muscle lengthening contractions in humans. *Muscle and Nerve*, *38*(5), 1434–1442. https://doi.org/10.1002/mus.21146

Oole, C. H. N. P., Oberts, M. I. D. R., Albo, V. I. J. D., & Underland, K. Y. L. E. L. S. (2011a). *M a r g e y o h s m b a t s e b*. *25*(2), 309–317.

Oole, C. H. N. P., Oberts, M. I. D. R., Albo, V. I. J. D., & Underland, K. Y. L. E. L. S. (2011b). *M a r g e y o h s m b a t s e b*. *25*(2), 309–317.

Ophoff, J., Van Proeyen, K., Callewaert, F., De Gendt, K., De Bock, K., Vanden Bosch, A., Verhoeven, G., Hespel, P., & Vanderschueren, D. (2009). Androgen signaling in myocytes contributes to the maintenance of muscle mass and fiber type regulation but not to muscle strength or fatigue. *Endocrinology*, *150*(8), 3558–3566. https://doi.org/10.1210/en.2008-1509

Petrella, J. K., Kim, J. S., Cross, J. M., Kosek, D. J., & Bamman, M. M. (2006). Efficacy of myonuclear addition may explain differential myofiber growth among resistance-trained young and older men and women. *American Journal of Physiology - Endocrinology and Metabolism*, *291*(5), 937–946. https://doi.org/10.1152/ajpendo.00190.2006

Phillips, S. M. (2014). *A Brief Review of Critical Processes in Exercise-Induced Muscular Hypertrophy*. *44*, 71–77. https://doi.org/10.1007/s40279-014-0152-3

Pierre Amarenco, Julien Bogousslavsky, Alfred Callahan 3rd, Larry B Goldstein, Michael Hennerici, Amy E Rudolph, Henrik Sillesen, Lisa Simunovic, Michael Szarek, K M A Welch, Justin A Zivin, S. P. by A. R. in C. L. (SPARCL) I. (2011). 需要引用的霍奇金第二肿瘤new England Journal. *New England Journal of Medicine*, *365*, 687–696.

Polanco, M. J., Parodi, S., Piol, D., Stack, C., Chivet, M., Contestabile, A., Miranda, H. C., Lievens, P. M.-J., Espinoza, S., Jochum, T., Rocchi, A., Grunseich, C., Gainetdinov, R. R., Cato, A. C. B., Lieberman, A. P., La Spada, A. R., Sambataro, F., Fischbeck, K. H., Gozes, I., & Pennuto, M. (2016). Adenylyl cyclase activating polypeptide reduces phosphorylation and toxicity of the polyglutamine-expanded androgen receptor in spinobulbar muscular atrophy. *Science Translational Medicine*, *8*(370), 370ra181-370ra181. https://doi.org/10.1126/scitranslmed.aaf9526

Proske, U., & Morgan, D. L. (2001). Muscle damage from eccentric exercise: Mechanism, mechanical signs, adaptation and clinical applications. *Journal of Physiology*, *537*(2), 333–345. https://doi.org/10.1111/j.1469-7793.2001.00333.x

Ratamess, N. A., Kraemer, W. J., Volek, J. S., Maresh, C. M., Vanheest, J. L., Sharman, M. J.,

Rubin, M. R., French, D. N., Vescovi, J. D., Silvestre, R., Hatfield, D. L., Fleck, S. J., & Deschenes, M. R. (2005). Androgen receptor content following heavy resistance exercise in men. *Journal of Steroid Biochemistry & Molecular Biology*, *93*, 35–42. https://doi.org/10.1016/j.jsbmb.2004.10.019

Sakakibara, I., Yanagihara, Y., Himori, K., Yamada, T., Sakai, H., Sawada, Y., Takahashi, H., Saeki, N., Hirakawa, H., Yokoyama, A., Fukada, S. ichiro, Sawasaki, T., & Imai, Y. (2021). Myofiber androgen receptor increases muscle strength mediated by a skeletal muscle splicing variant of Mylk4. *IScience*, *24*(4), 102303. https://doi.org/10.1016/j.isci.2021.102303

Sambasivan, R., Yao, R., Kissenpfennig, A., Wittenberghe, L. Van, Paldi, A., Gayraud-morel, B., Guenou, H., Malissen, B., Tajbakhsh, S., Galy, A., Sambasivan, R., Yao, R., Kissenpfennig, A., & Wittenberghe, L. Van. (2011). Erratum to Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration (Development (2011), 138 (3647-3656)). *Development*, *138*(19), 4333. https://doi.org/10.1242/dev.073601

Seale, P., Sabourin, L. A., Girgis-Gabardo, A., Mansouri, A., Gruss, P., & Rudnicki, M. A. (2000). Pax7 is required for the specification of myogenic satellite cells. *Cell*, *102*(6), 777–786. https://doi.org/10.1016/S0092-8674(00)00066-0

Sinha-Hikim, I., Cornford, M., Gaytan, H., Lee, M. L., & Bhasin, S. (2006). *Effects of Testosterone Supplementation on Skeletal Muscle Fiber Hypertrophy and Satellite Cells in Community-Dwelling Older Men*. https://doi.org/10.1210/jc.2006-0357

Sinha-Hikim, I., Roth, S. M., Lee, M. I., & Bhasin, S. (2003). Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *American Journal of Physiology - Endocrinology and Metabolism*, *285*(1 48-1), 197–205. https://doi.org/10.1152/ajpendo.00370.2002

Sinha-Hikim, I., Roth, S. M., Lee, M. I., Bhasin, S., & Indrani, S.-H. (2003). Testosterone-induced muscle hypertrophy is associated with an increase in satellite cell number in healthy, young men. *Am J Physiol Endocrinol Metab*, *285*, 197–205. https://doi.org/10.1152/ajpendo.00370.2002.-Testosterone

Sinha-hikim, I., Taylor, W. E., Gonzalez-cadavid, N. F., Zheng, W. E. I., & Bhasin, S. (2004). *Androgen Receptor in Human Skeletal Muscle and Cultured Muscle Satellite Cells : Up-Regulation by Androgen Treatment*. *89*(10), 5245–5255. https://doi.org/10.1210/jc.2004-0084

Snijders, T., Nederveen, J. P., Mckay, B. R., Joanisse, S., Verdijk, L. B., Loon, L. J. C. Van, Parise, G., & Boppart, M. Della. (2015). *Satellite cells in human skeletal muscle plasticity*. *6*(October), 1–21. https://doi.org/10.3389/fphys.2015.00283

Spiering, B. A., Kraemer, W. J., Vingren, J. L., Ratamess, N. A., Anderson, J. M., Armstrong, L. E., Nindl, B. C., Volek, J. S., Häkkinen, K., & Maresh, C. M. (2009). Elevated endogenous testosterone concentrations potentiate muscle androgen receptor responses to resistance exercise. *Journal of Steroid Biochemistry and Molecular Biology*, *114*(3–5), 195–199. https://doi.org/10.1016/j.jsbmb.2009.02.005

Tan, M. E., Li, J., Xu, H. E., Melcher, K., & Yong, E. L. (2015). Androgen receptor: Structure, role in prostate cancer and drug discovery. *Acta Pharmacologica Sinica*, *36*(1), 3–23. https://doi.org/10.1038/aps.2014.18

Timmerman, K. L., Lee, J. L., Fujita, S., Dhanani, S., Dreyer, H. C., Fry, C. S., Drummond, M. J., Sheffield-Moore, M., Rasmussen, B. B., & Volpi, E. (2010). Pharmacological vasodilation improves insulin-stimulated muscle protein anabolism but not glucose

utilization in older adults. *Diabetes*, *59*(11), 2764–2771. https://doi.org/10.2337/db10-0415

Tumasian, R. A., Harish, A., Kundu, G., Yang, J. H., Ubaida-Mohien, C., Gonzalez-Freire, M., Kaileh, M., Zukley, L. M., Chia, C. W., Lyashkov, A., Wood, W. H., Piao, Y., Coletta, C., Ding, J., Gorospe, M., Sen, R., De, S., & Ferrucci, L. (2021). Skeletal muscle transcriptome in healthy aging. *Nature Communications*, *12*(1). https://doi.org/10.1038/s41467-021-22168-2

Tyagi, R. K., Lavrovsky, Y., Ahn, S. C., Song, C. S., Chatterjee, B., & Roy, A. K. (2000). Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells. *Molecular Endocrinology*, *14*(8), 1162–1174. https://doi.org/10.1210/mend.14.8.0497

Verdijk, L. B., Gleeson, B. G., Jonkers, R. A. M., Meijer, K., Savelberg, H. H. C. M., Dendale, P., & Van Loon, L. J. C. (2009). Skeletal muscle hypertrophy following resistance training is accompanied by a fiber type-specific increase in satellite cell content in elderly men. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *64*(3), 332–339. https://doi.org/10.1093/gerona/gln050

Vingren, J. L., Kraemer, W. J., Hatfield, D. L., Volek, J. S., Ratamess, N. A., Anderson, J. M., Häkkinen, K., Ahtiainen, J., Fragala, M. S., Thomas, G. A., Ho, J. Y., & Maresh, C. M. (2009). Effect of resistance exercise on muscle steroid receptor protein content in strength-trained men and women. *Steroids*, *74*(13–14), 1033–1039. https://doi.org/10.1016/j.steroids.2009.08.002

Wageh, M., Fortino, S. A., McGlory, C., Kumbhare, D., Phillips, S. M., & Parise, G. (2021). The Effect of a Multi-ingredient Supplement on Resistance Training–induced Adaptations. In *Medicine & Science in Sports & Exercise: Vol. Publish Ah* (Issue February). https://doi.org/10.1249/mss.0000000000002641

Wang, R., & Brattain, M. G. (2007). The maximal size of protein to diffuse through the nuclear pore is larger than 60 kDa. *FEBS Letters*, *581*(17), 3164–3170. https://doi.org/10.1016/j.febslet.2007.05.082

Wang, X., & Proud, C. G. (2006). The mTOR pathway in the control of protein synthesis. *Physiology*, *21*(5), 362–369. https://doi.org/10.1152/physiol.00024.2006

West, D. W. D., Burd, N. A., Churchward-Venne, T. A., Camera, D. M., Mitchell, C. J., Baker, S. K., Hawley, J. A., Coffey, V. G., & Phillips, S. M. (2012). Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *Journal of Applied Physiology*, *112*(11), 1805–1813. https://doi.org/10.1152/japplphysiol.00170.2012

West, D. W. D., Burd, N. A., Tang, J. E., Moore, D. R., Staples, A. W., Holwerda, A. M., Baker, S. K., & Phillips, S. M. (2010). Elevations in ostensibly anabolic hormones with resistance exercise enhance neither training-induced muscle hypertrophy nor strength of the elbow flexors. *J Appl Physiol*, *108*, 60–67. https://doi.org/10.1152/japplphysiol.01147.2009.-The

Wierman, M. E. (2007). Sex steroid effects at target tissues: Mechanisms of action. *American Journal of Physiology - Advances in Physiology Education*, *31*(1), 26–33. https://doi.org/10.1152/advan.00086.2006

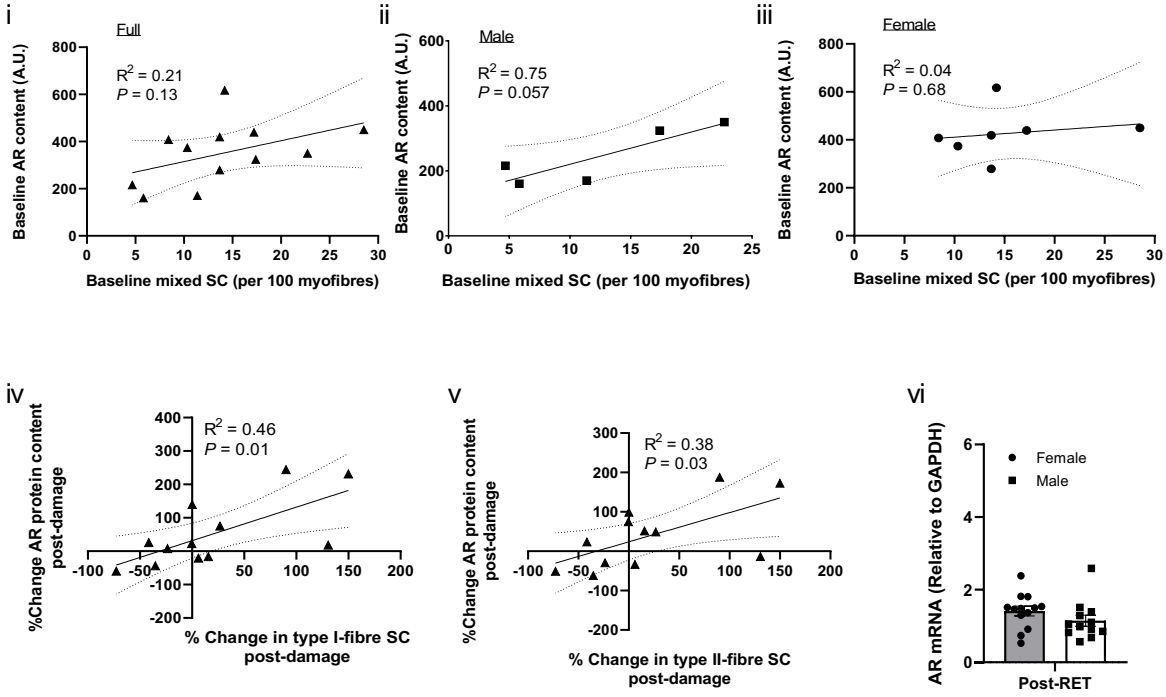
Willoughby, D. S., & Taylor, L. (2004). Effects of sequential bouts of resistance exercise on androgen receptor expression. *Medicine and Science in Sports and Exercise*, *36*(9), 1499–1506. https://doi.org/10.1249/01.MSS.0000139795.83030.D1

Yeh, S., Miyamoto, H., Shima, H., & Chang, C. (1998). From estrogen to androgen receptor: A new pathway for sex hormones in prostate. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(10), 5527–5532.

https://doi.org/10.1073/pnas.95.10.5527
Yin, H., Price, F., & Rudnicki, M. A. (2013). Satellite cells and the muscle stem cell niche. *Physiological Reviews*, *93*(1), 23–67. https://doi.org/10.1152/physrev.00043.2011
Yin, L., Lu, L., Lin, X., & Wang, X. (2020). Crucial role of androgen receptor in resistance and endurance trainings-induced muscle hypertrophy through IGF-1/IGF-1R-PI3K/Akt-mTOR pathway. *Nutrition and Metabolism*, *17*(1), 1–10. https://doi.org/10.1186/s12986-020-00446-y
Zaręba, K., & Sidorkiewicz, I. (2017). *CU R R E N T DE VE LOPME N T RECENT HIGHLIGHTS OF RESEARCH ON ANDROGEN RECEPTORS IN WOMEN: Vol. XXI*.

APPENDIX

Appendix A. AR gene expression, AR protein content and SC. Pearson's correlations between baseline mixed fibre Pax7^{+} cells per 100 myofibres and baseline AR protein content via WB and in i) Full group (n=12) ii) male (n=5) iii) female (n=7). Percent change in AR protein content post-damage (n=12) and iv) percent change in type-I fibre Pax7^{+} cells per 100 myofibres post-damage and v) percent change in type-II fibre Pax7^{+} cells per 100 myofibres post-damage. AR mRNA, relative to GAPDH, post-RET in females (n=13) and males (n=12).



Appendix B. AR protein content and CSA post-RET. Pearson's correlations between i) baseline mixed fibre CSA and percent change in AR nuclear content post-RET. Percent change in AR protein content post-RET via WB (n=12) and percent change in ii) mixed fibre CSA, iii) type-I fibre CSA, iv) type-II fibre CSA.