Human *α-actinin-3* genotype association with exercise induced muscle damage and the repeated bout effect

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ABSTRACT

Alpha-actinin-3 (ACTN3) is an integral part of the Z-line of the sarcomere. The *ACTN3* R577X (rs1815739) polymorphism determines the presence or absence of functional ACTN3 which may influence the extent of exercise induced muscle damage. This study aimed to compare the impact of, and recovery from, muscle-damaging eccentric exercise on subjects with or without functional alpha-actinin-3. Seventeen young men (20-33 years), homozygous for the R- (n=9) or X- (n=8) alleles, performed two bouts of stretch–shortening exercise (50 drop jumps) 2 weeks apart. Muscle soreness, plasma creatine kinase (CK) activity, jump height, maximal voluntary isometric torque (MVC), peak concentric isokinetic torque (IT), and electrically stimulated knee extension torques at 20 Hz and at 100 Hz were measured at baseline and a number of timepoints up to 14 days after each bout. There were no significant baseline differences between the groups. However, significant timepoint*genotype interactions were observed for MVC (p=0.021) and IT (p=0.011) for the immediate effect of eccentric exercise in bout 1. The RR group showed greater voluntary force decrements (RR v XX, MVC: -33.3% v -24.5%, IT: -35.9% v -23.2%) and slower recovery. A repeated bout effect was clearly observed but there were no differences by genotype group. *ACTN3* genotype modulates the response of muscle function to plyometric jumping exercise, although the differences are modest. *ACTN3* genotype does not influence the clearly observed repeated bout effect; however, XX homozygotes recover baseline voluntary torque values faster and thus may be able to undertake more frequent training sesssions.

Keywords: *Alpha-actinin-3*; R577X genoptye; eccentric exercise; muscle damage; repeated bout effect; elite athletes.

INTRODUCTION

Muscles are used to both drive movement (concentric contractions) and to act as breaks to movement (eccentric contractions). Vigorous exercise of any description can result in muscle damage (Clarkson et al. 1986); however, the most severe muscle damage is caused by unaccustomed eccentric exercise (Newham et al. 1983), that is when contracting muscles are actively lengthening. Such eccentric contractions are common during sport and are of importance because the ensuing muscle damage has been associated with muscle weakness, strains and injury (Proske et al. 2004). Eccentric contractions rarely occur in isolation in natural movements and are typically followed by a concentric action in what is known as the stretch–shortening cycle (Byrne et al. 2004). This has also been shown to elicit muscle damage, can result in severe stiffness and soreness of muscles, sarcomeric disruption and a reduction in peak isometric force for several days after an exercise bout (Byrne et al. 2004). Understanding the underlying contributors to this phenomenon will help to understand muscle function and have implications for the design of exercise programmes.

Interestingly, a second bout of eccentric exercise within days or weeks of the initial muscle damaging bout elicits markedly reduced symptoms of muscle damage compared with those experienced after the initial bout (Clarkson et al. 1992). This phenomenon, known as the repeated bout effect, is observed in both humans and animals (Mchugh 2003) although the underlying mechanisms remain to be fully elucidated. Neural adaptation to the initial exercise bout may be involved (Warren et al. 2000), however, the repeated bout effect has been demonstrated with electrically stimulated contractions in human elbow flexors (Nosaka et al. 2002), indicating that adaptations can occur independently of changes in central activation. Other hypotheses that have been suggested focus primarily on subcellular structural adaptations such as: increased collagen content (Lapier et al. 1995); increased serial sarcomere number (Butterfield et al. 2005; Butterfield & Herzog 2006; Lynn et al. 1998); and, increased desmin content (Barash et al. 2002; Peters et al. 2003).

It has been postulated that the sarcomeric disruption caused by eccentric exercise may drive the reduction in muscle functional capacity associated with eccentric exercise (Proske et al. 2004). Inter-individual differences in the vulnerability of muscle tissue to exercise induced damage may be in part genetically determined by variation in genes such as *alpha-actinin-3* (*ACTN3*). ACTN3 both anchors myofibrillar actin filaments to the Z-line in type II (fast) muscle fibers and is also implicated in metabolic and signalling pathways through its interaction with many types of protein (Lek et al. 2010; Mills et al. 2001). It is of interest here primarily because of its structural role in the sarcomere and a common nonsense polymorphism, R577X (rs1815739), which results in the complete absence of the protein in ~16% of the global human population (North et al. 1999). These XX homozygotes are, however, outwardly healthy due to functional redundancy with Alpha-actinin-2 (ACTN2) (North et al. 1999), although genotype associations with sprinting speed (Alfred et al. 2011; Moran et al. 2007; Yang et al. 2003) and strength (Walsh et al. 2008) show that the redundancy is incomplete.

There are a limited number of conflicting reports in the literature on ACTN3 and eccentric muscle damage (Clarkson et al. 2005; Norman et al. 2009; Vincent et al. 2010); here we aimed to provide a detailed assessment of muscle function before and after two bouts of muscle damaging exercise, two weeks apart, to highlight associations of genotype with differences in muscle damage, or function, and the repeated bout effect. We assessed jump height, a practical measure of muscle function, and both maximal voluntary (MVC, maximal voluntary contraction; and IT, isokinetic peak torque) and involuntary (P20, electrically stimulated knee extension torque at 20 Hz; and P100, electrically stimulated knee extension torque at 100 Hz) contractions to allow differentiation between maximal and submaximal stimulation of muscles. Muscle strength loss has previously been shown to be a more reliable indicator of muscle damage than muscle soreness or increases in plasma protein activity (Warren et al. 1999). We also assessed changes in both perceived extent of muscle damage (muscle soreness) and biochemical markers (plasma CK activity).

If R577X polymorphism influences the response to initial and / or repeated bouts of stretch-shortening exercise, its effects may be tested with the following hypotheses: firstly, due to ACTN3's structural role, that XX homozygotes may show an increased vulnerability to muscle damaging exercise in one or both bouts; secondly, due to its involvement in intracellular signalling, that the time over which indicators of such damage persist may be altered in the XX group in one or both bouts; and finally, that any changes between the repeated bouts in the extent or duration of damage, may be altered in the XX group. This would provide evidence that *ACTN3* genotype could account for inter-individual differences in the plasticity of skeletal muscles.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Lithuanian State Ethics Committee and is consistent with the principles outlined in the Declaration of Helsinki. Each subject read and signed a written informed consent form prior to participation.

Subjects

Eighteen healthy young men were recruited from a larger ongoing association study based on their *ACTN3* R577X genotype. This allowed selection of genotype groups of equal size (n=9). Only subjects homozygous for the R-allele (*i.e.* with a complete complement of ACTN3), or subjects homozygous for the X-allele (*i.e.* complete absence of ACTN3) were selected to take part. This was intended to provide the largest power to detect differences between genotype groups. One subject dropped out before completion of the study and was removed from all analyses. Subjects were moderately physically active (< 2 h/wk) but did not take part in any regular physical exercise program. They were asked to maintain their normal routine and refrain from any strenuous exercise for one month prior to, and throughout the duration of, the study. Subjects with any existing medical condition or taking medication that could affect natural muscle mass or function were excluded from the study. Subjects were also asked to refrain from taking any pain medication during the trial.

Genotyping

DNA was extracted from blood samples using the NucleoSpin® Blood kit (Macherey-Nagel, GmbH & Co KG, Düren, Germany) according to the manufacturer‘s protocol. *ACTN3* R577X genotype was determined using a PCR-RFLP method described in detail in the supplementary section of (Moran et al. 2007). Briefly, the primer sequences were 5’-CTGGGCTGGAAGACAGGAG-3' (forward) and 5’-AGGGTGATGTAGGGATTGGTG-3' (reverse). The 290 bp amplification product was digested with *DdeI* restriction enzyme (New England Biolabs UK Ltd., Hitchin, Hertfordshire, UK) and separated on a 2% agarose gel. RR homozygotes remained uncut; XX homozygotes had a single *DdeI* site and migrated as 192 bp and 98 bp bands. Subjects with RR or XX genotypes were recruited into the study.

Familiarisation and Warm Ups

A familiarisation session was performed one week before the initial drop jump bout (Table 1). Subjects were seated in the isokinetic dynamometer chair and asked to maximally activate their knee extensor muscles, and then to perform five submaximal drop jumps. Their psychological tolerance to electrical stimulation was assessed. All participants showed good compliance with the procedure.

A wam-up preceded all test sessions. It comprised 6–8 min of stationary cycling with the power set so that the Watts were approximately equal to the subject's body weight (kg), followed by some light stretching exercises.

Muscle Damaging Exercise

Two drop jump bouts, separated by two weeks, were performed (Table 1). Fifty drop jumps (with arms akimbo) were performed in each bout. Each drop jump comprised a jump from a 40 cm elevation to a squat of 90° at the knee joints, followed immediately by a maximal counter-movement vertical jump. A rest period of 20 s between jumps was chosen to minimise changes in energy metabolites that could affect muscle function (Vollestad et al. 1988). This protocol has previously been shown to cause measurable levels of muscle damage (Miyama & Nosaka 2007).

Muscle Functional Assessments

Muscle contractile properties were recorded before and immediately after (within 2–5 minutes) each drop jump bout then 2, 7, and 14 days into the recovery (Table 1) in the following order: P20, P100, MVC, IT, and five maximal drop jumps.

Since maximal effort was used to jump vertically after each of the drop jumps and this was performed on a contact mat (Newtest Powertimer Testing System, Oulu, Finland), the height of this jump (cm) was calculated with the formula: jump height = 122.625 × $(T_f)^2$, where T_f = flight time (s)

(Bosco et al. 1982). To obtain a robust estimate of jump height, the average height of five consecutive jumps was used for further analysis.

Measurements of voluntary and elecitrically stimulated torque were made on an isokinetic dynamometer (System 3; Biodex Medical Systems, Shirley, New York) calibrated according to the manufacturer’s service manual with a correction for gravity performed using the Biodex Advantage program (version 3.0). There was a 2 minute rest period between the electrically stimulated and voluntary contractions. The coefficient of variation for involuntary and voluntary torques over repeated tests was <5%.

MVC was measured at 120° knee angle (where 180° is full knee extension). Subjects were instructed to achieve and maintain maximal efforts of knee extension for 2–3 s. Each trace was visually inspected to ensure that there were no artefactual spikes at the start of the signal curve. Concentric IT of the knee extensors was measured at 30°s^{-1} (range of motion from 75° to 160°). IT at 30°s^{-1} produces similar results to MVC, but allows for differences in optimal force production angle (Kannus & Beynnon 1993; Skurvydas et al. 2010). Briefly, subjects were asked to extend their knee with maximal effort through the full range of motion, then relax and let their leg return to a neutral position (~90° knee angle). Arms were crossed on the chest (hands grasping the trunk supporting belts) during all tests on the dynamometer. To help ensure a maximal effort, standard vocal encouragement was provided during each jump and voluntary knee extension trial by the same experienced investigator. The subjects were asked to perform three attempts of each mode with a rest interval of 1 min. Only the best attempt was recorded. The highest peak torque attained in each mode and the knee angle at which peak torque was attained were recorded.

The equipment and procedure for electrically stimulated torque have been described previously (Skurvydas et al. 2010). Briefly, muscle stimulation was applied using flexible surface electrodes (MARP Electronic, Krakow, Poland) with one electrode (6 × 20 cm) placed transversely across the width of the proximal portion of the *quadriceps femoris* next to the inguinal ligament and the other electrode (6 × 11 cm) covering the distal portion of the muscle above the patella. A constant current electrical stimulator (MG 440; Medicor, Budapest, Hungary) was used to deliver 1 ms square-wave pulses at 150 V. Peak torques induced by a 1 s electrical stimulation at 20 Hz (P20; representing the steep section of the force-frequency relationship curve) and 100 Hz (P100; which is close to maximal force) were measured with the knee at 120° with a 3 s rest interval between electrical stimulations. Previous studies in humans have shown that eccentric contractions tend to affect muscle force more at low frequency (*e.g.* 20 Hz) than at high frequency (*e.g.* 100 Hz) stimulation and 20 and 100 Hz have previously been used in this context by others (Jones 1996). The change in the P20 to P100 ratio was used as a proxy for low-frequency fatigue (Jones 1996; Ratkevicius et al. 1998; Skurvydas et al. 2011).

Muscle Soreness and Plasma CK Assessments

Muscle soreness was reported subjectively daily throughout the study using an ordinal scale of 0-10, where 0 represented “no pain” and 10 represented “intolerably intense pain” (Ratkevicius et al. 1998). Participants rated the severity of soreness in their quadriceps when performing 2–3 squats just before the bout 1, and then daily at the same time of day, starting 24 h after the drop jump bout. Percentages for muscle soreness could not be calculated as all baseline values were zero. Therefore, raw muscle soreness data were used for analyses.

Samples of venous blood were collected, immediately centrifuged and analysed for plasma CK activity ($\mu kat \cdot L^{-1}$) using a Spotchem™ EZ SP-4430 biochemical analyser (Menarini Diagnostics, Reading, UK) with soft reagent strips (ArkRay Factory Inc., Shiga, Japan). Samples for CK activity were collected at baseline and 48 h, 1 week and 2 weeks after each exercise bout.

Statistical Analysis

Initial baseline values, for all phenoptyes, were normally distributed by the Ryan-Joiner test (Minitab 16.1.0). Baseline differences were assessed using unpaired t-tests. For repeated measures analyses, data (except for muscle soreness) were expressed as a percentage of bout-specific baseline values to account for baseline and bout variation. Prior to analyses, data were tested for normaility using the Ryan-Joiner test (Minitab version 16.1.0). For non-normal data, the best transformation was identified using a Box-Cox analysis and data were transformed to give a better approximation of the normal distribution. Means and 95% confidence intervals reported in Table 3, Figure 1 and Supplementary Table 1 are back-transformed onto the original scale for ease of interpretation. Further statistical analyses were carried out using SPSS 19. Data were analysed using general linear model repeated measures ANOVA with appropriate Greenhouse-Geisser corrections for sphericity as required. For muscle soreness, a Huynh-Feldt correction was applied for the analysis as muscle soreness was ordinal data (Stiger et al. 1998). Based on the average correlation between repeated measures of 0.310, we had 80% power to detect an effect size of 0.347 (G*Power v3.1.2). Where a significant effect was found, *posthoc* tests were performed using LSD tests. The level of significance was set at 0.05.

RESULTS

Baseline Values

There were no significant differences between genotype groups for the baseline values in any of the indices measured (Table 2). Nor were there any differences in age, height, body mass or body mass index (BMI) that could have confounded the data.

Immediate Response to Stretch-Shortening Exercise (Bout 1)

As predicted, the stretch-shortening exercise protocol produced measurable levels of muscle damage immediately after the initial exercise bout (Table 3). This was evidenced by a significant reduction in muscle function immediately after bout 1 whether measured by voluntary contractions (MVC and IT, $p<0.001$ for both; Figures 1A and 1B respectively) or involuntary contractions (P20 and P100, $p<0.001$ for both; Figure 1D-F). Significant differences in genotype response were detected for MVC and IT only (timepoint * genotype interaction terms respectively $p=0.021$ and $p=0.011$) with greater reductions in force in the RR group (Figures 1A and 1B respectively). The angle at which isokinetic peak torque was achieved was not significantly altered immediately after exercise (Figure 1C). The P20 / P100 ratio was reduced by exercise similarly in both genotype groups (Figure 1D-F) indicating that the exercise induced low frequency fatigue of similar magnitude in both genotype groups.

The immediate reductions in muscle function were followed by significant increases in muscle soreness ($p=0.001$; Figure 1G) and plasma CK activity ($p<0.001$; Figure 1H), and a significant decrease in jump height ($p=0.001$; Figure 1I). None of the responses differed by genotype in repeated measures ANOVA.

Timecourse of Stretch-Shortening Exercise Effects (Bout 1)

Peak torque of voluntary and involuntary contractions as well as the P20 / P100 ratio remained significantly lower than baseline for the duration of the post-exercise bout 1 period ($p<=0.003$ for all timepoints; Supplementary Table 1; Figure 1D-F). Muscle soreness ($p<0.001$), CK activity (p<0.001) and jump height (p=0.001) remained significantly different from baseline at 2 days but returned to baseline values by 7 days post exercise (Supplementary Tables 1 and 2; Figures G-I). The angle at which isokinetic peak torque (p=0.013) was achieved was significantly altered only 14 days after exercise (Figure 1C).

The jump height, IT and P100 of the XX group returned to baseline values quicker than the RR values (Supplementary Table 1; Figures 1B, 1D-F and 1I): jump height (p=0.021) and P100 (p=0.003) of the RR group were significantly lower than baseline at day 7 when XX's had returned to baseline values (p=0.718 and p=0.136 respectively). However, the SD of the XX group for P100 at day 7 was notably higher than the other days and this may have influenced this result. The IT of the RR group was significantly reduced from baseline at both days 7 (p=0.001) and 14 (p=0.001) when XX's had returned to baseline values (p=0.148 and p=0.199 respectively). The P20 / P100 ratio was significantly lower than baseline in the XX group (p=0.010) at day 14 when the RR group (p=0.104) had returned to baseline values.

For MVC, P20 and muscle soreness both genotype groups behaved similarly at all timepoints after bout 1 (Supplementary Tables 1 and 2; Figures 1A and 1D-F): MVC and P20 remained significantly reduced in both groups at all timepionts (p<=0.013 for all timepoints in both groups). Muscle soreness was significantly higher than baseline in both genotype groups at 2 days (RR, p<0.001 and XX, p=0.001) but returned to baseline values by 7 days.

Repeated Bout Effect (Bout 2)

The repeated bout effect is defined as the phenomenon whereby a second bout of muscle damaging exercise results in less damage than the intial bout (Clarkson et al. 1992). As expected, this effect can be seen clearly in the data: reductions in jump height, MVC, IT, P20 and P100 and increases in muscle soreness and CK activity are either significantly reduced or absent after the second bout of exercise (Supplementary Tables 1 and 2; Figures 1A-I) and significant interaction effects for bout * timepoint can be seen for all of these phenoptyes. Similar effects can be seen when considering immediate effects of exercise or effects over the entire 2 week timecourse (Supplementary Tables 1 and 2; Figures 1A-I). None of these effects differed significantly by genotype.

DISCUSSION

There are two important findings from this study. Firstly, differences between *ACTN3* genotype groups are modest and most easily detected in maximal contractions immediately after exercise, with XX homozygotes appearing to retain a higher proportion of their baseline voluntary torque values after stretch shortening exercise. Secondly, XX homozygotes also appear to return to baseline voluntary torque values faster than RR homozygotes. However, there were no baseline differences between the genotype groups or differences in their response to the repeated bout of stretch shortening exercise.

Muscle damage and *ACTN3* genotype

The prolonged decrease in muscle force-generating capacity and increased muscle soreness are consistent with classical muscle damage profiles observed after unaccustomed eccentric exercise (Byrne et al. 2004). Immediately post-exercise, RR homozygotes had a greater proportional loss of voluntary contraction torques (MVC and IT) and these reductions took longer to return to baseline levels. These findings are counter-intuitive and in conflict with our original hypothesis, however, it should be remembered that much of ACTN3's function is redundant with ACTN2 and differences between the ACTN3 genotype groups are perhaps better thought of as differences between ACTN2 and ACTN3 function in fast muscle fibres. Nonetheless, a mechanism that would explain why homozygosity for the X-allele (*i.e.* absence of ACTN3) confers better proportional maintenance of muscle function after stretch-shortening exercise is unknown.

No similar genotype differences were observed in the absolute values or dynamics of the indirect markers of muscle damage, such as jumping performance or muscle soreness score suggesting a minor role of alpha-actinin-3 in muscle resistance to exercise induced damage, as reported by others (Clarkson et al. 2005; McCauley et al. 2009). The lack of differences between genotypes in involuntary muscular tension in our study could be due to stimulation only evoking submaximal torques in relation to MVC: the observed tension was only about 50% and 63% of MVC during electrical stimulation at 20 Hz and 100 Hz, respectively, in both groups. These are common values in such types of study, but such stimulation may not be enough to stress the muscle or type II fibers sufficiently to reveal the difference in intrinsic inotropic state.

In a cross-sectional study of the general U.S. population, female XX homozygotes have previously been shown to have reduced baseline knee extensor peak torque (Walsh et al. 2008). Similarly, the *actn3* knock out mouse model has reduced grip strength (MacArthur et al. 2008). It is thus tempting to speculate that XX homozygotes have higher levels of underlying muscle sarcomere disruption produced by normal daily living and that the extent to which they can increase muscle damage and impair muscle function further may be limited. Concomitantly, a limited increase in disruption would allow a return to baseline levels to occur more quickly. Studies involving much larger numbers of individuals and highly detailed phenotyping would be required to address this possibility.

Recently, differential binding of Z-disk proteins by ACTN3 and ACTN2 has been demonstrated in yeast two-hybrid experiments, potentially altering the elastic properties of the sarcomere (Seto et al. 2011). A desmin-knockout mouse model also provides evidence of reduced vulnerability to muscle damage (Sam et al. 2000). In R-allele carriers (*i.e.* with alpha-actinin-3 present), the type II muscle fibers and whole skeletal muscle may generate slightly altered force transduction along the myofibrils during cross-bridge cycling, provided that the integrity of the sarcomeres are maintained. Thus alpha-actinin-3 itself may favor more profound Z-line streaming during intense muscular exertion because of stiffer (compared to XX) thin filament binding. This may explain our observation of a more pronounced decline in voluntary force production capacity of RR homozygotes after a bout of drop jumps; RR homozygotes may be more efficient at the expense of more depressed muscle function and prolonged recovery.

Vincent *et al* (2010) found a tendency for more pronounced reduction in dynamic concentric peak torque at high movement speed (200–300°s^{-1} change in knee angle) in an XX group after a single bout of muscle-damaging exercise. However, such high muscle contraction speeds generate submaximal tension and force production in type II muscle fibers which may account for the differences observed. They also did not find differences between genotypes in isometric or slow eccentric peak torques or, in contrast to the present study, in the extent of their decrease after muscle-damaging exercise. These discrepancies could be due to the different protocols used to induce muscle damage and the different assessments of muscle function.

The repeated bout effect

A protective effect of the initial exercise bout was evidenced by quicker recovery of skeletal muscle contractile function, less muscle soreness, and lower plasma CK activity after the repeated bout. Sarcoskeletal alpha-actinins (2 and 3) interact with a diverse range of proteins (Lek et al. 2010) and these interactions may affect the changes in type II muscle fibers after intense or damaging exercise. However, the improvement did not differ between genotype groups. On the other hand, it could be argued that the most important difference was in the length of the recovery period and that "trainability" may be modulated by *ACTN3* genotype through the faster recovery of muscle force in XX individuals after bout 1. This would allow XX homozygotes to train more frequently, benefiting their long-term adaptation. However, this is a speculative suggestion and would require confirmation in a larger study.

To understand the R577X variant, it is important to understand why the X-allele remains so common in the human population (Mills et al. 2001) and why both the R- and X-alleles appear to have persisted over such a long evolutionary period (Mills et al. 2001). Although some studies report an X-allele association with endurance phenotypes, most do not (Alfred et al. 2011) leaving 577X with no apparent advantage. In this scenario we would expect removal of the 577X allele from the population and fixation of 577R, which clearly has not occured. From the available data, it may be that the R-allele confers an advantage in concentric exercise, improving strength performance, whilst the X-allele protects against relative loss of function in the days following a bout of exercise involving eccentric contractions.

In conclusion, homozygosity for the ACTN3 577X allele may reduce the relative functional loss and provide a faster recovery of functionality to normal levels in response to unaccustomed plyometric exercise. However, the difference between genotypes is modest and the mechanism by which the difference is mediated remains elusive. Future studies in this area must involve significantly larger groups of subjects to ensure sufficient power to investigate what appear to be subtle differences.

Acknowledgements

The authors wish to thank all volunteers for their participation in this study. This work was funded by a grant from the Lithuanian Academy of Physical Education.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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TABLES

Table 1. Study testing protocol

		Day 0					Day 14					
	Day -7	baseline	exercise	post-ex	Day 2	Day 7	pre-ex	exercise	post-ex	Day 16	Day 21	Day 28
Familiarisation session	•											
Stretch shortening exercise bout			•					•				
Muscle soreness evaluation			*Measured daily throughout the study*									
Plasma sample for CK activity		•			•	•	•			•	•	•
Muscle functional assessments [1]		•		•	•	•	•		•	•	•	•

[1] Jump height, maximal voluntary isometric torque, peak concentric isokinetic torque and electrically stimulated knee extension torques at 20 Hz and at 100 Hz.

Table 2. Baseline values of anthopometric measurements, muscle damage and muscle function indices.

	RR (*n=9*)	*XX* (*n=8*)	p-value (unpaired t-test)
Age (years)	25.1 ± 1.5	25.8 ± 1.2	0.749
Height (m)	180.9 ± 1.9	181.7 ± 2.5	0.793
Body Mass (kg)	77.4 ± 2.4	79.8 ± 4.7	0.649
BMI ($kg·m^{-2}$)	23.7 ± 0.9	24.1 ± 1.0	0.797
Muscle soreness	0 ± 0	0 ± 0	NA
CK activity ($µkat·L^{-1}$)	2.9 ± 0.7	3.1 ± 0.8	0.897
Jump height (cm)	40.3 ± 2.5	37.8 ± 2.4	0.486
MVC (Nm)	292.1 ± 23.9	284.2 ± 19.2	0.802
IT (Nm)	252.0 ± 17.5	238.3 ± 11.4	0.532
ITA (degrees)	126.0 ± 2.6	121.9 ± 3.5	0.348
P20 (Nm)	146.5 ± 10.0	136.5 ± 10.5	0.500
P100 (Nm)	188.5 ± 15.2	171.3 ± 14.3	0.425
P20 / P100	0.79 ± 0.029	0.81 ± 0.033	0.658

Values given are mean ± standard error of the mean. BMI, body mass index; CK, plasma creatine kinase; MVC, maximal voluntary contraction torque; IT, peak isokinetic torque; ITA, angle at which IT was acheived; P20, P100, torques evoked by 20 Hz and 100 Hz electrical stimulation.

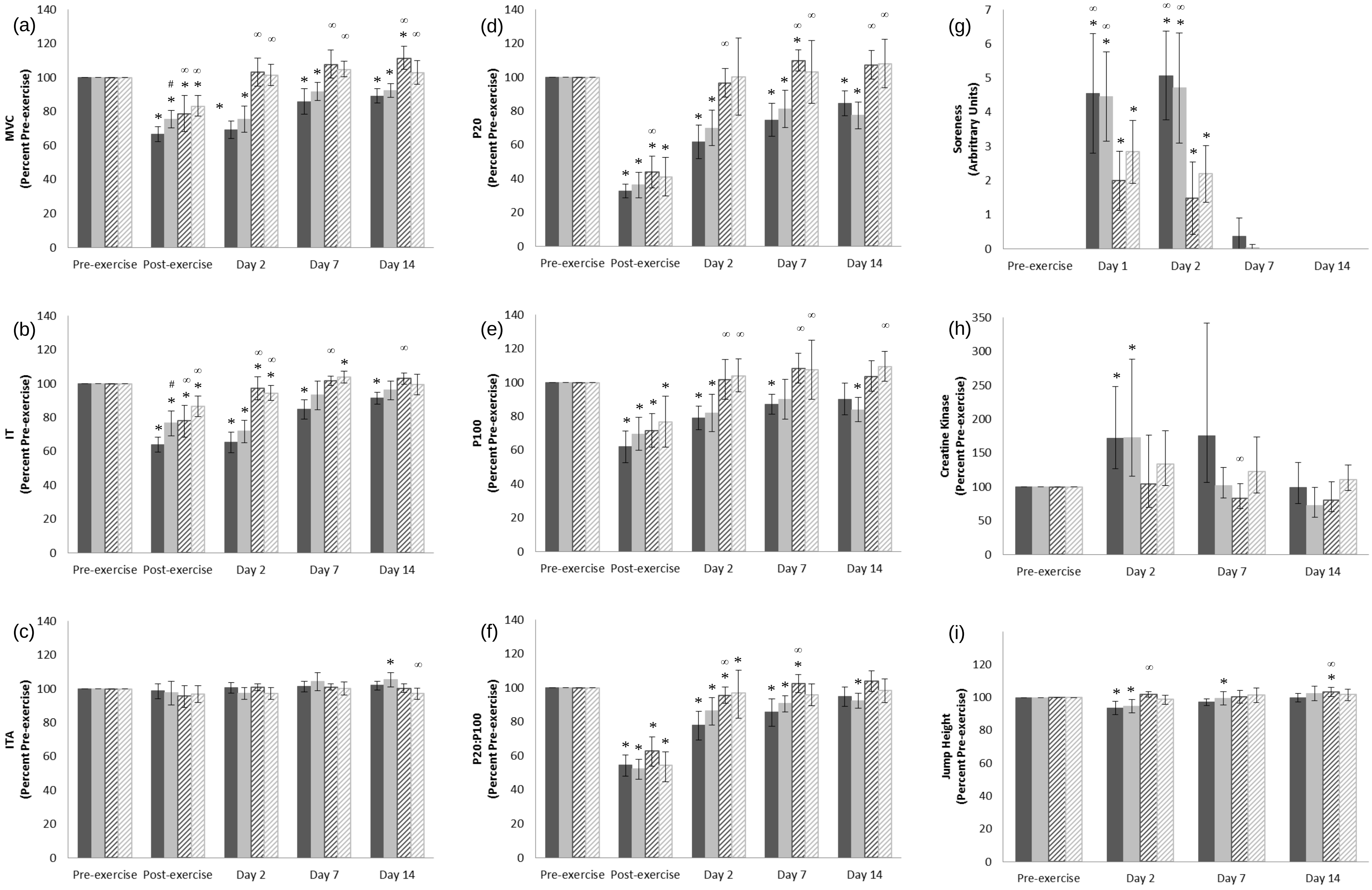
Table 3. Immediate bout 1 exercise induced changes in muscle damage and muscle function indices for both *ACTN3* genotype groups.

	Combined Group (n=17)	*p-value*[a]	*RR Group (n=9)*	*p-value*[b]	*XX Group (n=8)*	*p-value*[c]	*p-value*[d]
Muscle soreness	4.5 (3.4 to 5.6)	***<0.001***	4.5 (2.8 to 6.3)	***<0.001***	4.5 (3.1 to 5.8)	***<0.001***	0.938
CK activity†	72.5 (35.1 to 127.9)	***<0.001***	72.1 (26.4 to 147.7)	***0.008***	73.1 (15.3 to 188.4)	***0.040***	0.983
Jump height	-6.0 (-8.8 to -3.3)	***0.001***	-6.6 (-10.6 to -2.6)	***0.012***	-5.5 (-9.6 to -1.4)	***0.035***	0.712
MVC	-29.1 (-33.0 to -25.2)	***<0.001***	-33.3 (-37.6 to -29.0)	***<0.001***	-24.5 (-29.7 to -19.2)	***<0.001***	***0.021***
IT	-29.7 (-35.1 to -24.6)	***<0.001***	-35.9 (-40.4 to -31.8)	***<0.001***	-23.2 (-31.0 to -16.1)	***0.001***	***0.011***
ITA	-1.7 (-5.8 to 2.1)	0.398	-1.2 (-5.9 to 3.0)	0.595	-2.2 (-9.8 to 4.3)	0.544	0.813
P20	-65.7 (-69.8 to -61.6)	***<0.001***	-67.4 (-71.5 to -63.3)	***<0.001***	-63.8 (-71.4 to -56.2)	***<0.001***	0.408
P100	-34.4 (-41.2 to -27.6)	***<0.001***	-38.0 (-47.3 to -28.6)	***<0.001***	-30.5 (-40.3 to -20.6)	***0.001***	0.298
P20 / P100	-46.5 (-50.9 to -42.4)	***<0.001***	-45.4 (-52.1 to -39.5)	***<0.001***	-47.7 (-53.8 to -42.3)	***<0.001***	0.608

All values are mean change (95% confidence interval) from baseline to immediately post-exercise change as a percentage of baseline value except, CK activity and jump height which are baseline to 48 hour change and muscle soreness which is absolute change from baseline to 24 hours. CK, plasma creatine kinase; MVC, maximal voluntary contraction torque; IT, peak isokinetic torque; ITA, angle at which IT was acheived; P20, P100, torques evoked by 20 Hz and 100 Hz electrical stimulation. † N-values for RR and XX are 8 and 6 respectively for CK only. Post-hoc tests are t-tests, other p-values are from repeated measures ANOVAs: [a] exercise term, [b] Post-hoc exercise term for RR group only, [c] Post-hoc exercise term for XX group only, [d] Interaction between genotype group and exercise term.

FIGURES AND LEGENDS

Fig. 1 (a) Percentage change in MVC, (b) percentage change in IT, (c) percentage change in ITA, (d) percentage change in P20, (e) percentage change in P100, (f) percentage change in P20 / P100, (g) absolute change in muscle soreness, (h) percentage change in plasma CK activity, (i) percentage change in jump height, post-exercise bout 1 (solid fill; RR ■, and XX ■) and bout 2 (diagonal pattern fill; RR ▨ , XX ▨) in both ACTN3 genotype groups. Error bars are SEM. Post-hoc tests are t-tests, other p-values are from repeated measures ANOVAs: * $p<0.05$ post hoc compared with pre-exercise (paired t-test); $^{\infty}$ $p<0.05$ post hoc compared with equivalent timepoint and group in bout 1 (paired t-test); $^{\#}$ $p<0.05$ post hoc compared with RR at same timepoint and bout (unpaired t-test).


(a)
MVC (Percent Pre-exercise)
Pre-exercise
Post-exercise
Day 2
Day 7
Day 14
(b)
IT (Percent Pre-exercise)
(c)
ITA (Percent Pre-exercise)
(d)
P20 (Percent Pre-exercise)
(e)
P100 (Percent Pre-exercise)
(f)
P20:P100 (Percent Pre-exercise)
(g)
Soreness (Arbitrary Units)
Day 1
(h)
Creatine Kinase (Percent Pre-exercise)
(i)
Jump Height (Percent Pre-exercise)