Relationship between pre-exercise muscle stiffness and muscle damage induced by eccentric exercise

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Abstract

This study aimed to determine whether pre-exercise muscle stiffness is related to the amount of muscle damage induced by an eccentric exercise and to determine whether the post-exercise increase in stiffness is homogenously distributed between the synergist muscles. Fifty healthy participants were randomly assigned to an eccentric exercise group or a control group. The shear modulus (an index of stiffness) of rectus femoris (RF), vastus lateralis (VL) and vastus medialis oblique (VMO) was measured before, immediately after and at 48 hours after eccentric exercise. The maximal isometric voluntary knee extension (MVC) torque was also measured. Significant reduction in MVC torque was observed in the eccentric group both at post and 48 H when compared with pre-exercise (both $p < 0.001$). RF shear modulus increased significantly when assessed at 90° of knee flexion at post and 48 H after the eccentric exercise ($p = 0.004$ and 0.005, respectively). Slight but significant decrease in VL shear modulus was observed at post exercise for the eccentric group ($p = 0.002$). No change was observed in VMO. The decrease in MVC at 48 H was negatively correlated with the RF shear modulus measured at 90° of knee flexion before the exercise. Eccentric exercise induced a wide range of peak torque reduction and muscle-head specific modulation on muscle stiffness. Participants with stiffer RF muscles exhibited greater decrease in force-generating capacity at 48 H after eccentric exercise.

Key words: *Elastography, muscle tension, muscle damage, quadriceps femoris, ultrasound*

1. Introduction

During eccentric contraction, the muscle is forcibly lengthened leading to damage of its contractile and cytoskeletal constituents (LaStayo et al., 2003). Reduced maximal voluntary contraction (MVC) torque, delayed onset muscle soreness and increase in passive muscle stiffness are commonly used as non-invasive markers of muscle damage (Proske & Allen, 2005). Among them, the decrease in MVC is considered as one of the best indirect indicators of muscle damage in human (Paulsen, Mikkelsen, Raastad, & Peake, 2012). The magnitude of force reduction, however, is highly variable among individuals even when exposed to the same exercise protocol (Clarkson et al., 2005; Nosaka & Newton, 2002). The underlying mechanisms for these individual differences in strength loss are unclear. Muscle extensibility has been proposed as one of the factors that affect the force reduction associated with eccentric exercise (Chen et al., 2011; McHugh et al., 1999).

McHugh et al. (1999) first reported significantly less symptoms of exercise-induced force reduction in participants with more compliant hamstrings than those with stiffer hamstrings. In the same way, Chen et al. (2011) reported less eccentric exercise-induced force reduction after an 8-week flexibility training program on knee flexors. Further, the maximal range of motion of the hip joint (used as an index of flexibility of knee flexors) was negatively correlated with markers of muscle damage such as decrease in peak torque and plasma creatine kinase activity (Chen et al., 2011). However, the range of hip flexion is influenced by numerous factors including the stiffness of the muscle-tendon complex as well as the mobility of the hip joint making it difficult to draw a direct relationship between pre-exercise muscle stiffness and the amount of muscle damage. A more direct approach to assess muscle stiffness is needed.

Elastography can be used to estimate the shear modulus of a localized muscle region (Bercoff, Tanter, & Fink, 2004; Bouillard, Hug, Guevel, & Nordez, 2012; Lacourpaille et al., 2014). The shear modulus measured using ultrasound shear wave elastography is strongly linearly related to the Young's modulus (Eby et al., 2013). Using this technique, an increase in passive stiffness was observed after an eccentric exercise (Guilhem et al., 2016; Lacourpaille et al., 2012). The magnitude of this increased stiffness depended on muscle length, i.e., longer the muscle, larger the increase. Using magnetic resonance elastography, Green et al. (2012) observed a muscle-dependent change in stiffness after a bout of eccentric exercise. Specially, an increased stiffness was observed in the biarticular gastrocnemius muscle but not in the monoarticular soleus muscle. A recent study from Maeo et al. (2018) also reported a significant increase in muscle stiffness in the biarticular rectus femoris (RF) muscle but not in the two monoarticular vastus medialis (VM) and lateralis (VL) muscles during single-joint eccentric contraction of the knee extensors.

Considering the fundamental role of the quadriceps muscle group for daily and sports activities, we were interested in investigating whether pre-muscle stiffness of the quadriceps femoris would affect muscle damage induced by eccentric exercise. Specifically, the aim of this study was twofold: 1) to determine whether pre-exercise muscle stiffness is related to the force loss observed after a bout of eccentric knee extensions and 2) to determine whether the post-exercise increase in stiffness is homogenously distributed between the heads of the quadriceps.

2. Methods

2.1. Participants

Fifty-two healthy participants without any specific muscle training were recruited. None of the participants had known musculoskeletal disorder or leg injuries. Two participants from the control group did not complete the experiment and data are therefore reported for 50 participants. Participants were randomly assigned to either an eccentric group (n = 26 [15 males], 23.8 ± 3.3 years old, 58.6 ± 8.1 kg, 166.0 ± 9.2 cm) or a control group (n = 24, [12 males], 23.5 ± 3.4 years old, 56.1 ± 6.6 kg, 166.5 ± 8.0 cm) by drawing cards. There was no significant difference in age, body mass, and height between groups. However, BMI was significantly higher in the eccentric group (21.4 ± 1.6 $kg \cdot m^{-2}$) than the control group (20.2 ± 1.2 $kg \cdot m^{-2}$; $p = 0.014$). This study received ethical approval from the Human Subject Ethics Subcommittee of the Polytechnic University of Hong Kong, and all procedures adhered to the declaration of Helsinki. Participants provided informed written consent.

2.2.Study design

The study procedure is depicted in Figure 1. Resting muscle stiffness and peak torque achieved during maximal isometric knee extensions were measured before (pre), immediately after (post) and 48 hours (48 H) after the intervention (eccentric or control according to the group allocation). The order of the measurements was kept the same between the different time points. The intensity of muscle soreness was recorded at 48 H. Reduction in MVC torque and muscle soreness were used as indirect markers of the amount of eccentric exercise-induced muscle damage. Resting muscle stiffness was assessed using ultrasound shear wave elastography at different knee angles.

2.3. Maximal isometric knee extension force assessment

Participants sat in an isokinetic dynamometer (Cybex, Medway, Massachusetts, USA) with their hip was set at 85° (with supine position = 0°). The knee of the dominant leg was flexed at 60° (0°: knee fully extended). Participants first performed five sub-maximal isometric contractions as a warm-up. These contractions were followed by three maximal isometric contractions for 5 s (2 min rest in-between). The contraction with the highest peak torque was further considered. Peak torque $index_{post}$ and peak torque $index_{48}$ were expressed as percentage of pre-exercise torque.

2.4. Muscle stiffness measurement

Muscle shear modulus (an index of stiffness) of the dominant leg was measured by a trained examiner in muscle ultrasound examination who was blinded to the group allocation. An Aixplorer ultrasound scanner coupled with a 4-15 MHz linear transducer array (Aixplorer V4; Supersonic Imagine, Aix-en-Provence, France) was used in the shear wave elastography mode (general preset). Two-dimensional maps of shear modulus, with 1×1 mm spatial resolution, were obtained at 1 sample·s^{-1}.

During the image acquisition, the participants were positioned supine on the bed of the Cybex dynamometer with their hip in neutral flexion and rotation. The axis of the knee rotation was aligned with the rotation center of the dynamometer. The testing muscles were marked as follows: 1/5 of the distance from the midpoint of medial patella border to anterior superior iliac spine for VMO, 1/2 of the distance from anterior superior iliac spine to the midpoint of the superior tip of the patella for RF; 1/3 of the distance from the midpoint of lateral patella border to anterior superior iliac spine for VL (Xu, Hug, & Fu, 2016). The transducer was positioned within the direction of the muscle fibers for VL and VMO; and within the direction of the muscle shortening for RF (Bouillard et al., 2012). For VL and VMO, optimal image quality was achieved by slightly moving the transducer until several muscle fascicles could be seen without disconnection through the image (Blazevich, Gill, & Zhou, 2006). Ultrasound measurements lasted 5 s (i.e. 5 measurements at 1 sample·s^{-1}) for each muscle and each knee angle, i.e. 30°, 60° and 90° of knee flexion.

Videos of shear modulus maps were exported in mp4 format and sequenced into png images. Image processing was performed using a custom Matlab script (MathWorks, Natick, MA). First, each image was carefully inspected for artefacts (saturated values) or missing values (unfilled region within the elasticity map). If artefacts or missing values were present in any image, the region of interest (ROI) was reduced in size to exclude the area of artefact and/or missing values. The ROI was defined for each map as the largest muscle area that avoided fascia, bone and hypo-echoic regions. Image processing converted each pixel of the color map into a value of the shear modulus based on the recorded color scale. The shear modulus was averaged over the ROI for each image and the 5 shear modulus values corresponding to the 5 images were then averaged such that one single value was obtained for each measurement.

2.5. Muscle soreness assessment

Muscle soreness was assessed subjectively using a 10-cm visual analogue scale (VAS) anchored with ‘no pain’ at 0 and ‘worst pain imaginable’ at 10. Participants were asked to rate their maximal self-perceived pain while walking downstairs at 48 H after the eccentric exercise (Hafez et al., 2013).

2.6. Interventions

The eccentric group carried out a bout of eccentric contractions. Specifically, they seated on the dynamometer chair with their hip was set at 85° (with supine position = 0°). The range of knee motion was set between 30° to 110° of knee flexion to minimize knee discomfort (Skurvydas et al., 2011). A total of 75 maximal eccentric contractions were performed at an angular velocity of 0.26 $rad \cdot s^{-1}$ (Ballantyne & Shields, 2010; Child, Saxton, & Donnelly, 1998). Between each contraction the leg was passively repositioned at 30°. Each eccentric contraction was separated by a 6 s rest interval. The control group underwent passive movement of the knee joint at a velocity of 0.26 $rad \cdot s^{-1}$ from 30° to 110° for 75 repetitions.

2.7. Statistical analysis

Statistical analyses were performed with 21.0 SPSS software package (New York, USA). Normality testing (Kolmogorov-Smirnov) was used to assess normality of the outcomes. Unpaired-t tests were conducted to assess group differences in terms of age, body mass, height, and body mass index. Chi square analysis was conducted for comparing group difference on gender. A mixed design ANOVA with BMI as covariate was used to determine the effect of eccentric exercise on peak torque (between subject factor: group [eccentric, control]; within-subject factor: time [pre, post, 48 H]). Another mixed design ANOVA with BMI as covariate was used to determine the effect of the eccentric exercise on shear modulus of each muscle (between-subject factor: group [eccentric, control]; within subject factors: time [pre, post, 48 H] and knee angle [30°, 60°, 90°]). When significant interactions were detected, a *post-hoc* analysis was performed using the Bonferroni method. Partial correlation coefficient test was used to test the relationship between pre-exercise muscle shear modulus and peak torque $index_{post}$ or peak torque $index_{48}$ with gender as a factor to control the force difference between males and females. The level of significance was set at $P < 0.05$.

3. Results

We observed a significant group × time interaction ($p < 0.001$) on peak torque. Although there was no significant effect of time for the control group, peak torque decreased significantly at post ($p < 0.001$) and 48 H ($p < 0.001$) compared to pre-exercise for the exercise group (Table 1).

At 48 H, 24 (92%) participants of the eccentric group developed muscle soreness associated with stair descent (mean value = 2.8 ± 1.9). No participant in the control group indicated pain associated with stair descent (Table 1).

Figure 2 depicts shear modulus of the 3 muscles at pre-, post and 48 H post-exercise.

We observed a significant time × angle × group interaction ($p < 0.001$) on the RF shear modulus. Post hoc analysis indicated that when compared to pre-intervention, the RF shear modulus measured at 90° of knee flexion in the eccentric group significantly increased at post (37.3 ± 57.1%, $p = 0.004$) and 48 H (22.4 ± 35.5%, $p = 0.005$). Figure 3 depicts a typical elastography images of RF shear modulus measured at 90° of knee flexion before, immediately after and 48 hours after the eccentric exercise. The RF shear modulus did not change when measured at 30° and 60° of knee flexion ($p = 0.44$ and $p = 0.12$, respectively). No change in RF shear modulus was detected in the control group (all p values > 0.05). For the VL muscle, there was a time × group interaction on shear modulus ($p = 0.001$). Post hoc revealed a slight (< 10%) but significant decrease in VL shear modulus measured at post- compared to pre-intervention for the eccentric group ($p = 0.002$). No significant change of VL shear modulus was detected in the control group (all p values > 0.40). For the VMO muscle, there was neither a significant interaction nor main effects (all p values > 0.05).

When considering the eccentric group, a significant negative correlation was found between the pre-exercise RF shear modulus measured at 90° of knee flexion and the magnitude of torque reduction at 48 hours (r = -0.41, $p = 0.041$). It indicates that the lower pre-exercise RF shear modulus, the less reduction in peak torque induced by eccentric exercise. No correlation was found when considering the other knee angles and times for RF or the other muscles (VMO and VL; all |r| < 0.40 and all p values > 0.08). No significant correlation was found between shear modulus and muscle soreness (all p values > 0.09).

4. Discussion

Significant reduction in peak torque of knee extensors immediately after and at 48 H after exercise was observed in the eccentric group. The RF shear modulus measured at 90° of knee flexion increased immediately after and 48 H after the bout of eccentric exercise. The effect of eccentric exercise on stiffness was muscle-specific. In addition, the lower pre-exercise RF shear modulus, the less reduction in peak torque induced by eccentric exercise.

In the present study, we observed a decrease in peak torque of about 17% and 10% at post- and 48 H after an eccentric exercise, respectively. This is in line with previous studies that report a decrease in force of about 12% at 48 H after a knee eccentric exercise (Maeo et al., 2018). In accordance with previous work (Clarkson et al., 2005; Nosaka & Newton, 2002), we observed a large inter-individual variability in the reduction of peak torque (range: -3% to -33% at 48 H) despite that the participants performed the same eccentric exercise. This variability might be explained by different muscle mechanical properties. For example, the decrease in baseline isometric knee extension torque observed from 70° of knee flexion to 110° has been shown to be correlated with the strength loss observed after a damaging eccentric exercise (McHugh & Pasiakos, 2004). This is in line with the sarcomere strain theory of muscle damage in that muscles with a steeper descending limb on the length tension curve would experience greater sarcomere strain for a given exercise and would subsequently exhibit more myofibrillar disruption. In a similar way, the flexibility of the muscle-tendon unit has been suggested as an important factor that affect the susceptibility to exercise-induced muscle damage (Chen et al., 2011; McHugh et al., 1999). An 8-week static stretching program improved flexibility and attenuated the magnitude of muscle damage induced by a bout of eccentric exercise (Chen et al., 2011). It suggests that there might exist a relationship between tissue extensibility and exercise-induced force reduction.

Post-exercise increase in muscle stiffness has been observed in several muscle group after eccentric exercise, such as elbow flexors (Chleboun, Howell, Conatser, & Giesey, 1998; Howell, Chleboun, & Conatser, 1993; Lacourpaille et al., 2014), knee extensors (Hody et al., 2013), knee flexors (Matsuo et al., 2015) and plantarflexors (Hoang, Herbert, & Gandevia, 2007). Herein, we observed a significant increase in RF shear modulus by about 37% at post and by about 22% at 48 H when measured at 90° of knee flexion. In contrast to what was observed in RF, no increase in stiffness was observed in VMO but a slight decrease in stiffness was observed in the VL. This result is in line with a recent magnetic resonance imaging study, which reported muscle damage mainly localized on RF after eccentric knee extensions while VL was the least affected muscle (Maeo et al., 2018). Similar muscle-specific changes were observed in the triceps surae with increased stiffness of gastrocnemius but not of soleus muscle (Green et al., 2012). Using similar technology (ulltrasound elastography), Lacourpaille et al. (2017) reported a larger increase in stiffness for RF, than both VL and VMO after an eccentric exercise. Taking together, we believe that these results suggests that eccentric exercise-induced modulation on muscle stiffness is muscle-head specific. However, it remains to be demonstrated using direct measures *of muscle damage*.

Several reasons would be associated to the observed localized increase in RF shear modulus. First, because the fast twitch fibres are more susceptible to eccentric exercise induced muscle damage (Douglas, Pearson, Ross, & Mcguigan, 2017), it is possible that the predominance of fast twitch muscle fibers in the RF muscle makes it more prone to damage than the VMO and VL muscles (Friden & Lieber, 1998; Johnson, Polgar, Weightman, & Appleton, 1973). Second, the significant increase of muscle tension and high susceptibility to muscle damage during eccentric contractions in biarticular muscle compared with mono-articular muscle make RF more exposed to muscle damage than VMO and VL (Cross, Gibbs, Houang, & Cameron, 2004; Green et al., 2012). Furthermore, since the stretch effect produced by eccentric contraction may be lower in pennate muscle (Guilhem et al., 2016), the more pennate VMO and VL at measured site are in less risk of muscle damage by eccentric exercise compared with RF (Ema et al., 2013). Finally, since muscles with smaller size are more subject to eccentric contraction-induced muscle damage (Chen et al., 2011), RF，which exhibit the smallest cross-sectional area among the four heads of quadriceps (Arnold, Ward, Lieber, & Delp, 2010), would be more prone to be damaged.

ast, VL, which exhibit the largest cross-sectional area would be more susceptible to eccentric stress (Maeo et al., 2018). The change in VL stiffness was in a different direction than that observed for RF, i.e. a slight (about 10%) but significant decrease.

It should be kept in mind that post-exercise changes in stiffness are likely the cumulative consequences of various mechanisms with possible opposite effects. For example, increased muscle temperature induces a decrease in stiffness (Sapin-de Brosses et al., 2010) and muscle damage induces an increase in stiffness. It is therefore possible that the effect of muscle damage was much higher than the effect of muscle temperature for RF and that the effect of muscle damage was slightly lower than the effect of muscle temperature for VL.

Another essential finding of the present study is that the peak torque index at 48 H was negatively correlated with the pre-exercise RF shear modulus measured at its lengthened position. This result indicates that participants with higher RF stiffness exhibit larger force-loss at 48 H after an eccentric exercise. It suggests that passively stretch RF before exercise would cause a long-lasting attenuation of peak torque loss (Chen et al., 2011). This finding partially agrees with previous study which demonstrated a positive correlation between the pre-flexibility of knee flexors and muscle force 1 day to 5 day post eccentric exercise (Chen et al., 2011). As a lower flexible muscle appears to be stiffer, we speculated that the pre-stiffness of muscle could be one of predictors of decreased muscle force capacity after eccentric exercise. However, no correlation was found between pre-exercise muscle stiffness and immediately post-exercise force reduction in present study. One possible reason could be that immediately after a bout of eccentric contractions, the decrease of muscle force resulted from not only muscle damage, but also muscle fatigue (Faulkner, Brooks, & Opiteck, 1993). At 48 H after exercise, no fatigue exists for the recovery form fatigue completed 3 hours after exercise (Faulkner et al., 1993). Another reason may be the different index was used. The flexibility was measured through straight leg raise range of motion which is not only including the mechanical properties of muscle but hip and knee joint, tendon, fascias while direct measurement of muscle stiffness was made in present study. However, it should be noted that the force loss could only be explained partly by pre-exercise RF stiffness due to the moderate relationship (r = -0.041).

The present study requires the consideration of the following limitations. First, previous studies reported a length dependency of changes in muscle stiffness following eccentric exercise, i.e., longer the muscle, larger the increase in stiffness (Lacourpaille et al., 2017). It is therefore possible between-muscle and between-participant differences in stiffness changes were explained (at least partly) by different relative *muscle* length. Specifically, the absence of VL and VMO increased stiffness might be explained by the shorter relative length at which they were measured. However, regarding previous studies that suggested greater RF damage after eccentric knee extensions (Lacourpaille et al., 2017; Maeo et al., 2018) we believe that our results really reflects greater RF damage. Second, the magnitude of torque loss was relatively small (only 17% immediately after the exercise). If larger muscle damage was induced, the response of VL and VM might have been changed. Further study can be conducted with greater loading on the muscle.

In conclusion, we confirmed that a bout of eccentric exercise induced an increase in RF muscle stiffness when measured at its lengthened position immediately after and at 48 H after exercise. Such change in muscle stiffness was not observed in VMO and VL. The force generating capacity of knee extensors at 48 H was negatively correlated with pre-exercise muscle stiffness of RF muscle. Maintaining high force-generating capacity after eccentric contractions is essential for athletes who need to play continuously. Findings from the present study suggest the important role of muscle stiffness in the development of muscle damage. Prophylactic program of stretching should aim for decreasing RF muscle stiffness which may help to keep relative high level of force generating capacity in the quadriceps muscle at 48 H after eccentric exercise although the underlying mechanism remains to be further investigated.

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Disclosure statement

There are no conflicts of interest to declare.

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Table 1 Peak isometric knee extensor torque and muscle soreness before (Pre), after (Post) and 48 hours (48 H) after exercise

Group		Peak torque Pre	Peak torque Post	Peak torque 48 H	Muscle soreness at 48 H
Control	(N·m)	144.6 ± 33.9	142.1 ± 33.9	145.3 ± 33.8	0
	%pre		98.3 ± 6.1	100.6 ± 3.6	
Exercise	(N·m)	149.7 ± 32.8	124.0 ± 37.1*	134.8 ± 35.6*	2.8 ± 1.9
	%pre		83.2 ± 9.7#	90.5 ± 8.9#	

* Significant difference from the pre-exercise value. # Significant difference from the control group. Values are expressed in mean ± standard deviation.

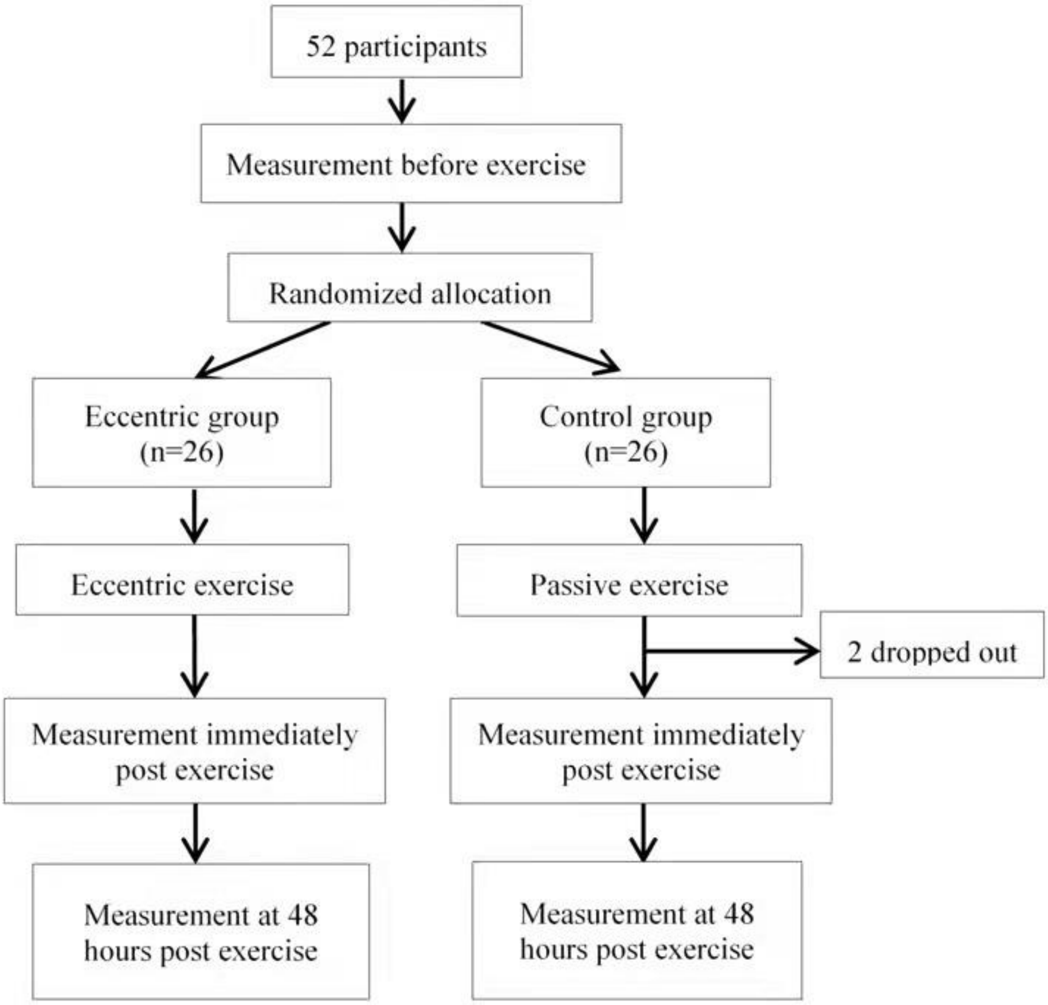

Figure 1 The study procedure.

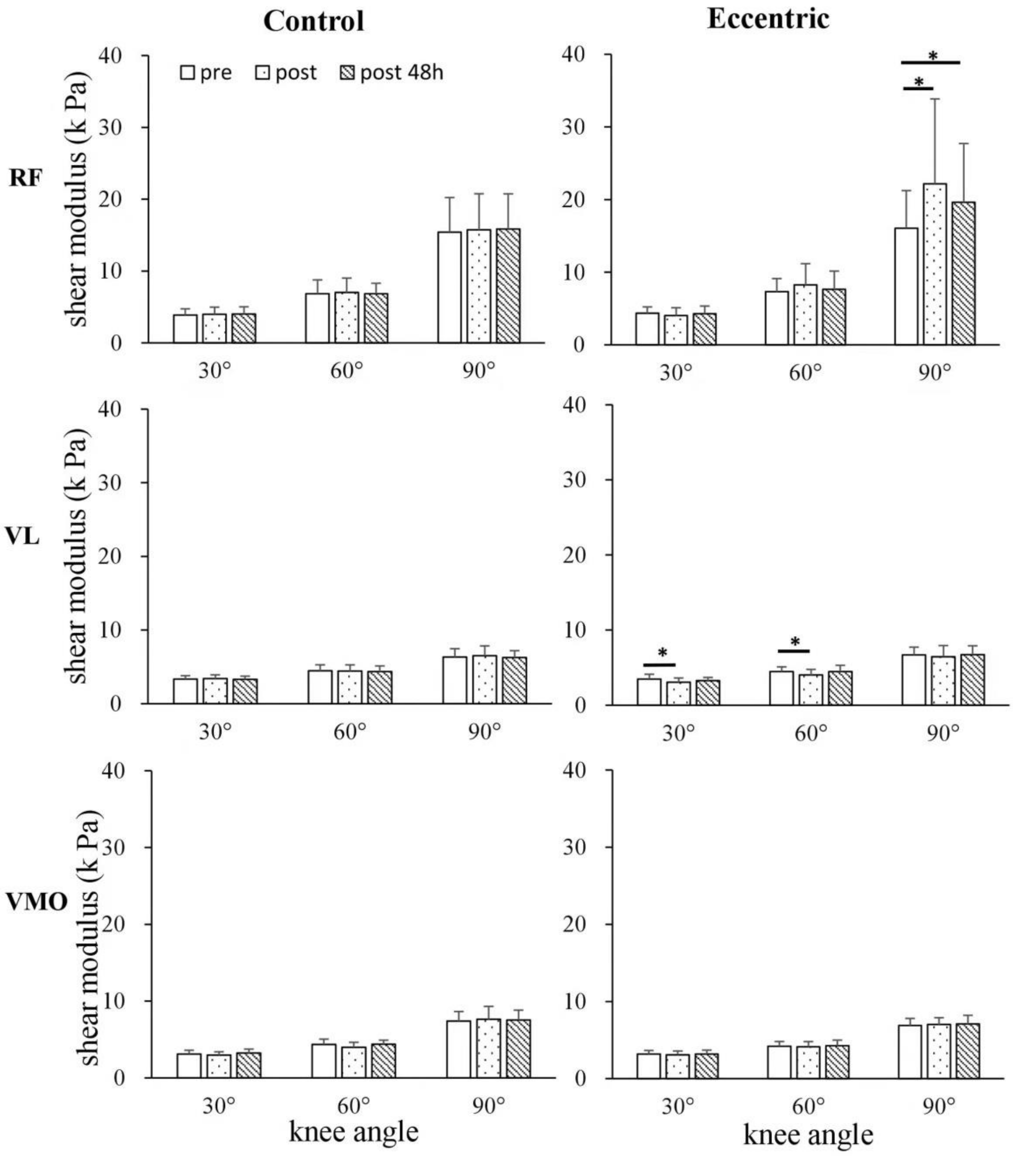

Figure 2 Muscle shear modulus measured at pre, post and at 48 H post- intervention. * indicates significant difference compared with pre-exercise. Note: RF: rectus femoris; VL: vastus lateralis; VMO: Vastus medialis oblique. * p ≤ .01.

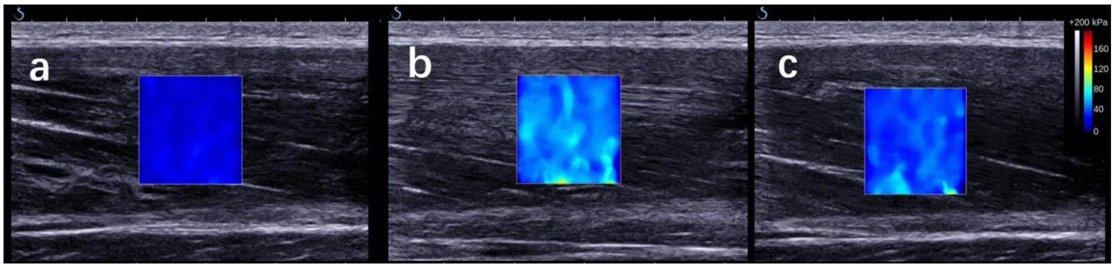

Figure 3 Typical elastography images of RF shear modulus measured at 90° of knee flexion before (a), immdiately after (b), and 48 H after (c) eccentric exercise.

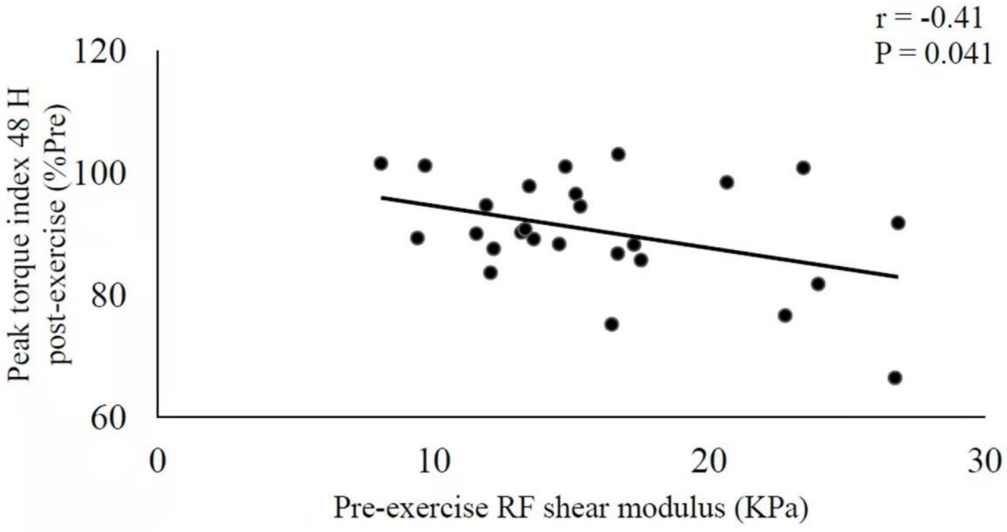

Figure 4 Plotting of peak torque index and RF shear modulus at 90° of knee flexion in participants receiving eccentric exercise. Note: RF: rectus femoris.