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ORIGINAL ARTICLE

Muscle damage and repeated bout effect induced by enhanced eccentric squats

SHORT TITLE: MUSCLE DAMAGE INDUCED BY ENHANCED ECCENTRIC SQUATS

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ABSTRACT

BACKGROUND: Muscle damage and repeated bout effect have been studied after pure eccentric-only exercise. The aim of this study was to evaluate muscle damage and repeated bout effect induced by enhanced eccentric squat exercise using flywheel device.

METHODS: Thirteen healthy males volunteered for this study. Creatine kinase blood activity (CK), quadriceps isometric peak torque and muscle soreness were used as markers of muscle damage. The dependent parameters were measured at baseline, immediately after and each day up to 96 hours after the exercise session. The intervention consisted of 100 repetitions of enhanced eccentric squat exercise using flywheel device. The same protocol was repeated after 4

weeks.
RESULTS: After the first bout, CK and muscle soreness were significantly greater ($P<0.05$) than baseline respectively up to 72 and 96 hours. Isometric peak torque was significantly lower ($P<0.05$) up to 72 hours. After the second bout, CK showed no significant increase ($P>0.05$), while isometric peak torque and muscle soreness returned to values similar to baseline after respectively 48 and 72 hours. All muscle damage markers were significantly lower after second compared to first bout.
CONCLUSIONS: The enhanced eccentric exercise induced symptoms of muscle damage up to 96 hours. However, it provided muscle protection after the second bout, performed four weeks later. Although it was not eccentric-only exercise, the enhancement of eccentric phase provided muscle protection.
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It is well known that unaccustomed eccentric-only exercise induces symptoms of muscle damage.[1-3] Exercise-induced muscle damage can be monitored using both invasive (*e.g.*, biopsy) and non-invasive measurements. Indirect markers have been proposed and are nowadays largely used in literature for evaluating the muscle damage. Increases in creatine-kinase blood activity (CK),[4] muscle soreness [5] and strength loss [6] are some of indirect muscle damage markers mainly used in literature.

After a second eccentric-only exercise bout, the markers of muscle damage dramatically decrease. Faster strength recovery, lower muscle soreness and lower CK blood activity have been measured after the second eccentric-only exercise.[7] It is well established that the first eccentric-only exercise bout protects muscle from muscle damage due to subsequent exercise sessions.[8] Such protection has been named "repeated bout effect". It has been shown to last up to six months, during which participants were not involved in any form of resistance exercise.[9] Even if the mechanisms underlying the repeated bout effect have not been totally clarified, inflammatory, neural or muscular explanations have been proposed, as previously reviewed.[10]

The previous studies that investigated the exercise-induced muscle damage and the repeated bout effect have used isokinetic [11] or dynamic constant external resistance [12, 13] eccentric-only exercises. Tesch *et al.* developed a flywheel device (YoYo® Technology, Stockholm, Sweden), in which concentric phase is weight-free and eccentric phase is enhanced by the inertia accumulated during the concentric phase.[15] Due to its gravity independence, it was originally designed for counteracting muscle atrophy and osteoporosis in spacemen involved in space flights.[15] However, further subsequent studies showed the effectiveness of enhanced eccentric training for inducing muscle strength and structure adaptations.[16, 17]

Recently, one study investigated muscle damage subsequent to enhanced eccentric a supine-squat exercise session.[18] However, in such study, only biochemical markers have been measured. In addition, the authors did not investigate the repeated bout effect. Therefore, the aim of the present study is to evaluate the exercise-induced muscle damage and the repeated bout effect following an enhanced eccentric squat exercise using a flywheel ergometer.

Materials and methods

This study was divided in two separate sessions and testing assessments. For investigating muscle damage, participants were tested at baseline, immediately after, and once a day up 96 hours after the exercise bout, for a total of 6 measurements (Figure 1). Then, to examine the magnitude of the repeated bout effect, the same protocol was repeated after 4 weeks. The length of the interval between the two bouts was selected according to the literature.[14] During such 4 weeks, the participants were strictly required to avoid any strenuous physical activity involving lower limbs. In order to get greater adherence to such instruction, these 4 weeks corresponded to off-season period. Each participant performed testing measurements at the same time, in order to avoid circadian parameters variations. Participants familiarized with both exercise and testing devices one day apart that preceded the first bout.

In order to investigate muscle damage induced by enhanced eccentric squat, muscle strength and soreness have been measured on quadriceps muscle. Although squat exercise involves several lower limb muscles, quadriceps has been used to estimate the effects of squat exercise.[19] In addition, greater quadriceps activation was found while squatting with flywheel device than conventional barbell squat.[16]

Participants

Thirteen healthy physically active young males, recruited among sport science students (mean age 21.7±3.1 years; mean weight 78.2±4.4 kg; mean height 1.80±0.2 m) were involved for this study. Knee, hip or ankle pains in the previous year were used as exclusion criteria, as well as any practice in regular resistance exercise in the previous six months. All subjects signed a written informed consensus and this study was previously approved by Ethical Committee of University of Verona. Finally, this study met the ethical standards of the journal.

Markers of muscle damage

BIOCHEMICAL MARKER

Creatine kinase (CK) blood activity was measured using capillary blood. Blood was collected in single 32 μL lithium heparin single use capillary pipette (Reflotron® PST, La Roche, Basel, Switzerland) after pricking a finger with a sterile single-use lancing device (Accucheck, La Roche). Heparinized blood was pipetted to the test strip (Reflotron® CK, La Roche) immediately after collection and then inserted in the device optical reader (Reflotron® Plus, La Roche). Test strips were stored in a refrigerator at +4°C and the optical reader was checked daily according manufacturer's instructions.

ISOMETRIC PEAK TORQUE

Participants performed peak torque measurements on isokinetic dynamometer (Cybex, Lumex, Ronkokoma, NY, USA). The device was calibrated and gravity correction executed according manufacturer procedures. Subjects were seated on dynamometer, with trunk, shoulders and tested knee secured by belts. Knee was properly aligned to the center of rotation. A lever immobilized the untested limb. Isometric peak torque was investigated at 60° of knee joint flexion, considering 0° as full knee extension. Participants received standardized encouragements by operator to maximally perform the test. Three repetitions were performed and the peak torque was inserted in data analysis. Only the dominant limb was tested.

MUSCLE SORENESS

A visual analogic scale was assessed to detect soreness during quadriceps palpation.[20] It consisted in a 100-mm line with "no pain" at the left margin and "extremely painful" at the right margin. Participants were seated and knee angle was 90°, while limb was totally relaxed. Palpation was standardized by operator at 50% of femur length. Participants were instructed to indicate their pain sensation marking this line. Subsequently, operator measured the distance between the left margin and the participants' answer and such distance was inserted in data analysis. Only the dominant limb was tested.

Intervention

Intervention consisted in 10 sets x 10 repetitions of squat using flywheel ergometer. Participants were instructed to perform the concentric phase as fast as possible and the eccentric phase until the knee angle was approximately 90°. Two mirrors were placed in front of participants and on their side, working as visual feedback for participant's self-monitoring squatting technique. In addition, an operator monitored each repetition. Peak and average power for each repetition were recorded using an encoder (SmartCoach™, SmartCoach Europe AB, Stockholm, Sweden) and the related software (SmartCoach™ v.3.1.8.0). A real-time monitor recorded the power output for each repetition and it was showed as visual feedback for exerting each repetition maximally. In addition, all participants were strongly and standardly encouraged to maximally perform each repetition. The recovery among sets was 60 seconds.

Statistical analysis

Sphericity assumption was analyzed used Mauchly's W test. Normality was analyzed using Shapiro-Wilk test. Dependent parameters changes were analyzed by two–way repeated measure ANOVA (time × bout) performed using SPSS v.20.0 (SPSS Inc., IBM Corp., Armonk, NY, USA). Post-hoc analysis using Bonferroni's correction was then calculated to investigate factors time (6 levels) and bout (2 levels). Difference in average power between first vs second bout were analyzed using a pair-wise *t*-test. Significance was set at $P<0.05$. Data are shown as mean ± SD. Post-hoc differences are shown using effect size (ES) and confidence intervals (95% CI). According the Cohen's standard, an ES=0.3 was considered as small, ES=0.5 as medium and ES=0.8 as large.

Results

Average power

No difference in average power resulted comparing first *vs*. second bout in concentric (respectively 248±83 and 275±92 W, P=0.536) and eccentric phases (respectively 462±121 and 498±137 W, P=0.741).

CK blood activity

The time-course of the CK blood activity values is shown in figure 2. No significant time x bout interaction (P=0.646) was found for the CK blood activity. As a within-bout comparison, after the first bout, compared to baseline, CK blood activity was significantly higher after 24 (ES=1.1, 95% CI: 0.3-1.9, P=0.006), 48 (ES=1.1, 95% CI: 0.1-2.0, P=0.003) and 72 hours (ES=0.9, 95% CI: 0.2-1.7, P=0.020) (Figure 2). Compared to baseline, after the second exercise bout, CK blood activity did not result in different values up to 96 hours (Figure 2). As a between-bouts comparison, Compared to the first bout, after the second bout, CK blood activity was significantly lower after 48 (ES=0.6, 95% CI: 0.1-1.1, P=0.018) and 72 hours (ES=0.6, 95% CI: 0.0-1.1, P=0.034) (Figure 2).

Isometric peak torque

The time course of isometric peak torque values is shown in Figure 3. A significant time × bout interaction (P=0.005) was found for the isometric peak torque. As a within-bout comparison, after the first bout, compared to baseline, isometric peak torque was significantly lower immediately after exercise (ES=2.8, 95% CI: 0.8-4.8, P=0.000), after 24 (ES=2.0, 95% CI: 0.5-3.5, P=0.000), 48 (ES=1.2, 95% CI: 0.2-2.2, P=0.001) and 72 hours (ES=0.7, 95% CI: 0.0-1.4, P=0.014) (Figure 3). After the second bout, compared to baseline, isometric peak torque was significantly lower immediately after exercise (ES=0.6, 95% CI: 0.3-0.8, P=0.000) and after 24 hours (ES=0.4, 95% CI: 0.0-0.7, P=0.049) (Figure 3). As a between-bouts comparison, compared to the first bout, after the second bout, isometric peak torque was significantly greater after 24 (ES=0.4, 95% CI: 0.0-0.6, P=0.029), 48 (ES=0.5, 95% CI: 0.2-0.8, P=0.001), 72 (ES=0.9, 95% CI: 0.5-1.4, P=0.000) and 96 hours (ES=0.6, 95% CI: 0.3-0.9, P=0.002) (Figure 3).

Muscle soreness

The time course of muscle soreness values is shown in Figure 4. Significant time x bout interaction (P=0.014) was found for the muscle soreness. After the first bout, compared to the baseline, muscle soreness was significantly greater after 24 (ES=5.8, 95% CI: 2.9-8.7, P=0.000), 48 (ES=6.6, 95% CI: 3.4-9.7, P=0.000), 72 (ES=4.6, 95% CI: 2.0-7.1, P=0.001) and 96 hours (ES=3.5, 95% CI: 1.0-6.0, P=0.004) (Figure 4). After the second bout, compared to baseline, muscle soreness was significantly greater after 24 (ES=3.1, 95% CI: 0.2-6.1, P=0.034) and 48 hours (ES=4.4, 95% CI: 0.3-8.4, P=0.030) (Figure 4) Compared to the first bout, after the second bout, muscle soreness was significantly lower after 24 (ES=1.0, 95% CI: 0.5-1.5, P=0.002), 48 (ES=1.0, 95% CI: 0.5-1.4, P=0.001), 72 (ES=1.1, 95% CI: 0.6-1.6, P=0.000) and 96 hours (ES=1.4, 95% CI: 0.9-1.9, P=0.000) (Figure 4).

Discussion

To the best of our knowledge, we evaluated for the first time muscle damage and repeated bout effect after enhanced eccentric squat exercise using a flywheel device. We showed that muscle damage markers significantly increased up to 96 hours after the first bout. However, after the second bout, significant decreases in muscle damage markers occurred.

Eccentric exercise induced muscle damage has been largely investigated in previous studies.[21-23] Data existing in literature showed that muscle damage markers resulted above the baseline up 96 hours or more.[24] It is generally accepted that muscle damage is induced only by lengthening contraction.[1] Indeed, when sarcomeres are on the descending limb of the force-length curve, they are instable and more prone to damage.[25] Confirming such hypothesis, no muscle damage was found after “concentric-only exercise”,[26] “concentric cycling” [27] or “step-up-only exercise”.[28] Interestingly, no muscle damage symptoms occurred after traditional “concentric-eccentric exercise”.[29] Even if the exercise in the present investigation was not an eccentric-only exercise, enhancing the eccentric phase led to increases in muscle damage markers. Similarly to our outcomes, increases in CK and lactate dehydrogenase blood activity up to 72 hours were found after one bout of supine squat exercise using flywheel device.[18]

After the first exercise bout, the CK blood activity was significantly higher than baseline up to 72 hours.[30] The increases in CK blood activity reflect fiber necrosis. After fiber disruption, the

CK is first released in the lymphatic system and then moved in the blood flow.[1] Although CK showed great inter-subjects variability,[31] our results were quite homogeneous. It could in part depends on the physically active population involved in the present investigation, which showed low CK blood activity values, as reported in previous studies.[14, 32] Indeed, when untrained participants underwent to eccentric exercise, CK blood activity values resulted two-fold greater.[4]

The isometric peak torque resulted significantly lower than baseline up to 72 hours. The mechanisms that affect the capacity of muscle to generate force are not fully understood. However, it has been proposed that inhomogeneous sarcomeres disruption could have led to a inability to develop maximal force using contractile elements.[33] Strength loss is one of the most valid and reliable muscle damage markers.[1] The amounts of strength deficit in this study were around 15% compared to baseline. Such strength deficits are lower than those reported in literature, in which participants experienced almost 50% of strength loss.[14] However, in such study, participants performed the eccentric-only exercise using single-joint isokinetic or isometric-load devices. Involving the squat exercise numerous lower limbs muscles, a sort of load turnover among quadriceps and other synergist muscles (*e.g.*, hip extensors) could have limited the quadriceps effort and consequently its strength loss.[19]

The muscle soreness remained significantly above the baseline up to 96 hours, as already reported in the literature.[24] It is thought that muscle soreness could derive from mechanical, rather than chemical, factors.[20] Indeed, the pain could be part of the mechanisms that protect muscle from a more dangerous damage subsequent to further maximal muscle contractions.[34] Confirming such hypothesis, it has been shown that nociceptors affected the motor cortex excitability by decreasing the motor evoked potential induced by transcranial magnetic stimulation. [35]

All the muscle damage markers, compared to the first bout, were significantly lower after the second bout performed 4 weeks later. Previous studies showed that the protective effect of eccentric-only exercise was effective after 4 weeks,[14, 36] and it is known that it can last up to 6 months.[9] The amounts of the initial muscle damage seemed to influence the magnitude of the repeated bout effect.[37] Interestingly, even a light eccentric-only exercise, not followed by muscle damage symptoms, protected muscle from a more intense eccentric-only bout.[13] In the present investigation, after the first bout, the muscle damage symptoms resulted smaller compared to the literature.[14] However, similarly to what previously reported,[13] the first enhanced eccentric exercise conferred protection to the muscles involved in a further subsequent enhanced eccentric bout. The repeated bout effect is typical of eccentric exercise. Indeed, when a concentric-only exercise was carried out before an eccentric-only exercise session, greater muscle damage symptoms were measured compared to eccentric-only exercise performed for the first time.[38] Therefore, enhancing the eccentric exercise generated a protective effect, which lasted for at least 4 weeks.

The mechanisms involved in the repeated bout effect are not fully understood. Three main hypothesis were reviewed for explaining the repeated bout effect.[10] Some authors suggested that an addition of in series-sarcomere after the first eccentric-only exercise can occur, which causes a rightward shift of the force-length relationship.[33] Therefore, the greater force produced in the descending limb can reduce the risk of sarcomeres overstretching.[39] Other authors suggested that the torque exerted during the eccentric-only exercise is initially distributed among a low number of motor units.[5] Based on a study that showed a selective recruitment of fast-twitch motor units during the eccentric contraction,[40] it was hypothesized that a greater recruitment of slow-twitch motor units could be recruited starting since the second eccentric exercise session.[5] Therefore, a torque exertion re-distribution could involve a higher number of motor units, decreasing the pro-fiber mechanical stress and consequently the risk of sarcomeres over-stretching. Finally, other authors hypothesized a re-arrangement of the non-contractile elements properties.[41] Particularly, the greater muscle stiffness found after eccentric exercise, can reduce the risk of muscle damage.[41] Although each hypothesis showed its strength and weakness points,[10] within this study design we are not able to indicate one of them as more likely.

Conclusions

In conclusion, the enhanced eccentric exercise induced symptoms of muscle damage up to 96 hours after the first bout. When, 4 weeks later, the same bout was performed, muscle damage symptoms were significantly smaller compared to the first bout. The enhanced eccentric exercise squat performed using a flywheel ergometer provided muscle protection from the second bout performed 4 weeks later.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Titles of figures

Figure 1.—The procedures for muscle damage evaluation are shown. The entire protocol was repeated after 4 weeks.

Figure 2.—CK blood activity time course is showed after first and second bouts. Differences within and between-subjects were analyzed by two-way repeated measures ANOVA.

*$P<0.05$ compared to baseline; $^{\#}P<0.05$ compared to the second bout.

Figure 3.—Isometric peak torque time course is showed after first and second bouts. Differences within and between-subjects were analyzed by two-way repeated measures ANOVA.

*$P<0.05$ compared to baseline; $^{\#}P<0.05$ compared to the first bout.

Figure 4.—Muscle soreness time course is showed after first and second bouts. Differences within and between-subjects were analyzed by two-way repeated measures ANOVA.

*$P<0.05$ compared to baseline; $^{\#}P<0.05$ compared to the second bout.